The role of *Phyllophora crispa* (HUDSON) P.S. DIXON 1964 mats (Rhodophyta) as hotspots for sessile invertebrate biodiversity in the Mediterranean Sea

Doctoral Thesis by

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"Beim Anblick des Meeres glaubt man,
einen alten Freund zu sehen, das Herz
öffnet sich, die Phantasie erblüht in
tausenderlei Vorstellungen menschlichen
Austauschs, der Erleichterung, der
Hoffung auf Freunde, auf Rückkehr unter
die Seinen"

- A. v. Humboldt

Abstract

The Mediterranean Sea, characterized by its geographically isolated location and geological history, harbors more than 17,000 eukaryotic marine species, most of which are concentrated in ecosystems recognized as biodiversity hotspots, such as seagrass meadows and coralligenous reefs. The associated benthic organisms of these biodiversity hotspots depend on some key species to build the surrounding habitat and create favorable niches for a thriving community. Hence, these species are also known as ecosystem engineers. One of the essential and best-investigated ecosystem engineers in the Mediterranean Sea is the seagrass *Posidonia oceanica,* which covers more than 1,000,000 ha along the Mediterranean shelf. Many well-studied and recognized hotspots are facing threats through global change and local impacts, e.g., in the case of *P. oceanica* meadows, leading to a decline of 34% in the last 50 years. Recently, persistent, mat-forming fleshy red algae, previously described for the Black Sea and several Atlantic locations, have also been observed to cover some areas in the Mediterranean Sea.

Pilot studies indicate that benthic mats formed by the scarcely investigated fleshy red alga *Phyllophora crispa* also host a high diversity of benthic organisms. For instance, some of the key sessile taxa recently reported for these mats are serpulid polychaetes and bryozoa, which live in dense epiphytic communities on the red algae thalli. Yet, a holistic overview of the associated taxa, comprising the sessile community, their abundance, species richness, and spatial variation, is missing. In addition, the distinct role of *P. crispa* as an ecosystem engineer and its capabilities to shape environmental conditions for benthic organisms is still unknown. To address these gaps and to evaluate the potential of *P. crispa* as a novel biodiversity hotspot, we conducted an *in-situ* study, encompassing a comparative analysis of associated sessile communities and assessed environmental gradients inside *P. crispa* mats and *P. oceanica* meadows in the northern Tyrrhenian Sea. The key environmental factors assessed were O_2 concentration, pH, Chl α, water movement, temperature, and light intensity. We comparatively and simultaneously measured in *P. crispa* mats, in neighboring *P. oceanica* seagrass meadows, on neighboring bare rocky substrates, and in the directly overlying water column.

We show that the biodiversity of sessile organisms in these red algae mats is high and exceeds that of neighboring seagrass meadows. We recorded 312 distinct sessile phenotypes (covering nine higher taxa) for both *P. crispa* and *P. oceanica*; 223 occurred on *P. crispa* mats and 179 on *P. oceanica* plants. Approximately 25% more phenotypes were found in *P. crispa* mats than in the neighboring *P. oceanica* seagrass meadow holobionts. The most abundant taxa on *P. crispa* were bryozoa, foraminifera, and serpulid polychaetes. Detailed analyses of these taxa confirmed significantly higher numbers of taxa and densities of individuals, resp., colonies identified in *P. crispa* mats than in *P. oceanica* meadows. The overall density of sessile organisms was three times higher on *P. crispa* than on *P. oceanica*.

Calculations of classical diversity indices further suggest *P. crispa* as a hotspot of sessile benthic diversity comparable to traditional biodiversity hotspots, such as seagrass or Mediterranean coralligenous reefs. We further show that *P. crispa* significantly reduces water movement and light levels compared to the overlaying water. Surprisingly, *P. crispa* increased the water temperature by 0.3 °C, while the water temperature in *P. oceanica* and on bare rocky substrates was reduced by 0.5 °C. *P. crispa* mats also showed higher O_2 and Chl α concentrations, whereas pH was lower than in *P. oceanica* meadows.

Our results imply that *P. crispa* mats host an exceptionally high diversity of associated sessile organisms equivalent to or even higher than those associated with seagrass meadows. In conclusion, fleshy red algae mats may act as alternative habitats and temporary sessile biodiversity reservoirs in times of environmental change and degradation of classical biodiversity hotspots. The supraregional distribution of *P. crispa* and further assessment of the associated mobile fauna remains to be addressed in future research efforts.

Zusammenfassung

Das durch seine geografisch isolierte Lage und geologische Geschichte gekennzeichnete Mittelmeer beherbergt mehr als 17.000 eukaryotische Meeresarten, von denen die meisten in Ökosystemen konzentriert sind die als Biodiversitäts-Hotspots anerkannt sind, wie z.B. Seegraswiesen und Coralligène. Die assoziierten benthischen Organismen dieser Biodiversitäts-Hotspots sind in der Regel auf Schlüsselarten angewiesen, welche Siedlungsraum bereitstellen und ökologische Nischen für eine diverse Gemeinschaft schaffen. Daher werden diese Arten auch als Ökosystemingenieure bezeichnet. Einer der wichtigsten und am besten untersuchten Ökosystemingenieure im Mittelmeer ist das Seegras *Posidonia oceanica*, welches mehr als 1.000.000 ha entlang des Mittelmeerschelfs bedeckt. Viele gut untersuchte und anerkannte Hotspots sind durch Klimawandel und lokale Auswirkungen bedroht, wie z.B. die oben genannten *P. oceanica* Wiesen, was zu einem Rückgang von 34 % dieses Lebensraums in den letzten 50 Jahren geführt hat. Kürzlich wurden in einigen Gebieten des Mittelmeeres ausdauernde, mattenbildende Rotalgen, die zuvor für das Schwarze Meer und mehrere atlantische Standorte beschrieben wurden, an verschiedenen Standorten beschrieben.

Pilotstudien weisen darauf hin, dass benthische Matten, die von der kaum untersuchten fleischigen Rotalge *Phyllophora crispa* gebildet werden, auch eine große Vielfalt an sessilen Organismen beherbergen. Beispielsweise sind einige der wichtigsten sessilen Taxa, die kürzlich in diese Matten gefunden wurden, serpulide Polychaeten und Bryozoen, die in dichten epiphytischen Gemeinschaften auf den Rotalgen-Thalli leben. Es fehlt jedoch ein ganzheitlicher Überblick über die zugehörigen Taxa, welcher die sessile Gemeinschaft, ihre Häufigkeit, ihren Artenreichtum und ihre räumliche Variation umfasst. Darüber hinaus ist die besondere Rolle von *P. crispa* als Ökosystemingenieur und seine Fähigkeiten, die Umweltbedingungen für benthische Organismen zu gestalten, noch unbekannt. Um diese Lücken zu schließen und das Potenzial von *P. crispa* als neuartigen Biodiversitäts-Hotspot zu bewerten, führten wir im nördlichen Tyrrhenischen Meer eine in situ Studie durch, welche zum ersten Mal eine vergleichende Analyse der damit verbundenen sessilen Gemeinschaften umfasste, sowie die Umweltgradienten innerhalb von *P. crispa* Matten und *P. oceanica* Wiesen analysierte. Die bewerteten Umweltfaktoren waren O_2-Konzentration, pH-Wert, Chl α, Wasserbewegung, Temperatur und Lichtintensität. Wir haben vergleichend und gleichzeitig in *P. crispa* Matten, in benachbarten *P. oceanica* Seegraswiesen, auf benachbarten unbewachsenen felsigen Substraten und in der direkt darüber liegenden Wassersäule gemessen.

Wir zeigen, dass die Biodiversität festsitzender Organismen in diesen Rotalgenmatten sehr hoch ist und die der benachbarten Seegraswiesen übersteigt. Wir haben 312 verschiedene sessile Phänotypen (zugehörig zu neun höheren Taxa) sowohl für *P. crispa* als auch für *P. oceanica* aufgezeichnet; 223

traten auf *P. crispa* Matten und 179 auf *P. oceanica* Pflanzen auf. Ungefähr 25 % mehr Phänotypen wurden in *P. crispa* Matten gefunden als in den benachbarten *P. oceanica* Seegraswiesen. Die am häufigsten vorkommenden Taxa auf *P. crispa* waren Bryozoen, Foraminiferen und serpulide Polychaeten. Detaillierte Analysen dieser Taxa bestätigten signifikant höhere Anzahlen von Phänotypen und Individuen- bzw. Koloniedichten in *P. crispa* Matten als in *P. oceanica* Wiesen. Die Gesamtdichte der sessilen Organismen war auf *P. crispa* dreimal höher als auf *P. oceanica*. Berechnungen klassischer Diversitätsindizes deuten ferner darauf hin, dass *P. crispa* ein Hotspot für sessile benthische Diversität ist, vergleichbar mit traditionellen Hotspots, wie Seegraswiesen oder Coralligène im Mittelmeerraum. Wir zeigen weiter, dass *P. crispa* die Wasserbewegung und die Lichtverhältnisse im Vergleich zum überlagernden Wasser signifikant reduziert. Überraschenderweise erhöhte *P. crispa* die Wassertemperatur um 0,3 °C, während die Wassertemperatur in *P. oceanica* und auf nackten felsigen Substraten um 0,5 °C reduziert wurde. *P. crispa* Matten zeigten auch höhere O_2- und Chl α-Konzentrationen, während der pH-Wert niedriger war als in *P. oceanica* Wiesen.

Unsere Ergebnisse implizieren, dass *P. crispa*-Matten eine außergewöhnlich hohe Diversität assoziierter sessiler Organismen beherbergen, die der von Seegraswiesen mindestens gleichwertig ist. Zusammenfassend lässt sich sagen, dass die untersuchten Rotalgenmatten in Zeiten von Umweltveränderungen und dem Verlust klassischer Biodiversitäts-Hotspots als alternative Lebensräume und temporäre Biodiversitätsreservoirs für sesshafte Organismen fungieren können. Die überregionale Verbreitung von *P. crispa*, sowie die weitere Untersuchung der assoziierten mobilen Fauna müssen in zukünftigen Forschungsanstrengungen angegangen werden.

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List of publications and manuscripts

Publications and manuscripts included in this thesis

Schmidt N, El-Khaled YC, **Rossbach FI**, Wild C (2021) Fleshy red algae mats influence their environment in the Mediterranean Sea. Front Mar Sci 8:1–12. doi: 10.3389/fmars.2021.721626

Rossbach FI, Casoli E, Beck M, Wild C (2021) Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes. Diversity 13:1–16. doi: 10.3390/d13060265

Rossbach FI, Merk B, Wild C (2021) High Diversity and Abundance of Foraminifera Associated with Mediterranean Benthic Red Algae Mats. Diversity 14:1–15. doi: 10.3390/d14010021

Rossbach FI, Casoli E, Plewka J, Schmidt N, Wild C (2022) New Insights into a Mediterranean Sea Benthic Habitat: High Diversity of Epiphytic Bryozoan Assemblages on *Phyllophora crispa* (Rhodophyta) Mats. Diversity 14:1–16. doi: 10. 3390/d14050346

El-Khaled YC, Daraghmeh N, Tilstra A, Roth F, Huettel M, **Rossbach FI**, Edoardo Casoli E, Koester A, Beck M, Meyer R, Plewka J, Schmidt N, Winkelgrund L, Merk B, Wild C (2022) Fleshy red algae mats act as temporary reservoirs for sessile invertebrate biodiversity. Comm Biol. doi: 10.1038/s42003-022-03523-5

Additional publications and manuscripts related to this thesis

Rossbach FI, Casoli E, Beck M, Wild C (submitted) A new hope? Mediterranean Algae mats are thriving with life. Front. Young Minds

Additional publications and manuscripts during the time of study

Rossbach S, **Rossbach FI**, Häussermann V, Försterra G, Laudien J (2021) *In situ* Skeletal Growth Rates of the Solitary Cold-Water Coral *Tethocyathus endesa* From the Chilean Fjord Region. Front. Mar. Sci. 8:757702. doi: 10.3389/fmars.2021.757702

Rossbach S, Arossa S, Geraldi NR, Klein SG, Kheng Lim K, Martin C, **Rossbach FI**, Shellard MJ, Steckbauer A, Valluzzi L, Duarte CM (in review) A New Geography of Ocean Hope. OneEarth.

Declaration of the contribution of the candidate to a multi-author article/manuscript, which is included as a chapter in the submitted doctoral thesis

The contribution of the candidate is given in % of the total workload (up to 100 % for each category)

Chapter 2 – Fleshy red algae mats influence their environment in the Mediterranean Sea

Experimental concept and design	100 %
Experimental work and data acquisition	80 %
Data analysis and interpretation	20%
Preparation of figures and tables	0 %
Drafting of the manuscript	20 %

Chapter 3 – Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes

Experimental concept and design	100 %
Experimental work and data acquisition	60 %
Data analysis and interpretation	90 %
Preparation of figures and tables	100 %
Drafting of the manuscript	80 %

Chapter 4 – High diversity and abundance of foraminiferans associated with Mediterranean benthic red algae mats

Experimental concept and design	100 %
Experimental work and data acquisition	60 %
Data analysis and interpretation	100 %
Preparation of figures and tables	100 %
Drafting of the manuscript	90 %

Declaration of the contribution of the candidate to a multi-author article/manuscript, which is included as a chapter in the submitted doctoral thesis

Chapter 5 – New Insights into a Mediterranean Sea Benthic Habitat: High Diversity of Epiphytic Bryozoan Assemblages on Phyllophora crispa (Rhodophyta) Mats

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Experimental work and data acquisition	60 %
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Chapter 6 – Fleshy red algae mats act as temporary reservoirs for sessile invertebrate biodiversity

Experimental concept and design	100 %
Experimental work and data acquisition	80 %
Data analysis and interpretation	10 %
Preparation of figures and tables	10 %
Drafting of the manuscript	20 %

Date:

Signature:

Chapter 1 – General introduction

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Introduction

Due to its unique historical and geographical situation, the Mediterranean Sea (lat. "sea between the lands") is well known to harbor a high level of biological diversity accompanied by a high rate of endemism, both accounting for the term "biodiversity hotspot"[1, 2, 3]. Hosting between 4 to 18% of the world's marine biodiversity, the Mediterranean Sea biome is among the 25 most important biodiversity hotspots[2,4]. The Mediterranean Sea's typical, highly diverse habitats are coastal benthic habitats, including seagrass beds, coralligenous assemblages, and other hard-bottom communities[1,5]. While total coastal areas lining the world's oceans comprise 7.6% of their surface area, the Mediterranean Sea shelf accounts for 20% of its surface, underlining the significance of the encompassed habitats[6]. Marine biodiversity is pivotal for the stability of the surrounding ecosystems[7]. Healthy ecosystems, in return, provide various economically important services for adjacent regions, ranging from fisheries to tourism and sources for pharmaceutic products[8–10]. Currently, the marine biodiversity in the Mediterranean Sea is threatened by the effects of global change and local factors, such as plastic pollution and habitat loss[11–16]. Protecting areas of important ecosystems can alleviate these adverse effects[17,18]. Therefore, identifying and investigating new potential biodiversity hotspots is crucial for efficient mitigation strategies.

Important communities & biodiversity hotspots in the Mediterranean Sea

Hard-bottom communities

Highly diverse and thriving communities are ubiquitous around the rocky bottoms of the Mediterranean Sea[19]. Their community assemblages change with depth, depending on abiotic factors, such as currents and light availability. Benthic assemblages are dominated by autotrophic macroalgae and sessile organisms comprising all phyla. While algae strongly depend on light to perform photosynthesis, the occurrence of sessile organisms is mainly determined by currents as part of their different feeding strategies. The effects of those two abiotic factors change with increasing depth and are responsible for a typical zonation along these gradients[20,21]. Sessile organisms are of paramount importance as they transfer biomass and energy from the water column to the benthic community by feeding on suspended food particles[22]. We can distinguish between two main groups: active and passive suspension feeders. Active suspension feeders (e.g., bryozoans, sponges) can whirl surrounding water to catch food particles (POM[a] and plankton), while passive filter feeders (e.g., corals) rely on currents that provide a constant supply of food[20,21]. Three main zones can be distinguished according to their specific community:

1) Shallow, light-flooded areas with strong, turbulent currents/wave action (upper infralittoral) are dominated by flat macroalgae turfs (e.g., *Cystoseira* spp.)[23,24]. Slow-growing, sessile animals only find a place in shaded crevices and show encrusting growth to avoid damage by the strong water movement (e.g., bryozoans, sponges, small scleractinians). Exceptions are cnidarians with symbiotic algae (e.g., *Eunicalla singularis* and *Cladocora caespitosa*) that can prevail next to the macroalgae[19,25].
2) Intermediate slopes with occasionally strong but laminar currents and declining light conditions (lower infralittoral), where slower-growing macroalgae like *Halimeda tuna* share the surface with sessile invertebrates[26,27]. Numerous macroalgae species serve as a substrate for sessile invertebrates, vastly increasing sparse settling ground and sustaining an entire epifauna community[23,28,29]. Passive filter feeders like Gorgonians (mainly *Eunicella cavolinii* and, in deeper habitats, the sciaphilic[b] *Paramuricea clavata*)[30] and Sabellidae grow with flexible structures into the water column to catch food from the currents[31–33]. Gorgonians can form dense forests that foster benthic biodiversity and ecosystem stability[30,34].

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[a] POM: **P**articulate **O**rganic **M**atter

[b] Sciaphilic: “shade-loving“; plants and algae that grow in shady habitats. Ancient Greek σκῐά (skiá, “shade”) + φιλιά (philia, “love“).

3) Only slow-growing, sciaphilic algae can assert themselves to create a unique habitat of their own (see Coralligenous assemblages) in the darker circalittoral zone. Predominant sessile organisms are active suspension feeders (mainly sponges and bryozoans)[35] that do not rely on currents to transport food and oxygen. The absence of turbulence and currents enables many species to build delicate and fragile calcareous structures into the water column[35].

Seagrass meadows

Meadows of the endemic seagrass species *Posidonia oceanica* (L.) DELILE cover vast areas around the Mediterranean coastline, ranging from 1 – 40 m in depth, with each meadow covering up to several km^2. This habitat substantially shapes the Mediterranean Sea and is home to more than 400 plant species and thousands of animals that rely on it as a spawning ground, nursery, or permanent habitat[36,37,38]. The occurrence of the dependent organisms can be subject to changes along temporal and spatial gradients[39], and thus numbers vary between less than 100[27] and more than 300[40] species with seagrass as their natural habitat. The main groups of vagile fauna within the meadows are mollusks, polychaetes, and crustaceans[41,42,43].

Macroalgae beds

Fleshy macroalgae of the Laminariales and Fucales orders are important key species, dominating many hard-bottom communities in temperate areas. In the northwest Mediterranean Sea, the structural complexity of *Cystoseira* species provides shelter and secondary settling grounds to a diverse invertebrate community with alpha diversity of up to 174 invertebrate species per 400 cm^2 [23]. Benthic macroalgae are important primary producers, although limited to coastal areas and continental shelves[44]. In contrast to solitary macroalgae, turf systems are complex structures with distinct layers of different functions and biogeochemical properties[23,45]. Turf-forming macroalgae provide additional ecosystem services as bioengineers for diversity hotspots[46], particle filters, and have recently received some attention regarding carbon burial ("blue carbon")[47,48].

Coralligenous assemblages

Coralligenous assemblages are mainly built by encrusting red macroalgae in company with calcareous and eroding invertebrates[49]. The resulting reef structures are characterized by a high micro spatial variability and are typically located between seagrass beds and coastal muddy bottoms. Conservative estimations based on various studies suggest a total number of up to 1,666 species, including macroalgae (315)[46], invertebrates (1,241), and fish (110 – 125)[50]. Hence, this structurally complex habitat is home to one of the crucial biodiversity hotspots in the Mediterranean Sea.

Ecosystem engineers

Many of these communities and biodiversity hotspots depend on ecosystem engineers — organisms that create, modify or destroy their habitat and consequently affect the availability of resources to other species. Autogenic engineers alter their environment by modifying themselves (e.g., trees, kelp), while allogenic species actively change their physical environment (e.g., beavers)[51]. With their actions, ecosystem engineers can extend a species range across environmental gradients, altering their fundamental niche[52] (for instance, beavers changing meadows and running streams into ponds and wetlands, shifting the environment to a more favorable place for aquatic species). To create a biodiversity hotspot with increased species richness, the engineer species must create surroundings that are unavailable elsewhere and host specialized species that cannot thrive elsewhere than in the engineered areas[53].

Coralline red algae as bioengineers

The main species of coralline red macroalgae in the Mediterranean Sea are *Mesophyllum lichenoides* (Ellis) Lemoine & *Pseudolithophyllum expansum* (sensu Lemoine) - slow-growing, sciaphilic species and thus typically found in greater depths (30 – 70 m[54]) or fringing shaded caverns and overhangs. However, the coralligenous assemblages can reach an impressive thickness of 3 – 4 m and are widely distributed all around the Mediterranean coastline (with a possible exception along the Lebanese and Israeli coasts)[55]. The growth rate of *M. lichenoides* has been estimated to be between 0.11 and 0.26 mm y^{-1} [56,57]. Net growth rates of recent constructions range from 0 to 0.42 mm y^{-1}, mainly depending on light availability (depth) and sedimentation effects[50]. The constructing red algae are not subject to significant seasonality, while the activity and biomass of the related community (soft algae and invertebrates) change throughout the year. Although the biodiversity is directly affected by the accompanied soft algae (and thus varies with depth), the framework (up to 400 % surface coverage[50]) of the structural base is built up by encrusting red algae as an essential ecosystem engineer.

Gaps of knowledge

In the northwest Mediterranean Sea, the red macroalgae *Phyllophora crispa* (HUDSON) P.S. DIXON, 1964[58] (see Box 1) has been identified as an essential mat-forming species and potential biodiversity hotspot[29,59,60] (Figure 1 A – C). Fields of these macrophytes have been proven to host high biodiversity in the Black Sea before[61]. In the Mediterranean Sea, the sciaphilic *P. crispa* has been observed to form dense mats (Figure 1 B) of up to 100 % coverage at depths between 15 – 35 m[29]. These mats have been reported to harbor diverse communities of sessile organisms, including serpulidae[72], foraminifera, and bryozoa[29], among the most dominant groups (Figure 1 C). The Gorgonian forests, sharing the same substrate, have recently been reported to suffer from climate change-induced mass dieoffs[13,15,62,63], leading to potential phase shifts in this particular habitat[13,64]. It remains unclear to what extent the macroalgae *P. crispa* hosts biodiversity across different phyla and how the inhabiting communities compare to neighboring established hotspots (i.e., *P. oceanica* meadows). Knowledge about how *P. crispa* functions as a bioengineering species and its particular influence on the surrounding water parameters is missing.

Box 1 — Biology of *Phyllophora crispa*

Phyllophora crispa is an epilithic[c] rhodophyte[65] with up to 15 cm long cartilaginous Thalli[66,67] growing from a short, round holdfast[68] (Figure 1 A). These Thalli form a characteristic dichotomous[d] growth with rounded, light red tips[68]. The reported depth range of *P. crispa* is between 1 – 100 m, while the occurrence and length of the Thalli increase with depth[65,67]. Dense populations of *P. crispa* have been reported from the northern Atlantic (Iceland, Norway, Portugal), the Black –, and the Mediterranean Sea[23,29,60,61,68]. The individual algae are perennial[e;66,67]; reproduction periods have been observed to occur from November to January in the northern Atlantic[69]. While the reports from the Atlantic and the Black Sea describe loose laying aggregations of *P. crispa*[61,70], observations from the Mediterranean Sea describe attached mats on rocky substrates[29,60,71,72].

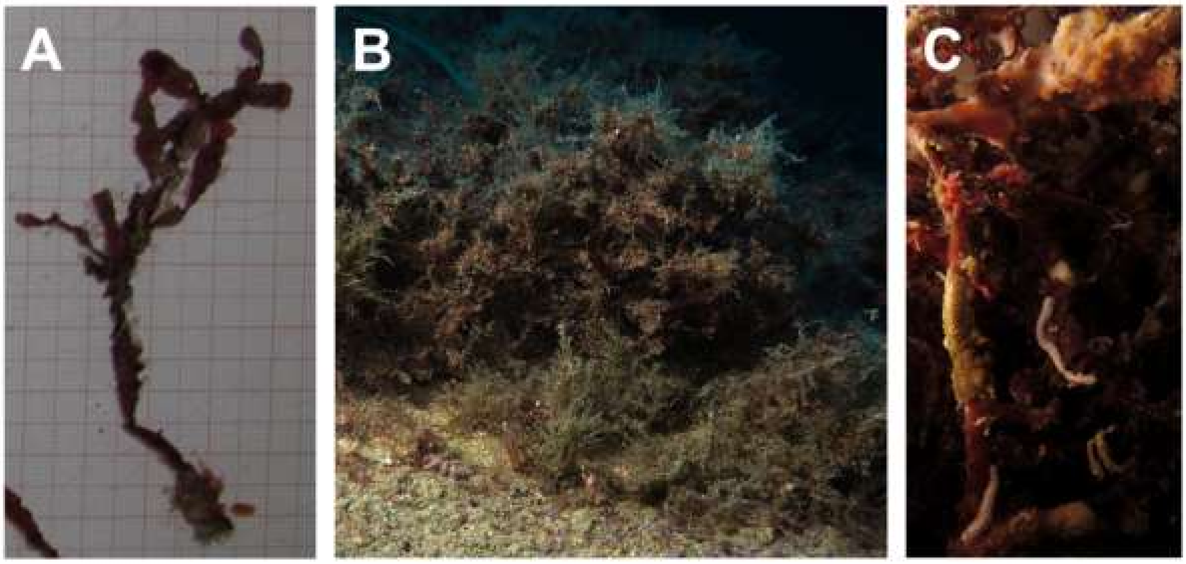

Figure 1: Single Thallus of Phyllophora crispa (A), outer rim of a P. crispa mat (B), and close-up showing epiphytic organisms (C).

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[c] Epilithic: growing on stone or stone like material. Ancient Greek ἐπί- (epi-, “above”) + λίθος (lithos, “stone“).
[d] Dichotomous: branching into two equal branches. Ancient Greek δίχα (dícha, “double”) + τομή (tomé, “cut“).
[e] Perennial: plants or algae that live more than two years and reproduce multiple times (once per year in general).

Aims and approach

Aims

Bioengineering species are essential for healthy marine ecosystems, and understanding their role in hosting rich biodiversity is of particular interest to tailor efficient conservation strategies. We aimed to describe the biodiversity associated with *P. crispa* mats in relation to the well-established biodiversity hotspot of *P. oceanica* meadows. Further, we aimed to assess the capability of *P. crispa* to shape local environmental factors to facilitate a prosperous epiphytic[f] community. To address these aims, we sought to answer the following research questions:

1. What are the abundances and phenotype richness of sessile organisms inside *P. crispa* mats compared to *P. oceanica* meadows, and can *P. crispa* mats be considered biodiversity hotspots?
2. What are the effects of *P. crispa* mats on water parameters compared to the overlaying water body, and does *P. crispa* fulfill ecosystem engineer functions?
3. Can *P. crispa* mats potentially serve as a biodiversity refuge in times of biodiversity loss and degradation of established hotspots in the Mediterranean Sea?

Approach

All data collection was carried out in the northern Tyrrhenian Sea, around the coast of Giglio Island, where the “Institut für Marine Biologie” (IfMB) is located. In the western Tyrrhenian Sea, *P. crispa* has been reported to grow in dense mats[60,73], and recent studies confirmed high abundances of sessile epifauna on *P. crispa* mats around Giglio Island[29,72,74]. Our approach encompassed the evaluation of epiphytic biodiversity on *P. crispa* mats and *P. oceanica* meadows as a reference and *in situ* measurements of environmental parameters in both habitats. The setting allowed us to analyze fresh samples of algae material and seagrass samples for all Phyla of epiphytic invertebrates and sessile Foraminifera. Furthermore, we monitored water parameters inside the algae mats and seagrass meadows, including water movement, temperature, light intensity, pH, Oxygen concentration, and Chlorophyll α–like fluorescence.

Thesis structure and outline

This thesis comprises five studies, all of them investigating different aspects of the role of *P. crispa* mats as biodiversity hotspots and their influence on environmental factors. The topic is treated in seven chapters, with the first chapter representing a general introduction and the last chapter a

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[f] Epiphytic: growing on a plant or algae. Ancient Greek ἐπί- (epi-, “above”) + φυτόν (phutón, “plant”).

general discussion. The five intermediate chapters consist of published manuscripts that contribute to understanding the role of *P. crispa* as habitat-forming species in the Mediterranean Sea and the implications for its role as a biodiversity hotspot amidst reported losses of established habitats and species. These chapters can be further summarized in three sections:

Section 1: The effects of *P. crispa* mats on environmental parameters

Chapter 2 addresses the presence of environmental gradients inside *P. crispa* mats compared to neighboring *P. oceanic* meadows. We describe the effects on water movement, light intensities, and temperature throughout the mats and meadows related to the overlaying water. Therefore, we adapted a gypsum dissolution technique to evaluate relative changes in water movement and placed arrays of combined light and temperature loggers inside the respective habitats.

Section 2: Key phyla of sessile organisms among the epiphytic community on *P. crispa*

Chapter 3 analyzes the composition of serpulid polychaetes, their richness, and abundance on *P. crispa* mats compared to *P. oceanica* meadows. This analysis includes evaluating shared and unique species in their respective habitats. The spatial variability of serpulid polychaetes associated with *P. crispa* mats is also presented. Chapter 4 analyzes the abundance and diversity of epiphytic foraminifera on *P. crispa* and *P. oceanica* reference material. The chapter includes an in-depth investigation of the composition of foraminifera morphotypes concerning their ecological function. Chapter 5 analyses the abundance and diversity of the epiphytic bryozoan community inside *P. crispa* mats compared to *P. oceanica* meadows and the spatial variability on *P. crispa*. Furthermore, we describe which families were most abundant and which were solely found on *P. crispa*.

Section 3: General analysis of the epiphytic community on *P. crispa* related to environmental gradients inside the mats

Chapter 6 provides a holistic overview of the sessile communities found in both investigated habitats. Beyond the direct comparison of both investigated habitats, we related our findings to worldwide established biodiversity hotspots based on literature data. Furthermore, assessments of environmental gradients present in *P. crispa* mats and *P. oceanica* meadows are presented. Observed patterns are then analyzed to conclude the role of *P. crispa* as a bioengineering species and potential refuge habitat for threatened biodiversity in neighboring habitats.

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Chapter 2 – Fleshy red algae mats influence their environment in the Mediterranean Sea

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Abstract

In the Mediterranean Sea, the fleshy red alga *Phyllophora crispa* forms dense mats of up to 15 cm thickness, mainly located on rocky substrates in water depths below 20 m. Because of the observed density of these mats and some first observations, we hypothesize that *P. crispa* is a yet undescribed ecosystem engineer that provides a multitude of ecological niches for associated organisms along small-scale environmental gradients. Therefore, we conducted an in-situ pilot study in the Western Mediterranean Sea to assess potential influence of the algae mats on the key environmental factors water movement, temperature and light intensity. We comparatively and simultaneously measured in *P. crispa* mats, in neighbouring *Posidonia oceanica* seagrass meadows, on neighbouring bare rocky substrates without algae mats, and in the directly overlying water column. We used several underwater logging sensors and gypsum clod cards. Findings revealed that *P. crispa* significantly reduced water movement by 41 % compared to the overlying water column, whereas water movement was not affected by *P. oceanica* meadows and bare rocky substrates. Surprisingly, *P. crispa* increased the water temperature by 0.3 °C relative to the water column, while the water temperature in *P. oceanica* and on bare rocky substrates was reduced by 0.5 °C. Light intensity inside the red algae mats was reduced significantly by 69 % compared to the water column. This was similar to measured light reduction of 77 % by *P. oceanica*. These findings highlight the strong influence of the dense red algae mats on some key environmental factors. Their influence is obviously similar or even higher than for the well-known seagrass ecosystem engineer. This may be a factor that facilitates associated biodiversity similarly as described for *P.oceanica*.

Keywords: macroalgae, seagrass, *Posidonia oceanica*, phytal habitat, hard-bottom communities, environmental gradients

Introduction

Ecosystem engineers are defined as organisms that control the availability of resources either directly or indirectly by their ability to change the physical state of abiotic or biotic material[1,2]. Therefore, ecosystem engineers can create or modify habitats[3] by affecting environmental parameters, such as light, temperature, pH, oxygen and availability of substrate. Jones et al.[1,2] define two types of bioengineering organisms: 1) autogenic engineers which modify their environment by endogenous processes, such as growth (e.g., trees) and 2) allogenic engineers which modify other living or non-living material in their surrounding (e.g., beaver).

Corals are a prominent example of structural engineers, a subgroup of autogenic engineers, that physically create substrate, control resources and shape their environment[2,4]. For example, the metabolism of both corals and their symbiotic algae (i.e., respiration and photosynthesis) cause oxygen micro-gradients near reefs[5] that can affect a range of associated species[6,7]. Mangroves are another example of structural ecosystem engineers that create habitats for numerous aquatic species[8,9]. The complex structure of their roots provides shelter from predators[10,11]. Moreover, kelp algae modify their surroundings' physical state through their morphology in coastal environments worldwide[2]. Aggregations of such macroalgae form a unique habitat for marine organisms[12] by dampening water currents[13,14] and diminishing light[15–17].

In the Mediterranean Sea, a member of the seagrass family Posidoniaceae, the endemic *Posidonia oceanica* (LINNAEUS DELILE 1813) acts as a well-known biodiversity hotspot[18,19] and an autogenic ecosystem engineer. It forms wide meadows that can, among others, affect the hydrodynamics of currents and waves[20,21]. Further, *P. oceanica* promotes sediment stability by buffering resuspension[22–24].

Another potential habitat-building species in the Mediterranean Sea is the fleshy red alga *Phyllophora crispa* (HUDSON 1964). In water depths below 20 m, the alga forms discontinuous mats of at least 5 cm thickness, mainly on rocky substrates, that can cover large parts of the seafloor[25]. A massive development of these red-algae mats lead to it becoming a dominant benthic habitat around the Island of Giglio since 2012[25].

Bonifazi et al.[25] provided a first description of the associated fauna of *P. crispa*, and its role as a habitat forming species at the Island of Giglio, Italy, and came to the conclusion that biodiversity of mobile and sessile invertebrates associated with the mats is high. A reason for this finding is likely that *P. crispa* mats create a range of micro-niches by influencing key environmental parameters such as water current exposure, temperature and light availability. Because of the observed density of these mats and some first observations, we hypothesize that *P. crispa* is a yet undescribed ecosystem engineer that provides a multitude of ecological niches for associated organisms along small-scale environmental gradients. This study thus aimed to investigate the potential role of *P. crispa* as an ecosystem engineer and answer the following research question: What are the differences in water movement, temperature and light availability between *P. crispa* mats, *P. oceanica* meadows and rocky substrates without any overlying mats? To answer this question, we conducted a series of comparative and simultaneous in-situ measurements in the Mediterranean Sea.

Materials and methods

Study Site and Habitat Characterization

The study was performed in September and October 2019 on the Island of Giglio, Tuscany, Italy. All observational surveys were conducted *in situ* via scuba-diving at a depth of 28 – 30 m at the dive site "Punta del Morto" (42°23'22.2"N 10°53'24.3"E), which is located at the northern coast of Giglio. The study area was on the west side of the dive site, where, starting at a depth of 20 m, granite blocks cover most parts of the seafloor. In the Mediterranean Sea, the occurrence of small patches of bare granitic rock at water depths of 30 m is common. Additionally, dense *Posidonia oceanica* seagrass meadows, characterized by straight, single leaves, and dense *Phyllophora crispa* mats of robust, branched, and entangled structure are present at the study site. All three habitats (i.e., *P. crispa* mats of at least 5 cm thickness, *P. oceanica* seagrass meadows of at least 20 cm height, bare rocky substrate, Supplementary Fig. S1) were found at the same water depth in an area of about 250 m^2. Limiting the study to this one sampling site allowed for a direct comparison by quantifying environmental conditions within these three habitats, as we expect that all habitats experienced identical external conditions (e.g., temperature, salinity, insolation, hydrodynamics, etc). The rhizome part of *P. oceanica*, above and below ground, was not included in this study.

Environmental Parameters

Effects on water movement relative to the water column by *P. crispa* and the other two ecosystem types were measured simultaneously using the gypsum dissolution technique[26,27]. While this method is not appropriate to measure absolute water flow, it is well suitable for relative comparison of water movement[28–30]. Gypsum of smooth consistency (Quick-mix gips, toom # 3050388, $CaSO_4$) was poured into muffin cups. Air bubbles were removed with continuous finger snapping for 30 seconds. After a drying period of 24 h at air temperature, gypsum balls were taken out of the cups and dried to constant weight in an oven at 60 °C before being glued to plastic cards avoiding gaps between both the cards and the gypsum balls. Then, clod cards were numbered and weighed. Clod cards were placed at heights of 0 cm (seafloor), 1 cm (within *P. crispa* mats), 5 cm (algae-water interface), and 20 cm (above the algal mats) by attaching them to a 30 cm long metal rod with cable ties (Supplementary Fig. S2). Replication was done primarily temporal, with rods being deployed at four different time points. A total of eleven rods was placed in *P. crispa* at all four timepoints. The same set-up was used at one timepoint on rocky substrates, resulting in two replicates. Since seagrass meadows grow higher than the red algae mats, a metal rod of 80 cm length was used and an additional clod card was placed 30 cm from the ground. This setup allowed selecting the same positions that were used for *P. crispa* and rocky substrates: 0 cm (seafloor), 1 cm (within *P. oceanica* meadow), 5 cm (within *P. oceanica* meadow), 20 cm (meadow-water interface) and 30 cm (above the meadow). A total of three rods was deployed at three different time points in *P. oceanica*, resulting in a replication of three. The size of the gypsum balls was similar for all environments. The set-ups were prepared before deploying *in situ* and stayed in the water for 6 to 7 days after deployment. The set-ups were then transported out of the water, clod cards were carefully removed from the metal stick, rinsed with fresh water and placed on a drying rack. Then, clod cards were dried in an oven at 60 °C until a constant dry weight was reached. The relative weight loss of gypsum balls (presented as a reduction of daily average weight loss of gypsum balls relative to the overlaying water [%]) was used as an indicator for the strength of relative water movement. We used this relative approach to exclude effects of habitat volume differences on the results. Calculations were made as follows:

$$weight\ loss\ [\%\ day^{-1}] = \frac{w1}{\left(\frac{w1}{t}\right) \times 100}$$

reduction of daily average weight loss relative to the overlaying water

$$[\%] = 100 - \left\{100 - \left[\left(\frac{100}{mean\ (wlw)}\right) \times mean(wlp)\right]\right\}$$

with w_1 = weight before water exposure, wl = total weight loss [g], t = time in the water [days], wlw = weight loss at position [% day^{-1}], wlp = weight loss at position [% day^{-1}].

For light and temperature measurements, the same set-up as for the water movement measurements was used simultaneously in *P. crispa*, *P. oceanica* and on rocky substrates using Onset HOBO Pendant Data Loggers (part # UA-002-64, accuracy temperature: ± 0.53 °C, light unit: lux). These loggers are designed for measurements of relative light intensity differences, as they are not accurate for measuring absolute light intensities[31]. For *P. oceanica*, the loggers were positioned at 0 cm, 1 cm, 5 cm, 20 cm, 35 cm and 55 cm distance from the seafloor to increase the vertical resolution of measurements. A total of seven rods were placed in *P. crispa* mats at four time points, a total of three rods in *P. oceanica* meadows at three time points, and a total of eight rods were placed at four time points on rocky substrates. Data loggers were exchanged and placed alternately in different habitats and in different heights above the seafloor for each observation to avoid any systematic error. Data collection intervals were set at 15 sec, and loggers collected data for 5 consecutive days. The resulting replication for temperature and light observations in the different habitats is shown in Table S1 (Supplementary). Loggers were read out with the program "HOBOware" (version 3.7.17).

This study was conducted simultaneously in the three investigated habitats, which means that all habitats experienced identical environmental conditions. Our findings therefore reveal differences in environmental factors resulting directly from the different characteristics of the investigated habitats.

Statistical Analysis

Statistical analysis was conducted using the statistic program "R" (version 3.5.1) and the package "car"[32]. One-Way-ANOVA was used to calculate significant differences between data sets when the sample size was three or higher. Due to the small sample size, differences regarding weight loss of gypsum balls on rocky substrates were calculated with a t-test. Differences were considered as significant at p-values below 0.05.

For temperature and light analysis, data points used for calculations correspond to the mean of single data points over a period of five minutes ($\text{data point}_{5\text{min}} = \text{mean}\ (20 * \text{data point}_{15\text{sec}})$). Light data of light hours, from 6 am to 8 pm, was used for the analysis.

Results

Water Movement

The weight loss of gypsum clod cards was used as an indirect measurement of water movement. Overall, results show a reduction in weight loss of gypsum clod cards relative to the water column by 41 ± 8 % in *Phyllophora crispa* mats, 25 ± 16 % in *Posidonia oceanica* seagrass meadows, and 13 ± 8 % on the rocky substrates (Fig. 1). Only in *P. crispa* mats, the weight loss was significantly different among heights above seafloor (Supplementary Table S2a). Gypsum balls placed on the substrate surface inside the *P. crispa* mats showed a significantly lower weight loss of 41 ± 8 % compared to the gypsum balls in the overlaying open water (ANOVA: $F_{1,21} = 26.28$; $p \leq 0.001$).

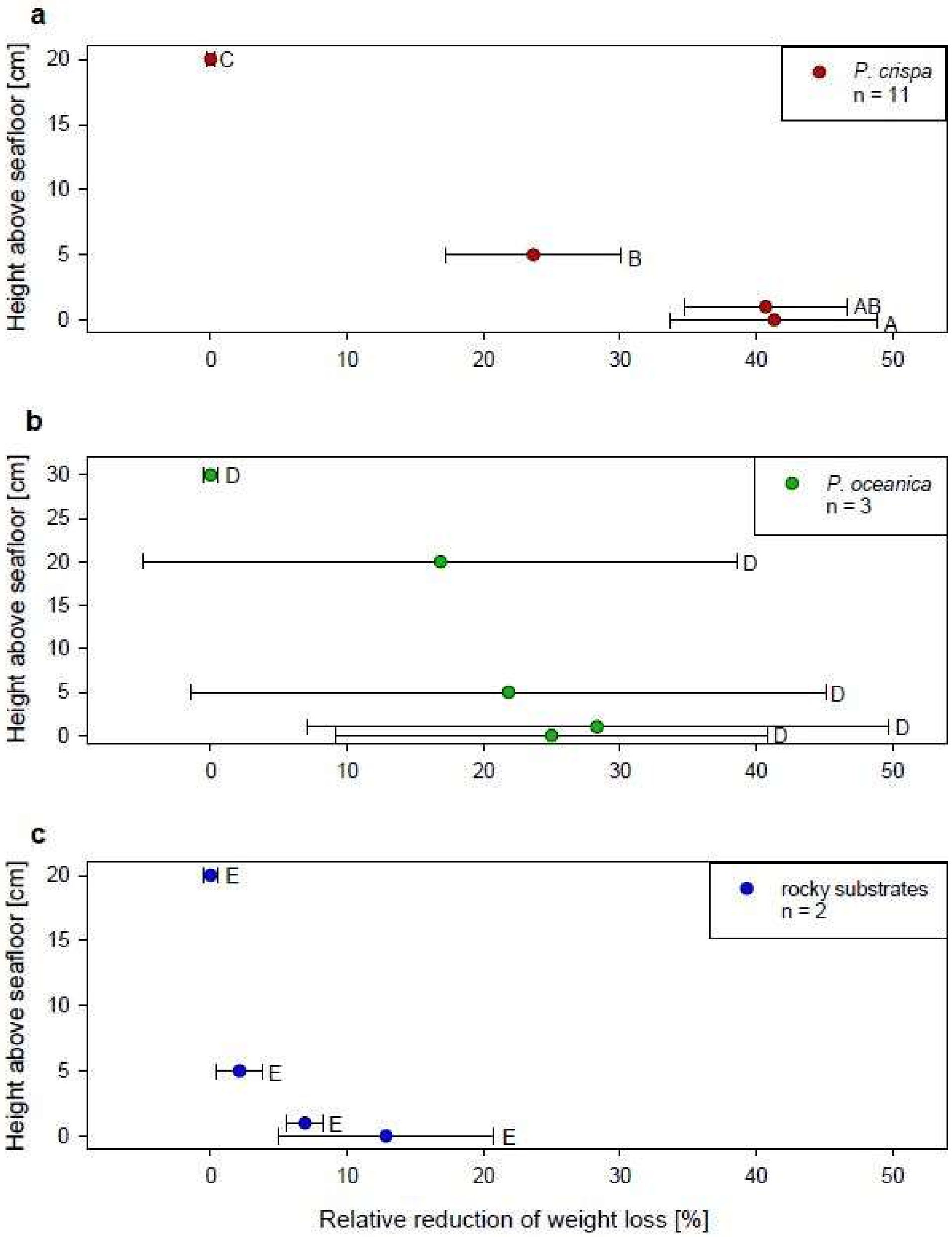

Figure 1: Reduction of daily average weight loss of gypsum balls in Phyllophora crispa (a), Posidonia oceanica (b) and on rocky substates (c) relative to the overlaying water (0 %). Displayed are mean values and standard error. n = number of rods deployed in situ. No significant differences are indicated by the same letter, significant differences by different letters.

Temperature

The daily temperature cycle was similar in all three habitats, as increasing temperatures from morning (6 am) to evening (6 pm) were observed. Then, temperatures decreased until midnight and remained stable until the morning (Figure 2). The temperature in *P. crispa* mats ranged from a minimum of 18.6 °C to a maximum of 20.4 °C, from 17.2 to 19.3 °C in *P. oceanica*, and from 18.2 to 20.0◦C on rocky substrates. Furthermore, temperature stratifications varied within the investigated habitats. Temperatures at the bottom of *P. crispa* mats (0 cm) were significantly higher than those measured at

1, 5, and 20 cm distance from the seafloor (Supplementary Table 2B), which all were in a similar range (Figure 2A). For *P. oceanica*, the temperatures observed at the seafloor were significantly (ANOVA: F1,575 = 156.98; $p \leq 0.001$) lower than temperatures found 35 and 55 cm above the seafloor (Figure 2B). On rocky substrates, temperatures measured at the seafloor (i.e., 0 cm) were significantly lower compared to the other positions (Figure 2C). The water temperature was 0.31 °C higher at the seafloor compared to the water column for *P. crispa*. For *P. oceanica* and the bare rocky substrates, the water temperature was 0.5 °C lower at the seafloor compared to the water column, respectively,

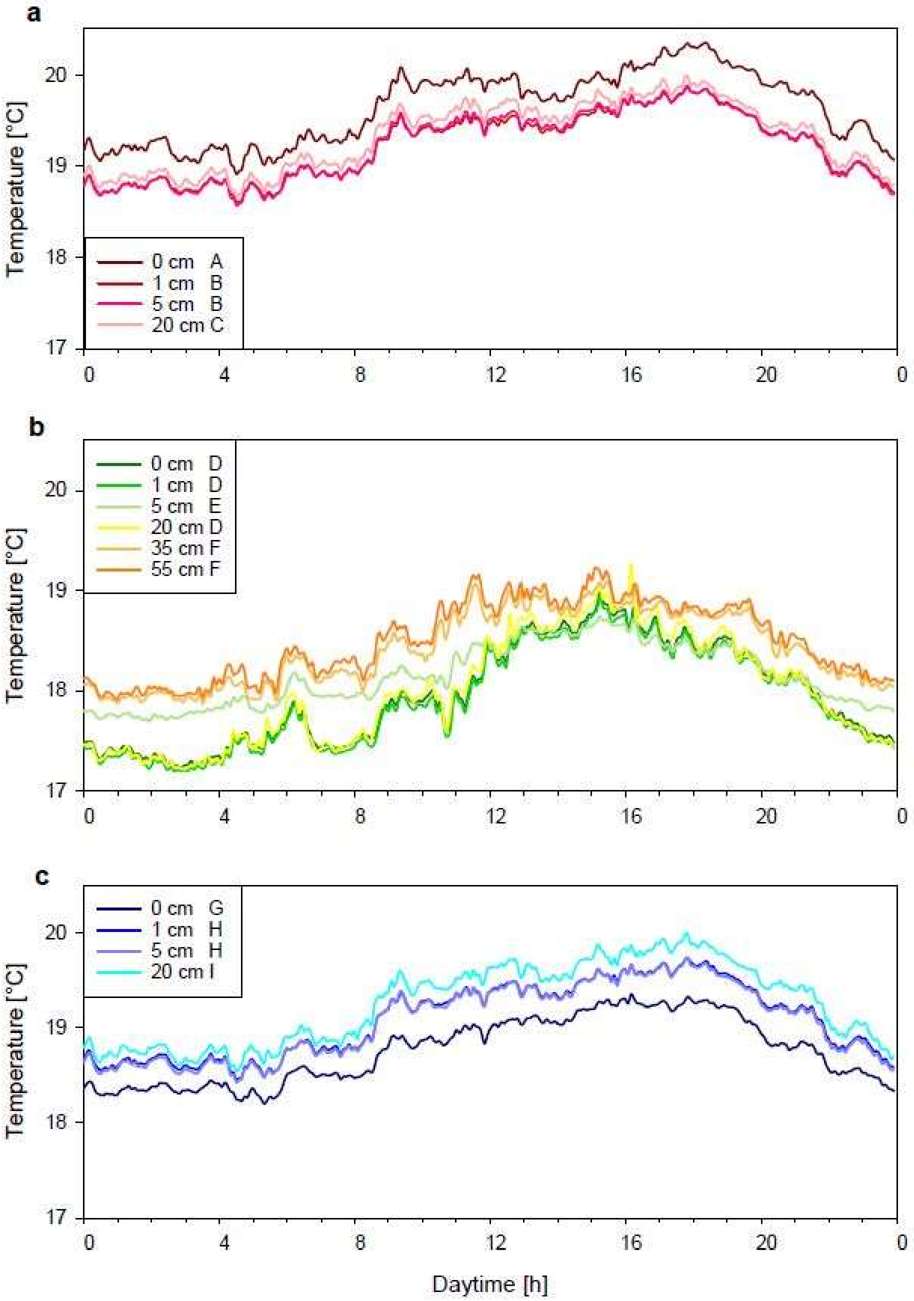

Figure 2: Average temperature in Phyllophora crispa (a), Posidonia oceanica (b) and on rocky substrates (c). Displayed are mean values. No differences are indicated by the same letter, significant differences by different letters.

Light Intensity

Light stratification was different for the three investigated habitats (Fig. 3). In *P. crispa* mats (Fig. 3a), light intensity was consistent at 0 cm and 1 cm above the seafloor, i.e., within the mat, while the light intensity was two-fold higher at 5 cm and 20 cm above the seafloor (Supplementary Table S2c). Close to the seafloor, a relative light intensity of 31 ± 0.3 % compared to the overlying water was recorded in this habitat. Similar light values were recorded for *P. oceanica* (Fig. 3b) 0 cm, 1 cm and 5 cm above the seafloor. Higher light intensities occurred 20 cm above the seafloor, while the highest light values were noted 35 cm and 55 cm above the seafloor. Lowest values of minimum 23 ± 0.2 % light intensity relative to the overlying water were recorded close to the seafloor in seagrass. On rocky substrates (Fig. 3c), light intensity showed an irregular increase with increasing distance from the seafloor. A reduction of light intensity in the absence of plant canopies, on rocky substrates, was less intense, i.e., 58 ± 0.3 % relative to the logger placed furthest away from the seafloor at 20 cm height. Overall, a reduction in light intensity from the highest to the lowest position of 69 ± 0.3 % for *P. crispa*, 77 ± 0.2 % for *P. oceanica* and 43 ± 0.3 % for rocky substrates was noted.

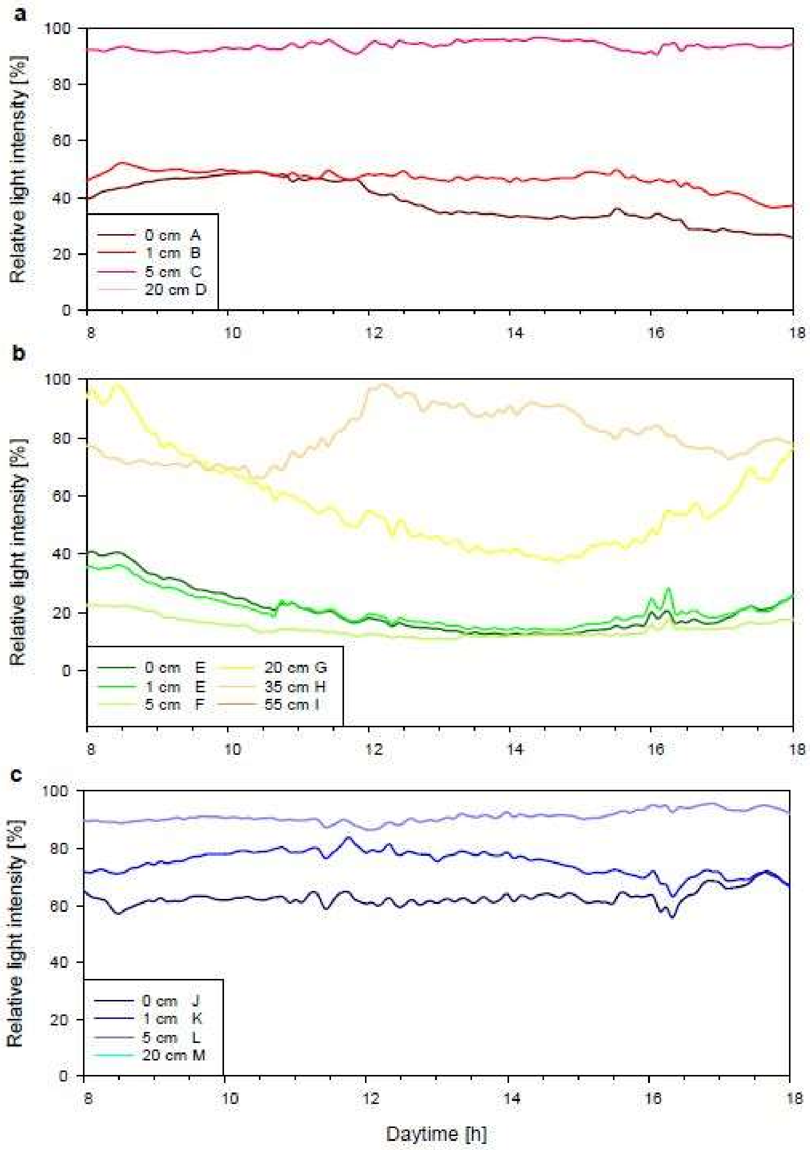

Figure 3: Relative light intensity compared to loggers placed above canopy [%] in Phyllophora crispa (a), Posidonia oceanica (b) or at similar water depth on rocky substrates (c). Displayed are mean values observed during light hours from 8 am to 6 pm. References (100 %) are included in the legend for the display of statistical differences. No statistical differences are indicated by the same letter, significant statistical differences by different letters.

Discussion

It has recently been shown that the red alga *Phyllophora crispa* harbours many associated organisms in the north-western part of the Mediterranean Sea[25,33–35]. In this study, we investigated the influence of *P. crispa* on the environmental factors water movement, temperature and light intensity. Furthermore, we related our finding of *P. crispa* to neighbouring *Posidonia oceanica* meadows and bare rocky substrates. Our results indicate a potential role of *P. crispa* as an autogenic ecosystem engineer.

Characteristics of Investigated Habitats

The water movement was reduced by 41 % at the deepest part of the *P. crispa* mats compared to the water movement 20 cm above the seafloor. Studies on soft-sediment macro algae canopies with a similar structure found similar results, showing reduced water flow and mixing in the lower part of the canopy[36]. Both, rocky substrates and *P. oceanica* meadows showed a less intense decrease (13 % and 25 %, respectively) in relative water movement towards the substrate, which was not statistically significant, potentially due to low replication. Previous studies[37,38] found significant reductions of water currents inside seagrass meadows that were located at a water depth of 10 m (e.g.,[21,39,40]). However, investigated seagrass meadows in the present study were located at a depth of 28 meters, in which current is usually weaker than in more shallow areas, which hypothetically explain the absence of statistically significant effects. Water movement in the water column directly above *P. crispa* and *P. oceanica* was similar to rocky substrates, which indicates that the differences between the three investigated habitats are likely caused by the lack of topography of the rocky substrates compared to the structural complexity of the plant canopy habitats as shown for i.e., seagrass[20,41]. Structural complexity or heterogeneity of a habitat is characterized by the spatial arrangement and diversity of substratum types[42] and has previously been shown to correlate with lowered water flow[43,44] and the degree of protection from predation[45]. A stronger reduction of water movement was observed in the rather thin *P. crispa* mats over a shorter distance, compared to the *P. oceanica* meadows, suggesting a higher structural complexity of the thalli, which have a relatively high surface area within a small volume compared to seagrass leaves.

Temperatures were significantly higher at the bottom of the algae mats, on average by 0.31° C. Although observed differences were within the range of accuracy of the data logger, all replicates showed similar differences. Since a temperature gradient was observed on the bare rocky substrates with decreasing temperatures towards the seafloor, the observed differences in *P. crispa* likely do not originate from potential temperature differences within the water column. It has been shown for seagrass, that their leaves retain organic matter from the water[37]. As the structural complexity seems higher in *P. crispa* mats, the filter capacity may also be higher, resulting in potentially high microbial activity. Habitat complexity also promotes micro-scale heterogeneity[46,47] and additionally provides a large surface area for high microbial activity. It has been shown that *P. crispa* enlarges the seafloor surface by a factor of 4.9 (YC El-Khaled et al., unpublished data), which could potentially lead to high microbial activity. High rates of microbial replication and activity have been observed to create a heat output in laboratory experiments[48]. Potential warming underneath the algae mat could therefore likely be a result of the intense respiration activity of associated (micro) organisms.

Temperatures in *P. oceanica* meadows and on rocky substrates decreased with decreasing distance to the seafloor. The combination of less self-shading, limited water exchange and enhanced microbial activity could cause the temperature differences in *P. crispa* mats as opposed to *P. oceanica* meadows and rocky substrates (Table 1[38]). Although an increase in temperature as indicated for *P. crispa* could not be observed for seagrass leaves, it may potentially occur in the rhizomes, as water current reduction is expected, and the habitat structure becomes more complex[49].

Table 1: Environmental gradients in different habitats observed in this study [*] and previous reports by [1] Bacci et al., 2016, [2] Arumugam et al., 2013, [3] Dalla Via et al., 1998. No significant influence on environmental factor is indicated by a horizontal arrow, an increase by an arrow heading upwards, a decrease by an arrow heading downwards.

	Posidonia meadow	*Phyllophora* mat	Rocky substrates
Water currents	[*] → [1] ↓	[*] ↓	[*] →
Temperature	[*,2] ↓	[*] ↑	[*] ↓
Light	[*,3] ↓	[*] ↓	[*] →

Overall, a statistically significant gradient in light intensity occurred on rocky substrates, and in the observed plant canopy habitats of *P. crispa* and *P. oceancia*. Light intensity gradients observed on rocky substrate were presumably the same as in the open water for all substrates. *P. crispa* mats decreased the light intensity by 69 % from the overlaying water to the bottom of the mat, which is a strong environmental light gradient across the relatively thin mat of about 5 cm thickness. For *P. oceanica* meadows, a decrease in light intensity of 77 % compared to the overlaying water was observed. Previous studies also showed strong light gradients in habitats of macrophyte dominance, i.e., *P. oceanica* meadows, of up to 70 % and confirm self-shading effects[50]. These structures reduced the incidence of the blue, green and red wavelengths by respectively 57 – 58 %, 61 – 63 %, and 51 – 53 % within the seagrass stands, additionally to light attenuation of the water column. A third interconnected light gradient occurs due to direct shading of macrophyte structures caused by epiphytes[50,51], which can reach a thickness of up to 6 cm on *P. crispa* mats[25]. In combination with self-shading induced by the entangled thalli of the alga and horizontally lying blades, this can explain the exceptional strong light gradient.

Potential relevance for ecosystem functions

Based on several studies, lowered water movement inside *P. crispa* mats potentially promotes sediment trapping and reduces resuspension, as described for seagrass meadows[22,24,52]. Subsequently, this could affect the associated biodiversity, as large amounts of sediment trapped within *P. crispa* mats provide a heterogeneous habitat for infauna species and offer building material and food in form of organic and inorganic matter for tube-building species[53]. Previously, the global occurrence of algal turfs has been related to stressful habitats[54–56] and their structural characteristic of sediment trapping[56]. However, the coast of Giglio island is affected by sediment deposition, which consists of fine terrigenous particles controlled by rainfall and larger particles derived from erosion of granite rocks[25]. Sedimentary processes in this area are therefore a natural scenario rather than the result of anthropogenic stressors, such as pollution[25].

Moreover, the community composition can be affected by reduced water movement within the algae mat. A similar pattern has been observed for the eelgrass *Zostera marina* (L.), where lowered water movement enhances the accumulation of planktonic organisms, such as invertebrate larvae and protozoans[57]. The settlement of invertebrate larvae is greatly affected by small-scale fluid dynamics[58] and thus by changing these parameters, *P. crispa* mats consequently change the community composition. Reduced water movement could favor the settlement of e.g., certain bryozoan species [59,60] and other sessile invertebrates. Experimental studies have shown that low water turbulence and velocity enhance the settlement of some species of e.g., hydroids[61], bryozoans[61–63], barnacles[61,64], bivalves[65] and polychaetes[62], while it decreases the settlement of other species of the same taxa[61,62,65]. In general, we expect a high number of settling invertebrate larvae in *P. crispa*, as low water movement increases the acceptance of surfaces by post-settled larvae and therefore the attaching of certain species, e.g., barnacles[64,66,67]. Further, high numbers of mobile organisms, particularly larvae and juveniles, may be attracted to this habitat as it offers protection from water turbulences.

Observed higher temperatures at the bottom of *P. crispa* mats could potentially indicate a thermal insulation effect of *P. crispa*. However, future studies are required to confirm this hypothesis. While many studies focused on the effect of temperature on algae growth (e.g.,[68–70]), the effects of algae on the habitat's temperature are so far unstudied. Studies of another important ecosystem engineer however report similar effects on temperature: shallow water scleractinians increased water temperatures in their microenvironment, due to solar radiation and water holding capacities by surface heterogeneity[71,72]. While solar radiation plays only a minor role in the deep layer of *P. crispa* mats, we showed that water movement is low in the algae mats, which could indicate a long water residence time and explain a similar effect on the thermal microenvironment of this habitat.

P. crispa provides a habitat that differs in light intensity to bare rocky substrates, as the plant structures create shade. The alga's phylloids hinder light to reach the seafloor, which limits requirements for primary production within the red algae mats. Light availability is most important for photoautotrophic organisms and therefore an important factor for overall oxygen availability. Hence, in seagrass meadows, oxygen availability generally increases with the distance to the rhizome[73]. Other macroalgae mats have been shown to inhabit a high abundance of microphytobenthos along a vertical gradient, which is a food source for associated organisms[74–76] and causes high phototrophic activity. In the present study, we found a 1.1-fold stronger light gradient over a shorter distance in *P. crispa* mats, which implies that the layer of high phototrophic activity is in closer proximity to the deeper layer of heterotrophic activity. This could lead to higher oxygen availability for heterotrophic organisms, especially heterotrophic microbes, and hence fuel the potential warming effect of microbial activity inside the mats. High microbe abundance is beneficial for suspension feeders, such as certain Porifera[77], ascidians[78,79], bryozoans (e.g.,[80–82]), bivalves (e.g.,[83–85]), cnidarians[86], crustaceans[87,88] and polychaetes[89,90], that feed on microbes. The aforementioned active benthic filter feeders create their own currents, e.g., by using their cilia or muscles[85,91], and are therefore not affected by the lowered water movement inside *P. crispa* mats.

Ecological implications

P. crispa widely covers rocky substrates and increases in abundance with increasing water depth beyond 25 m[25,92]. Loss of *P. oceanica* meadows and rocky substrate communities threaten the overall high biodiversity in the Mediterranean Sea[93]. *P. crispa* potentially recolonizes these degraded habitats following the concept of algal phase shifts. The importance of *P. crispa* as an autogenic ecosystem engineer was stated by Zaitsev[94] in the Black Sea, where *P. crispa* is a key species along the north-western rocky coasts. More than 100 species of invertebrates and 40 species of fish, some of which are endangered or rare[95], inhabited the *P. crispa* mats in this area. This associated community benefits from the algal habitat as a food source, substratum for spawning, material for the construction of nests, or shelter from predators[94]. Provision of food or protection for rich associated communities are also characteristics of other algal habitats, such as *Cladophora* mats[74–76] or *Cystoseira* beds[96].

The term 'ecosystem engineer' is often associated with large-scale bioengineers, such as corals, mangroves, and seagrasses, which create extensive contiguous habitats covering wide areas (e.g.,[97–99]). However, referring to Jones et al.[1,2], scale is not a parameter defining 'ecosystem engineers'. Bivalves for example, such as mussels or oysters, are well-recognized structural small-scale bioengineers that form dense beds or reefs[100,101] , which create habitats of smaller volume and act mainly on a local scale[102]. Their shells provide substrate for other organisms to colonize[103], alter the local water flow regime[104], as well as the local dynamics of sediment[103]. This study showed that *P. crispa* influences its environment in a similar way, i.e., by reducing water movement and creating habitat. Despite reaching heights of only about 15 cm, this red alga therefore acts as a small-scale ecosystem engineer, forming dense but thin mats, that can cover areas of up to 10,000 km^2, as shown for the Black Sea[94].

Our study showed that key environmental parameters are shaped by *P. crispa*, thus a complex and valuable habitat is likely provided in the Mediterranean Sea. As the complexity of a habitat increases biodiversity[105] and *P. crispa* mats show highly complex structures and environmental gradients, they could act as a biodiversity hotspot in the Mediterranean Sea, similar to the Black Sea, as already indicated by the pilot study of Bonifazi et al.[25]. A total of 99 non-colonial invertebrate taxa were collected in *P. crispa* mats in the Mediterranean Sea, while polychaetes were the most abundant group, followed by Mollusca and Crustacea[25]. Additionally, that study reports 37 taxa of colonial invertebrates, which were dominated by Bryozoa and Porifera, but also included Tunicata and Anthozoa. Further, Bonifazi et al.[25] state that *P. crispa* mats significantly increase species richness and abundance of sessile filter feeders in contrast to shallower areas without the red algae mats. This could be the result of enhanced invertebrate larvae settlement due to reduced water movement in *P. crispa* mats observed in this study, which has been previously shown for seagrass meadows[106]. The dominance of sessile filter feeders within *P. crispa* mats observed[25] strengthens our hypothesis of high oxygen availability due to high microphytobenthos abundance in the mats that enhances overall microbial abundance resulting in a higher food availability for active filter feeders.

This study provides the first attempt to investigate the potential role of the red alga *P. crispa* as an autogenic ecosystem engineer, which may harbor high associated biodiversity. Our results indicate the possibility that *P. crispa* provides resources for associated organisms by retaining sediment, providing food, and creating habitat. While we were able to show that *P. crispa* serves a habitat by influencing the key environmental factors water movement and light intensity, its effect on temperature needs to be studied further.

Future studies should investigate the diversity of infauna in Mediterranean *P. crispa* mats to understand their role as potential biodiversity hotspots and also confirm the abundance of *P. crispa* in other regions of the Mediterranean coast. Further, studies on the turnover rate of *P. crispa* mats are needed to understand the ability to generate refuge for organisms, which may suffer from habitat loss due to anthropogenic pressure and climate change.

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Chapter 3 – Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes

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Chapter 3 – Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes

Abstract

The Mediterranean Sea harbors more than 17,000 eukaryotic marine species, with several ecosystems recognized as biodiversity hotspots, such as *Posidonia oceanica* meadows. Recent research indicates that benthic mats formed by the fleshy red alga *Phyllophora crispa* are also associated with high species richness. Among key groups found in these mats are sessile polychaetes, which live as epiphytes on the red algae thalli. Knowledge of abundance, species richness, and spatial variation of polychaetes associated with these habitats is still scarce. We carried out a comparative assessment focusing on serpulid polychaetes within samples from *P. crispa* mats and neighboring *P. oceanica* meadows at six different sampling sites around Giglio Island (Tyrrhenian Sea, Italy). A total of 17 serpulid taxa were identified. The abundance of serpulids (5665 individuals m^{-2} of *P. crispa* mat) were similar to neighboring *P. oceanica* meadows (2304 individuals m^{-2} leaves and 5890 individuals m^{-2} shoots). The number of serpulid taxa was significantly higher in *P. crispa* mats (average 6.63 ± 1.32 taxa) compared to *P. oceanica* beds (average 1.56 ± 0.63 and 1.84 ± 1.04 taxa in leaves and shoots, respectively). Within habitat type, there were no significant differences in species richness between sites. The most abundant species found was *Josephella marenzelleri* (61 % of individuals), while *Vermiliopsis* spp. and *Bathyvermilia* sp. were exclusively found in *P. crispa* samples. Our results highlight that *P. crispa* mats host an exceptional diversity and that these habitats should be included in conservation strategies. Further research should focus on the significance of other important taxonomic groups within these mats and evaluate the distribution of *P. crispa* in different regions of the Mediterranean Sea.

Keywords: *Phyllophora crispa*; phytal habitat; hard-bottom communities; ecosystem engineer; Serpulidae; Mediterranean Sea; sessile epifauna

Chapter 3 – Mediterranean Red Macro Algae Mats as Habitat for High Abundances of Serpulid Polychaetes

Introduction

With almost 17,000 described eukaryotic species[1], the Mediterranean Sea harbors high levels of biological diversity accompanied by a high rate of endemism (estimated 20 %) accounting for the term “biodiversity hotspot”[1–3]. Its relatively small size compared to the world’s oceans (less than 1 %), in combination with this prevalent endemism, underlines the Mediterranean Sea’s ecological relevance[4]. In particular, many Mediterranean biogenic habitats’ structural complexity (e.g., *Posidonia oceanica* seagrass meadows) facilitates their role as biodiversity hotspots by providing spawning grounds, nurseries, and permanent settling space for many species[5–9]. Furthermore, this structural complexity results in a wide range of ecological niches formed by temporal and spatial gradients (e.g., light and temperature). These gradients are often created by engineering or foundation species[10] that modify their environment through their growth (e.g., macroalgae), while others alter local conditions collectively (e.g., tropical corals). This habitat modification often results in a shift of ecological zonation (e.g., seaweeds providing wet layers in tidal zones) by mitigating stressors for inhabiting species[11]. Finally, associated mobile species often enhance the engineer’s growth by driving out competitors or protecting against grazers[12,13].

Some of the most studied biodiversity hotspots in the Mediterranean basin include *Posidonia oceanica* (L.) DELILE, 1813 meadows (Figure 1C–E) and coralligenous habitats. The marine angiosperm *P. oceanica* promotes high biodiversity and provides nursery grounds for commercially important vertebrate species[14]. Furthermore, the structural complexity of the meadow serves as a secondary substrate for diverse communities[15] of sessile invertebrates (e.g., Serpulidae)[5]. Coralligenous habitats are bioconstructions primarily built by encrusting red algae and secondarily by calcifying invertebrate species, which also build a structurally complex habitat and provide biogenic substrate for a wide range of epibiota [7].

An additional potential and relatively unexplored biodiversity hotspot is the habitat created by mat-forming, fleshy red alga *Phyllophora crispa* (HUDSON) P.S.DIXON, 1964 (Figure 1A,B)[16,17]. In the Black Sea, *P. crispa* mats harbor a rich community of associated fauna, including a diverse invertebrate epifauna[17]. The documented importance of these habitats led to the establishment of a marine reserve[18]. Marine polychaetes are one of the main groups of associated invertebrate epifauna of these mats[19].

P. crispa mats colonize light-exposed rock surfaces all around Giglio Island. Together with *P. oceanica* meadows and coralligenous reefs, it represents one of the most abundant biogenic habitats in this area. Hemisciaphilic assemblages dominated by *P. crispa* were reported in the Tyrrhenian Sea, both along Giglio Island[16,17] and the North-East coast of Sardinia[20,21]. While differences in diversity and abundance of associated epifauna between *P. oceanica* meadows and other sciaphilic (adapted to low light conditions) hardbottom communities have been reported[22,23], only a few studies describe the associated biodiversity of *P. crispa* mats.

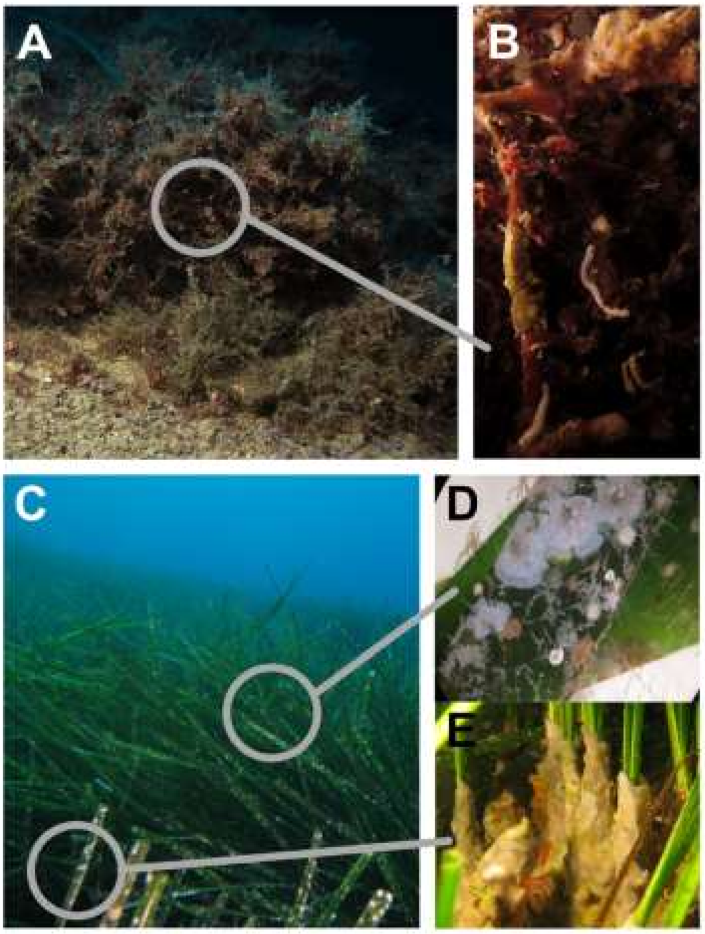

*Figure 1: Top: P. crispa mat in full view (**A**) and close up including sessile polychaetes and other epifauna (**B**). Bottom: P. oceanica mat as seen from the water column (**C**) and close-ups of the leaves (**D**) and shoots (**E**), showing different sessile epifauna. Pictures: F. I. Rossbach.*

Polychaetes are among the most diverse metazoan groups, with an estimated number of 2481 species in the Mediterranean Sea, accounting for 20.5 % of the 12,088 species reported globally[24]. They show a wide functional variety and adaptation to different environmental conditions[25–27]. Polychaete abundance and diversity are often used to assess benthic communities' states and dynamics[28].

Serpulid polychaetes are sessile organisms that colonize various marine habitats, from the shallow infralittoral to abyssal depths[29] and contain great taxonomic diversity[30]. Key drivers for the abundance and diversity of sessile polychaetes are environmental gradients (e.g., light and depth), as well as suitable space for larval settlement[19,31]. They contribute a considerable amount of carbonate bioconstructions from tropical to boreal latitudes, and shape the seafloor by acting as secondary builders[32,33]. Their ability to precipitate carbonate emphasizes their pivotal role as bioengineering species[34]. In the Mediterranean Sea, serpulids have been relatively well-studied[35–38], and several species have been recognized as lessepsian migrants (i.e., immigrated species from the Red Sea)[30,39–41]. Furthermore, the habitats’ structural heterogeneity provided by the algae influences serpulid assemblages (i.e., by offering settling ground and accumulating food particles), as demonstrated by Casoli et al. (2016)[19]. Therefore, the study of serpulids distribution as proxy groups for the occurrence of spatial microhabitats[42,43] is pivotal to understanding the biological diversity hosted in poorly investigated habitats, such as *P. crispa* mats. However, the distribution and role of serpulid worms in Mediterranean *P. crispa* mats are still not well understood. With this work, we aim to answer the following research questions:

(1) What are the abundance and species richness of serpulid polychaetes in *P. crispa* mats compared to *P. oceanica* meadows?

(2) Which species are found in both habitats and which species are unique to *P. crispa* mats?

(3) What is the spatial variability of serpulid polychaetes associated with red algae mats?

Materials and methods

Study Area and Sampling Activities

The study was carried out at five sites distributed along the North-eastern and North-western coasts of Giglio Island, in the Tuscan Archipelago National Park (42°21'19.4" N 10°54'06.1" E, Tyrrhenian Sea) (Figure 2). The islands' underwater seabeds are characterized by granite slopes, alternating with sand bottoms, where *P. oceanica* meadows, *P. crispa* mats and coralligenous habitats colonize infralittoral seabeds. All samples were collected by SCUBA divers at a water depth of 30 m. Samplings were carried out at three sites with *P. crispa* mats (Site PC1, PC2 and PC3), one with only *P. oceanica* meadows (Site PO), and one site (Site mix) with both habitats being present (Figure 2).

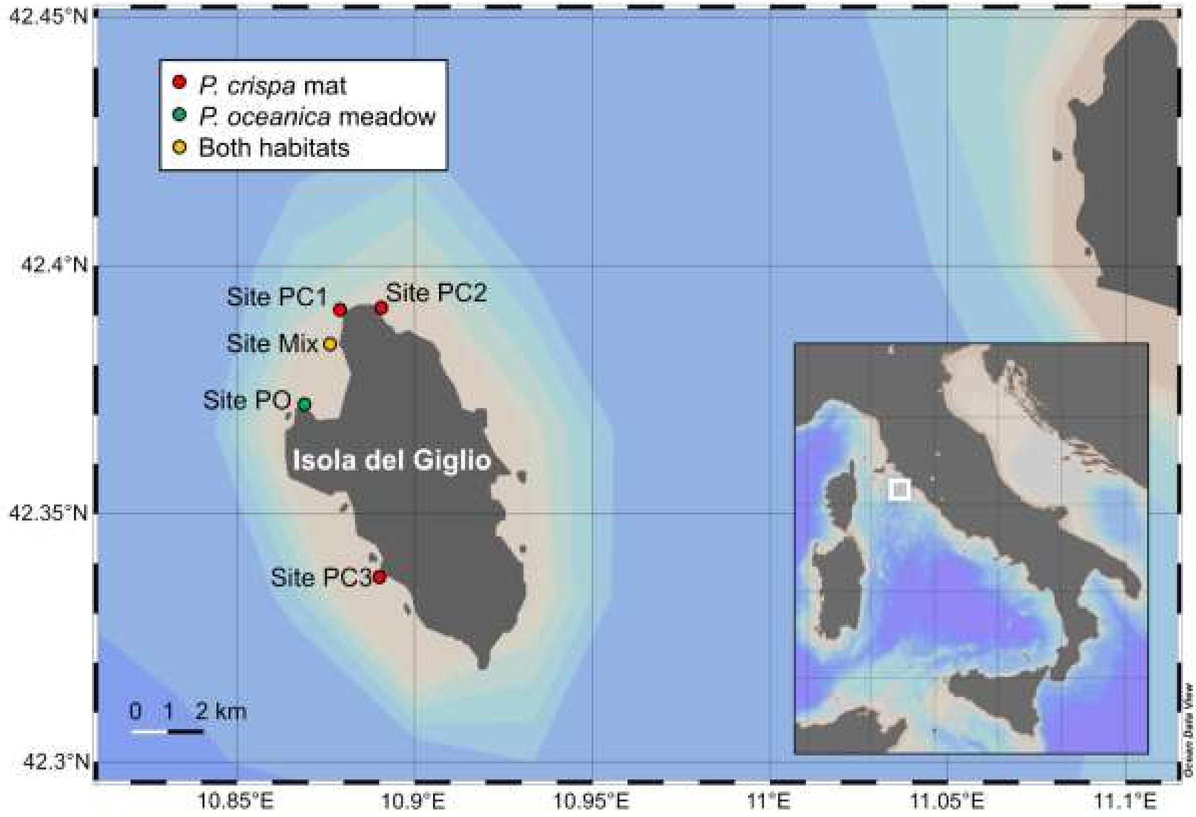

Figure 2: Location of Giglio Island in the northern part of the Tyrrhenian Sea and sampling sites, for P. crispa mats (red), P. oceanica meadows (green) and sites where both habitats were sampled (yellow).

Temporally randomized sampling took place during late spring, between May and July 2019. For sampling *P. crispa* mats, a standardized maximum mat thickness of 5 cm was defined. After randomly defining a sampling spot in the target area, a metal frame (size 30 × 30 cm) was placed in the mat and all algal thalli within, including the holdfasts, were carefully removed using a spatula. Sampled material was then placed into 1 L Kautex jars (each holding approx. 1/3 algae, 2/3 seawater). Every site was sampled four times, resulting in a total of 16 replicate samples for this habitat.

In total, 19 *P. oceanica* shoots and 17 leaves (site PO: 10 shoots and 9 leaves; site mix: 9 shoots and 8 leaves) were sampled separately into 1 L Kautex jars. The leaves were cut at the sheath of the shoot and shoots were cut at the rhizome node. Samples were immediately transferred into seawater holding tanks at the Institute for Marine Biology (IfMB, Campese, Italy) and kept at a constant temperature of 18 °C. Shoots and leaves were treated as separate subhabitats taking into account their different ecological traits, particularly regarding their longevity as a fundamental trait for serpulid settlement[44,45]. With this approach, we followed recent studies on *P. oceanica* epifauna[46–48]. The number of samples at each of the sites represents the whole epiphytic community, as demonstrated by previous studies on epiphytic communities associated with *P. oceanica* meadows that used 15 shoots as a significative sampling effort[49,50]. We used this number as a minimum goal for our sampling efforts. Sample completeness was confirmed using the approach of Chao et al. (2016) (Appendix A Figure A1).

In addition, we counted the number of *P. oceanica* shoots per m² (total *n* = 74 quadrats; using a plastic tube frame of 40 × 40 cm) and leaves per shoot (total *n* = 32 shoots) in the sampling area.

Species Identification

For the analysis of *P. crispa* associated serpulid polychaetes, a subsample of approximately 10 g wet weight was taken from the main sample. All replicated samples were processed within three days after collection. Algal thalli were transferred into small bowls and cut into single phylloids for analysis under a stereomicroscope (maximum 40× magnification). Following the analysis, the wet weights of the main and subsamples were measured after removing adherent water. The *P. oceanica* shoots were analyzed as a whole under a stereo microscope, while leaves were cut into pieces of approximately 8 cm for easier handling and to avoid double counting.

All specimens were identified using relevant literature (Appendix A Table A1) and crosschecking with online resources (WORMS; marinespecies.org, accessed on 31.07.2019. The number of individuals per species were also recorded for quantitative analysis. Counting and sample processing were standardized, and every observer was trained to a high level of taxonomic proficiency prior to processing the samples.

The data were then correlated to the surface area, as individuals per m^2 surface of *P. crispa* and *P. oceanica*. Thalli of *P. crispa* were placed in a bowl of water on a laminated graph paper sheet and flattened with a glass pane. Pictures were taken from a 90° angle using a Canon G12 camera and a tripod stand to ensure a constant distance and angle to the sample. The surface area was then calculated from the picture with ImageJ (version 1.52o, https://imagej.nih.gov/ij/, accessed on 23.04.2019) and multiplied by two to account for both sides of the thalli. The measured surface area was then extrapolated to the main sample, using the wet weight measured after the analysis. This relation allowed for the extrapolation of the density of serpulids to the surface area of underlying rock (Appendix A Formula (A1)). For *P. oceanica* shoots, we assumed a cylindrical shape and calculated the surface area using diameter and length. For *P. oceanica* leaves, we assumed a rectangular shape, calculating its surface area with length and width multiplied by two to account for both sides of the leaf. The total surface area of *P. oceanica* substrate was then extrapolated using the field observations of leaves per shoot and shoots per m² (Appendix A Formulas (A2) and (A3)), to assess the number of serpulids per m² of seafloor.

Diversity Descriptors

Diversity was assessed using four descriptors: total numbers of serpulid taxa per site and habitat, total abundances of individuals per m² of substrate, Shannon diversity index[51] and Pielou evenness index[52]. The descriptors were calculated as means per site and then reported with the respective standard error.

Statistical Analysis

Pairwise Wilcoxon–Mann–Whitney tests were carried out to assess differences in diversity descriptors among sites and habitats (Appendix A, Table A2). Differences in the composition of serpulid assemblages among sites and habitats were tested through multivariate permutational analysis of variance (PERMANOVA[53]). The northern sites' data were pooled to compare among habitats and further analyze differences among *P. crispa* sites (Table 1). Pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test. Data was not transformed to stress the importance of the abundance of taxa in determining the differences among habitats. The Serpulid assemblages were hierarchically clustered with Spearman ranked correlation (average linkage) using the software 'heatmapper'[54] to reveal differences in the species composition among sites and visually highlight variances in the serpulid assemblages among habitats. Analyses and plots were made with R (version 3.5.3)[55].

Table 1: Results of permutational multivariate analysis of variance (PERMANOVA) of Serpulid assemblages among P. oceanica subhabitats (shoots and leaves), and pairwise comparison of all habitats on the northern sites (site PC1, PC2, PO and mix). Significant results are indicated in bold.

Serpulid Assemblages on *P. oceanica* Subhabitats

Source	Df	SS	R2	F	*p*
Habitat	1	6.1885	0.5350	50.946	**0.001**
Site	1	0.2829	0.0267	2.329	0.094
Habitat:Site	1	0.2474	0.2333	2.037	0.117
Residuals	32	3.8871	0.3665		
Total	35	10.6059	1.0000		

Pairwise Comparison (All Habitats, N Sites)

Pairs		*p*	*p* adj
P. crispa mat	*P. oceanica* leaf	**0.001**	**0.003**
P. crispa mat	*P. oceanica* shoot	**0.001**	**0.003**
P. oceanica leaf	*P. oceanica* shoot	**0.001**	**0.003**

Results

Diversity Descriptors

A total of 2403 Serpulidae specimens belonging to 17 taxa were collected. Overall abundances of individuals m^{-2} and numbers of taxa were significantly higher in *P. crispa* mats than in *P. oceanica* shoot and leaf samples (Figure 3).

The density of individuals was highest in *P. oceanica* shoots at site mix (average 8197 ± 1549 individuals m^{-2}), and lowest in *P. oceanica* leaves at site PO (average 1714 individuals m^{-2}). The comparison with *P. oceanica* leaves showed significantly higher densities for *P. crispa* mats ($p < 0.001$). The shoots of *P. oceanica* hosted a similar number of individuals (average 5890 ± 815 individuals m^{-2}) compared to *P. crispa* samples (average 5664 ± 622 individuals m^{-2}) (Figure 3A). The calculated numbers of serpulids per m^2 seafloor showed a similar trend of highest values for *P. crispa* and *P. oceanica* shoots (average 1,239,728 ± 784,455 and 864,444 ± 1,081,419, respectively), and a lower density for *P. oceanica* leaves (average 238,567 ± 179,055). The combined averages of both *P. oceanica* sub habitats add up to 1,103,011 ± 1,096,142 individuals per m^2 seafloor.

All of the 17 identified taxa were found in *P. crispa* samples and only 10 taxa were found in *P. oceanica* samples. The highest number of *P. crispa* associated taxa were found in the samples from Site PC2 (average 8 ± 1 taxa) and the lowest in samples from site mix (average 6 ± 0 taxa) and site PC3 (average 6 ± 2 taxa). In comparison, *P. oceanica* samples harbored 2 ± 1 different taxa on the leaves and shoots, respectively (Figure 3B).

Shannon diversity index was higher in *P. crispa* mats (average 1.1 ± 0.09) compared to *P. oceanica* habitats (average leaves: 0.2 ± 0.06; average shoots: 0.2 ± 0.07; Figure 3C). Site PC3 showed a significantly lower diversity compared to other *P. crispa* samples ($p < 0.05$).

Pielou evenness index was highest in *P. crispa* samples (average 0.6 ± 0.05) compared to *P. oceanica* habitats (average leaves: 0.4 ± 0.09; average shoots: 0.4 ± 0.04; Figure 3D). Site PC3 showed a significantly lower evenness compared to other *P. crispa* samples ($p < 0.05$).

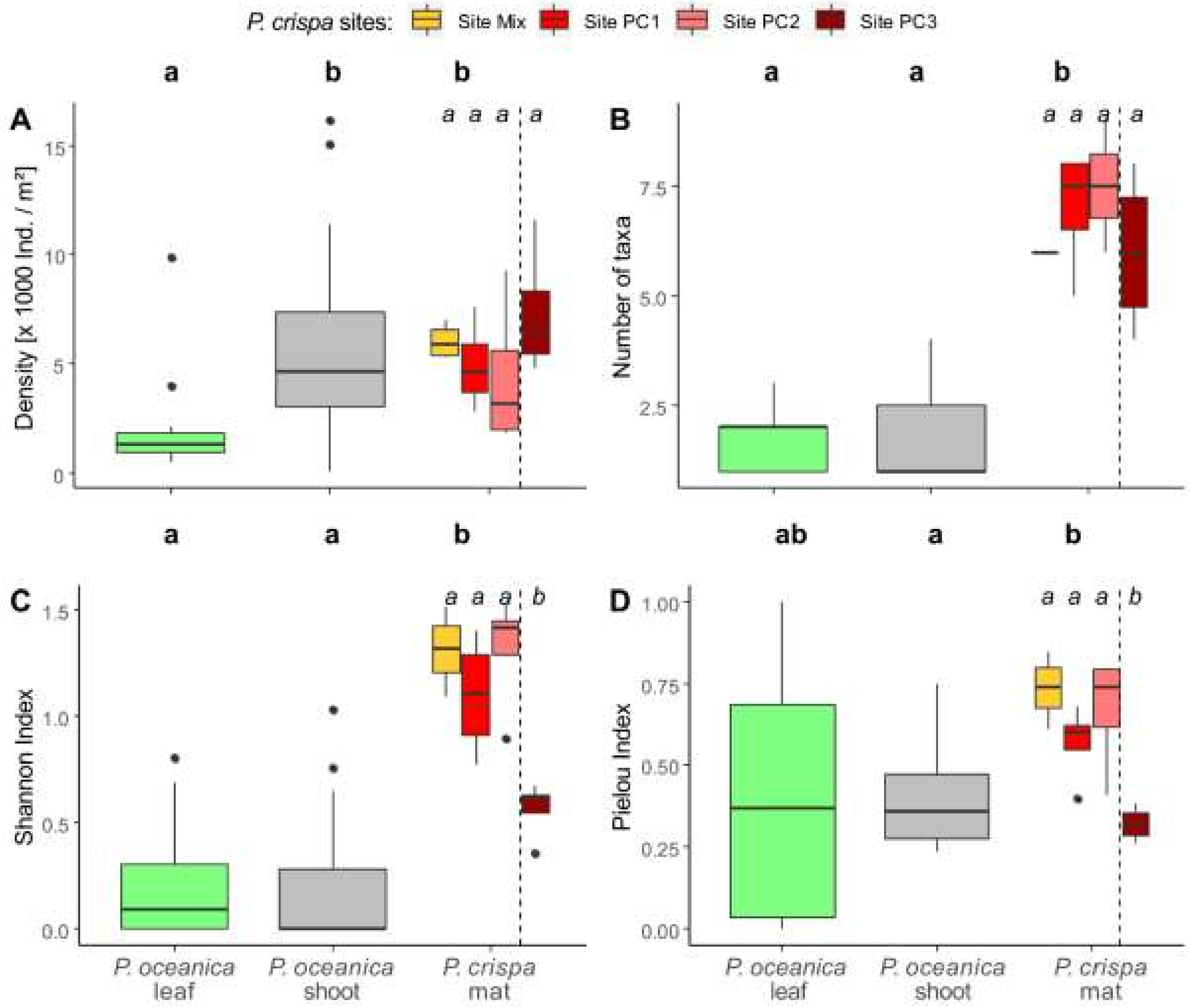

*Figure 3: (**A**) Total numbers of identified species per site, (**B**) mean densities of serpulids per m^2 substrate per site, (**C**) Shannon diversity index and (**D**) Pielou evenness index reported for the investigated P. crispa (red) sites in comparison to P. oceanica leaves (green) and shoots (grey). Letters a and b indicate the results of pairwise Wilcoxon–Mann–Whitney tests performed for comparison among habitats in northern sites (excluding PC3; bold letters) and among all P. crispa sites. Black dots resemble outliers.*

Analysis of Serpulid Assemblages

The multivariate analyses (PERMANOVA) showed significant differences among the two *P. oceanica* habitats without an effect of the site (Table 1). The analysis of *P. crispa* sites showed a significant difference among sites; however, the pairwise comparison did not confirm this result (Table 2). We further analyzed differences among the habitats for the northern sites only, avoiding potential effects of the location around the island. The cluster analysis confirms these results and shows differences in the species composition of the different habitats (Figure 4). The dendrogram of Spearman rank correlation across sites and habitats shows clustering according to habitat, with a lower coefficient for *P. crispa* and *P. oceanica* shoots ($r < 0.6$) than *P. oceanica* leaves compared to *P. oceanica* shoots and *P. crispa* mat ($r > 0.8$).

Table 2: Results of permutational multivariate analysis of variance (PERMANOVA) and pairwise comparison of Serpulid assemblages among P. crispa sites. Significant results are indicated in bold.

Serpulid Assemblages on *P. crispa* Mats

Source	Df	SS	R2	F	*p*
Site	3	0.9182	0.4066	2.7407	**0.012**
Residual	12	1.3401	0.5934		
Total	15	2.2583	1.0000		

Pairwise Comparison (*P. crispa* Sites)

Pairs		*p*	*p* adj.
Mix	PC1	0.444	1.000
Mix	PC2	0.032	0.192
Mix	PC3	0.033	0.198
PC1	PC2	0.351	1.000
PC1	PC3	0.104	0.624
PC2	PC3	0.062	0.372

The most frequent species found in all samples of *P. crispa* mats was *Josephella marenzelleri*. This species was also largely present on *P. oceanica* shoots, however, it was not found on *P. oceanica* leaves. In contrast, the most abundant species on the leaves was *Janua* sp., which also occurred in 13 *P. crispa* replicates and on 7 investigated *P. oceanica* shoots. In addition, *J. marenzelleri* was also the most abundant species in terms of individuals found m^{-2} of *P. crispa* and *P. oceanica* shoots, while *Janua* sp. showed the highest density on *P. oceanica* leaves. Out of the 17 species, two were exclusively found on *P. crispa* samples: *Bathymermilia* sp. and *Vermiliopsis labiata* (Figure 4). The species *Pileolaria militaris* showed similar abundances in *P. crispa* and *P. oceanica* samples.

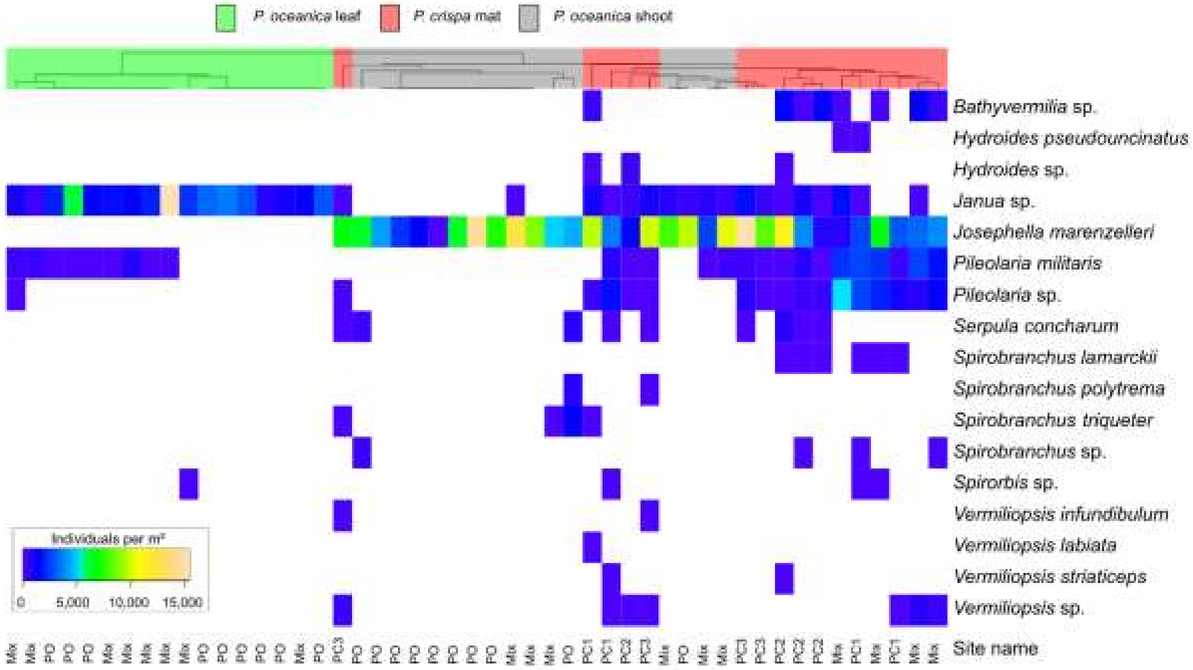

Figure 4: Hierarchical clustering (average linkage, Spearman ranked correlation) of taxa abundances among sites (individuals per m²) found in P. crispa (red) and P. oceanica habitat northern sites (green = leaf; grey = shoot).

Discussion

This study highlights the high diversity and density of serpulid polychaetes for all investigated habitats. The total number of species found associated with *P. crispa* mats (17 species) was consistent with previous studies from other areas in the Mediterranean Sea [37,56], including the results of Casoli et al. (2016), which investigated *P. crispa* mats along a water depth gradient and found 16 species (Table 3) [19]. Here, we focused on the water depth range with the highest *P. crispa* mat development and identified potential differences among sites. Species richness and abundance were high across all sites; however, the southernmost site (Site PC3) showed a lower diversity and evenness than the northern sites.

The extrapolated abundances per m² seafloor were comparable between *P. crispa* (average 1,239,728 ± 784,455) and *P. oceanica*, (1,103,011 ± 1,096,142) with the shoots mainly contributing to these numbers. However, the calculations likely cause some bias due to the extrapolation based on average values, as indicated by the large standard deviations.

Comparing *P. crispa* mats and neighboring *P. oceanica* meadows revealed significant differences in serpulid assemblage composition. By modifying the substratum through the creation of spatial microhabitats and influencing feeding habits, seagrass beds and algae mats appear to favor the establishment of different polychaete assemblages [43,57,58]. Habitat formation and the substantial improvement of food source accessibility are pivotal ecological drivers that influence the epiphytic serpulid assemblages' diversity and structure. Overall abundance, species richness, diversity and evenness were significantly lower in most *P. oceanica* samples compared to *P. crispa*. Exceptions are *P. oceanica* shoots, where similar densities of individuals were found compared to *P. crispa* mats. The species composition was also similar, with the same dominant species (*J. marenzelleri*), which resulted in a shared cluster disparate from the *P. oceanica* leaf samples (Figure 4). This clustering could be related to the two habitats' semi-hemisciaphilious conditions, which leads to reduced competition with algal epiphytes and influences rhizome communities[31]. Lower light conditions inside *P. crispa* mats and *P. oceanica* shoots are also reflected by the lower numbers of the photophilic Spirorbinae (e.g., *Janua* sp.)[59], mostly found on the leaves of *P. oceanica*. The perennial *P. crispa* thalli provide an extension of colonizable surfaces that are less flexible and long-lived compared to *P. oceanica* leaves. Constant motion and a one-year life cycle favor species with well-developed strategies to cope with stresses on *P. oceanica* leaves, such as Spirorbidae (*Janua* sp.). These are recognized as pioneer species, characterized by fast and cinsistent recruitment on smaller surfaces[60,61]. Thus, dense red algae mats composed of both prostrate and erect thalli (heterotrichous species)[17] constitute a sheltered and long-lived habitat rather than oscillating structures of shallow algae or *P. oceanica* leaves.

In comparison to previous reports of neighboring (i.e., coralligenous reefs) habitats, we see that *J. marenzelleri* is a ubiquitous species that is found from the shallow infralittoral to deeper coralligenous habitats (Table 3). This species is known to be a pioneer species with the ability to colonize available settling grounds quickly and efficiently[36]. Furthermore, *J. marenzelleri,* with its articulate tube, is particularly adapted to colonize rigid algal thalli as epiphytes, being found both on *Cystoseira* spp. canopy and encrusting red algae[59,62]. Other widely distributed taxa characterized the serpulid assemblage on *P. crispa* samples. However, the presence of species with dark or deep-habitat affinity (*Bathyvermilia* sp. and *V. infundibulum*) confirmed the hemisciaphilic conditions provided within the algal mat.

In contrast, serpulids with a larger and more erect growth form are only found in coralligenous habitats (e.g., *Protula* sp., Table 3). These slow-growing species are sensitive to water movement and prefer hard settling grounds over flexible algae and plants[59]. However, the overall high abundance of serpulids on *P. crispa* thalli supports their rigid structure and long-term stability as key characteristics for the establishment of these species. The laminal thalli of *P. crispa* represent a living substratum suitable for the colonization of sessile solitary species, increasing the surface available for settlement. Sedimentation processes on horizontal and gently sloped sea beds, where *P. crispa* is abundant, can help understand the patterns described in this study[17]. Serpulidae comprise exclusively filter-feeding species that can benefit from organic and inorganic particle sedimentation as trophic sources.

Communities of the brown algae *Cystoseira* sp. in the Ionian Sea host a notably higher diversity of serpulids (Shannon of 2.4) than we found in *P. crispa* mats (average Shannon of 1.1)[59]. This shows dominance of a few species in the *P. crispa* mats, as also seen in the abundance values. Interestingly, the abundances exhibit a trend of higher numbers on the southern site (PC3) while diversity and evenness are significantly lower (Figure 3). This variation could reflect the serpulid assemblage's heterogeneity over a larger spatial scale due to the physical exposure of different *P. crispa* mats. While the diversity was still considerably high in both areas, it also points out that the western Peninsula (Figure 2) separates the southern *P. crispa* mats from the northern patches. This effect could be driven by the rather exposed situation of PC3 to the prevalent southern currents, while the northern sites are situated on the leeward side of the island[63]. Thus, this situation could also impact larval as well as food particle supply.

Table 3: Serpulidae species found in the samples compared to other habitats, x indicates presence; Data from (1) Casoli et al. (2016)[19] and (2) this study (grey columns).

Species	Shallow Infralittoral (1)	*P. crispa* Mat (1),(2)		Coralligènous Habitat (2)	*P. oceanica* Meadow (2)
Bathyvermilia sp			x		
Filograna implexa (Berkeley)	x	x			
Hydroides spp. (Gunnerus)	x	x	x	x	x
Janita fimbriata (Delle Chiaje)				x	
Janua sp	x		x	x	x
Josephella marenzelleri (Caullery and Mesnil)	x	x	x	x	x
Metavermilia multicristata (Philippi)				x	
Pileolaria spp	x	x	x	x	x
Protula sp. (Risso)				x	
Semivermilia crenata (O. G. Costa)		x		x	
Semivermilia cribrata (O. G. Costa)				x	
Serpula spp	x	x	x	x	x
Serpula vermicularis (Linnaeus)		x		x	
Spiraserpula massiliensis (Zibrowius)				x	
Spirobranchus spp	x	x	x	x	x
Spirorbis spp			x	x	x
Vermiliopsis spp		x	x	x	

We conclude that *P. crispa* mats harbor a rich serpulid assemblage, comparable to or even exceeding other neighboring biodiversity hotspots (i.e., *P. oceanica* meadows). Furthermore, we found two species that were unique to this habitat in our study. This high diversity underlines the vital function of *P. crispa* as a habitat-forming species and host for exceptional biodiversity. Therefore, we recommend evaluating this habitat for future conservation actions to prevent habitat and biodiversity loss. Future research should also address the role of other taxonomic groups associated with the *P. crispa* mats and assess differences in the composition of infauna communities on a regional scale to confirm its role as an essential benthic habitat in the (western) Mediterranean Sea. Our results revealed potential regional differences in the *P. crispa* associated fauna that need to be further addressed by sampling other areas along the Mediterranean coast. This information is crucial to explore how connected or isolated the different patches of *P. crispa* mats are in terms of exchange of biodiversity and larval settlement. Potential drivers of connectivity for serpulid communities are prevalent water currents[33] as indicated by the differences between the northern sites and the rather exposed southern site[63].

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Chapter 4 – High Diversity and Abundance of Foraminifera Associated with Mediterranean Benthic Red Algae Mats

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Abstract

The Mediterranean Sea comprises habitats such as *Posidonia oceanica* seagrass meadows that exhibit high associated biodiversity of sessile organisms. Recent pilot research indicates that benthic mats formed by the scarcely investigated fleshy red alga *Phyllophora crispa* also host a high diversity of benthic fauna. Among the key taxa found in these mats in the recent pilot studies are benthic foraminifera that live as epiphytes on the red algae thalli. Knowledge about their abundance and species richness associated with this habitat in relation to reference habitats is missing. We thus carried out a comparative assessment focusing on foraminifera within samples from *P. crispa* mats and neighboring *P. oceanica* meadows on five different sampling sites around Giglio Island in the Tuscan Archipelago (Tyrrhenian Sea, Italy). A total of 104 different foraminiferal taxa were identified, of which a total of 85 taxa were found in *P. crispa* samples (46 exclusively in this habitat). This biodiversity was higher compared to other studies on phytal habitats in the Mediterranean Sea. The number of foraminiferal taxa associated with *P. crispa* was significantly higher (average 27.5 ± 8.1 taxa) compared to *P. oceanica* (leaves average 7.0 ± 3.6, shoots average 7.9 ± 3.4 taxa). The abundance of foraminifera (12,000 individuals m^{-2} surface area of *P. crispa* mat) was also higher than in the neighboring *P. oceanica* meadows (7,792 individuals m^{-2} leaf and 8,171 individuals m^{-2} shoot surface area). The most frequently found taxa across habitats were *Miniacina miniacea*, *Lobatula lobatula,* and *Sejunctella* sp. (24%, 20%, and 6% of the total population, respectively). Our results imply that *P. crispa* mats host an exceptional diversity of associated foraminifera that is even higher than those associated with seagrass meadows. Red algae mats built by *P. crispa* may thus be considered as potential refuge habitats and biodiversity reservoirs in management and conservation.

Keywords: *Phyllophora crispa*, phytal habitat, hard-bottom communities, ecosystem engineer, Mediterranean Sea, epibionts

Chapter 4 – High Diversity and Abundance of Foraminifera Associated with Mediterranean Benthic Red Algae Mats

Introduction

The Mediterranean Sea harbors almost 17,000 described eukaryotic species[1], a high amount of biodiversity that is accompanied by a high rate of endemism (estimated 20%) resulting from its somewhat enclosed geographical position[2]. Together with this high rate of endemism, this high biodiversity marks the region as a "biodiversity hotspot"[1,3,4]. On a smaller scale, the structural complexity of many Mediterranean habitats (e.g., *Posidonia oceanica* seagrass meadows) facilitates their role as biodiversity hotspots by providing spawning grounds, nurseries, and permanent settling space for a variety of sessile and mobile species across habitat borders[5–9]. The ecological niches required for this high biodiversity are often created by spatial or temporal gradients (e.g., light and temperature, as recently described for *P. crispa* mats[10]) formed by an engineering species[11]. Engineering species may alter their environment by their growth, while others actively change local conditions to favor inhabiting species. The resulting modification of the habitat may result in shifts of ecological zonation (e.g., algae accumulating debris as a food source for detritivores) by mitigating stressors for depending species[12]. Often, also the inhabitant mitigates stress for the engineer, e.g., by fending off predators or cleaning out competitors[13,14].

Well-known habitats of high diversity in the Mediterranean Sea are *Posidonia oceanica* (L.) Delile, 1813 meadows, and coralligenous habitats. The seagrass species *P. oceanica* is a refuge for exceptional biodiversity and holds commercial value, e.g., by providing nursery grounds for important fish species[15]. The structural complexity of the meadow facilitates diverse communities of sessile invertebrates by providing secondary settling grounds and enhancing the settlement of planktonic life stages[5,16]. The calciferous bioconstructions of coralligenous habitats are mainly built by encrusting red algae and secondarily by calcifying invertebrate species, building a structurally complex matrix, and providing biogenic substrate for a wide range of associated biota[7]. Both are thus habitat-forming species and can be considered ecosystem engineers.

Recently, the mats of the red macroalgae *Phyllophora crispa* (Hudson) P.S.Dixon, 1964 have gained some attention as engineering species and potential hotspots of sessile epifauna biodiversity in the Mediterranean Sea[17–22]. In the Black Sea, these habitats facilitate prosperous communities and have led to establishing a marine reserve in the Bay of Odesa[23]. In the Mediterranean Sea, assemblages of different macroalgae have been shown to host diverse epiphytic foraminiferal communities[24,25].

In the Mediterranean Sea, dense mats of *P. crispa* have been reported to form hard-bottom communities in the Tyrrhenian Sea, around the islands Giglio[19,20] and Sardinia[17,18]. However, only e few studies describe the associated biodiversity in these mats, even though the composition of associated biodiversity differs between classical hotspots (i.e., *P. oceanica* meadows) and sciaphilic (shade-loving) hardbottom communities[26,27].

Foraminifera are unicelled protists with a high taxonomic diversity and cosmopolitan distribution across all marine ecosystems, brackish, and rarely freshwater habitats[28,29]. Foraminifera inhabit pelagic and benthic environments; the latter may be colonized by recent offspring or settlement of pelagic propagules[30]. Especially vegetated bottoms form suitable habitats for epiphytic foraminifera, and primarily seagrass meadows have been studied for their foraminiferal assemblages[31–37]. Some studies have also shown the importance of macro-algae as particular habitat for epiphytic foraminifera[38,39]. Many taxa of foraminifera host different symbionts, such as red- / green algae, diatoms, or dinoflagellates[40]. Long-lived, symbiont-bearing benthic species larger than 3 mm³ are summarized as larger benthic Foraminifera (LBF) [41,42]. Especially LBF species are important calcifiers[43], and been recognized as essential indicators for water quality and healthiness of marine ecosystems[44–46]. Key features for this function are their relatively short lifespan and reproductive cycle compared to benthic epifauna, their ubiquity, and sensitivity to biotic and abiotic perturbations, leading to rapid community composition changes after environmental disturbances[47–49]. The distribution patterns and role of foraminiferal communities in Mediterranean *P. crispa* mats are relatively unstudied to date. With this work, we aim to answer the following research questions:

1. What is the abundance and diversity of epiphytic foraminifera in *P. crispa* mats in relation to *P. oceanica* meadows?
2. What is the composition of epiphytic foraminifera morphotypes in *P. crispa mats*?

To answer these questions, we conducted a comparative field study of *P. crispa* mats and *P. oceanica* meadows along the coast of Giglio Island in the Tuscan Archipelago (north-western Mediterranean Sea). We assessed the quantity of foraminifera occurrence and species composition at five different sampling sites.

Materials and methods

Sampling activities

The study area is located along the North-eastern and North-western coasts of Giglio Island, in the Tuscan Archipelago National Park (42°21'19.4"N 10°54'06.1"E, Tyrrhenian Sea) (Figure 1). Granite slopes characterize the benthic infralittoral around the island, alternating with sand bottoms, where *P. oceanica* meadows, *P. crispa* mats, and coralligenous habitats colonize the sea bed. Five sampling sites were chosen where *P. crispa* mats, and *P. oceanica* meadows occur in similar target water depths. SCUBA divers conducted sampling at a water depth of 30 m, where previous observations confirmed dense occurrences of *P. crispa*. Samples of *P. oceanica* were taken randomly between 20 and 30 m depth, a few meters above the lower boundary, to avoid bias by sampling at the ecological limit of the plant. The sampling sites included three locations with *P. crispa* mats (Site PC1, 2, and 3; Figure 1), two with only *P. oceanica* meadows (Site PO and 6), and another site (Site mix) with both habitats being present. Sampling activities took place on a timely randomized schedule between May and July 2019. Mats of dense *P. crispa* stands with a minimum thickness of five cm were chosen randomly. A handful of whole algae was carefully removed with the holdfast from the rock surface using a spatula. Each site was sampled four times, resulting in 16 *P. crispa* mat samples. All sampling was done with a spatula or scissors to avoid dropping of the specimen due to shaking movements (e.g., by just ripping off material) before immediately transferring the material into a sampling jar as described in previous studies[24,33]. We decided for this method to focus on the living, epiphytic foraminifera and avoid loss of fragile taxa by drying or sieving methods.

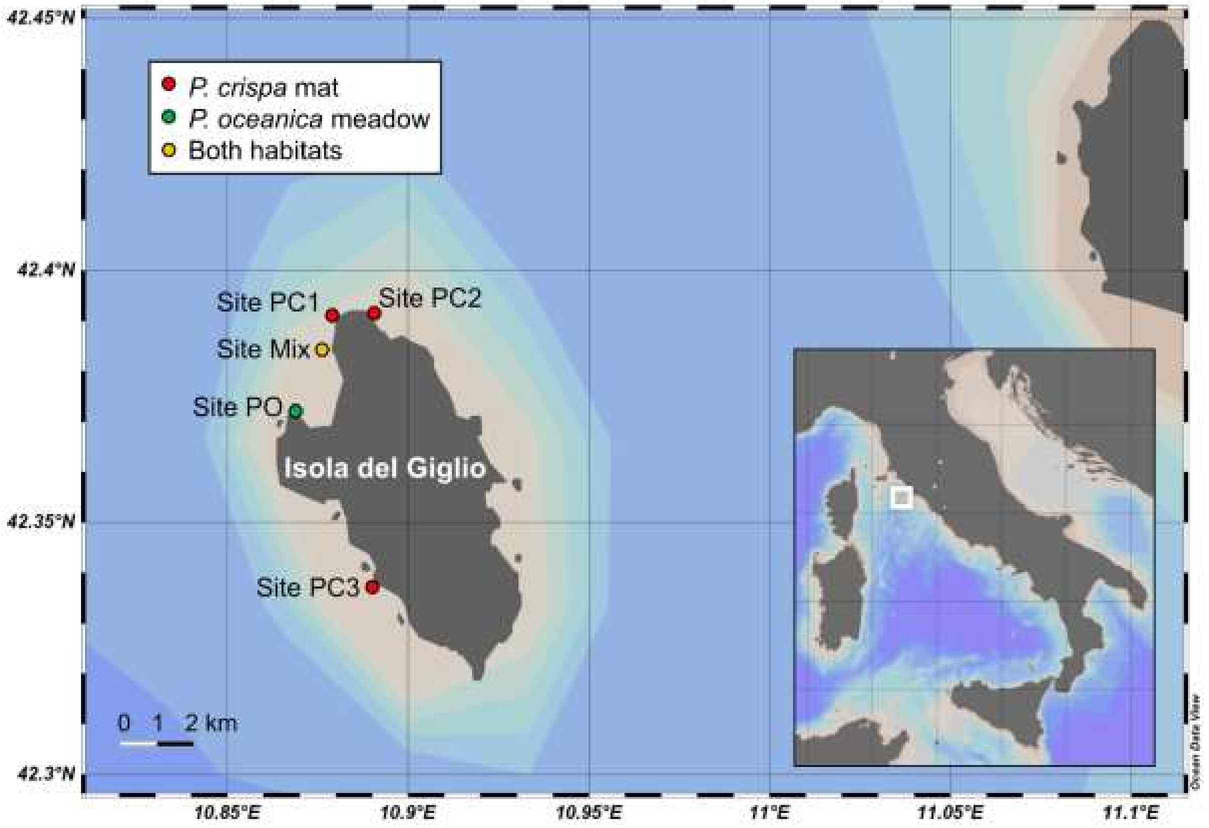

Figure 1: Location of the study area in the Tyrrhenian Sea with sampling sites around Giglio Island marked in red for Phyllophora crispa mats, green for Posidonia oceanica meadows, and yellow where both habitats were sampled. Made with OceanDataView [50].

In total, 20 *P. oceanica* shoots and 18 leaves (site PO: 10 shoots and 9 leaves; Site Mix: 10 shoots and 9 leaves) were sampled at the respective sites. Shoots were cut at the lowest point to the rhizome node, and leaves were cut off at the sheath. All samples were transferred into one-liter PVC containers, allowing enough water inside to keep a constant temperature and sufficient oxygen supply for the biota (approximately 1/3 sample material and 2/3 seawater). After each dive, samples were directly transferred into husbandry basins at the Institute for Marine Biology (IfMB, Campese, Italy) and kept at constant temperature (18 °C, equivalent to *in situ* temperature), with bubbling stones for oxygen supply. Processing of samples took place within three days after sampling. Shoots and leaves were treated separately because they are widely recognized as two sub-habitats with different ecological traits[51,52]. The sample completeness was confirmed with the rarefaction and interpolation method described by Chao et al. (2016) (Appendix, Figure A1)[53]. We also opted for this sampling approach to examine a similar surface area for each habitat and site (see results section).

In addition to the biodiversity assessments, we measured daily cycles of environmental parameters (i.e., oxygen and pH) on site PC2, where *P. oceanica* meadow (n = 12), *P. crispa* mat (n = 22), and bare rock bottom (n = 12) were found in close vicinity (< 10m distance). Oxygen concentration (mg / l) and pH were measured at the bottom of each habitat using Eureka Manta loggers (GEO Scientific Ltd.), set to one-minute measuring intervals. The obtained data were plotted as average values per hour ± SD (Figure 4).

Species identification

All samples were examined as a whole in water bowls containing seawater under stereo magnifiers (maximum 45x magnification) or microscope (maximum 400x magnification) as needed. The leaves of *P. oceanica* were cut into pieces of 8 cm for easier handling under the stereomicroscope and to avoid double-counting. The shoots were analyzed as a whole. Algae thalli of *P. crispa* were carefully separated into single phylloids for the analysis. This study focused on living epiphytic foraminifera > 300µm to avoid juveniles and abundance bias due to short-lived, opportunistic species.

All foraminiferal specimens found in the samples were counted and identified to the lowest possible taxonomic level using relevant identification literature[32,36,54,55] and online resources (marinespecies.org, mindat.org, accessed on 16.06.2021) as a cross-reference. Species were then further categorized into morphotypes regarding their ecological features. We followed the categories proposed by Mateu-Vicens et al. (2014) in adaptation for the Mediterranean Sea, based on the original concept of Langer (1993)[35,37]. In this adaptation, five morphotypes were used: A* and SB for long-lived (lifespan of 1 year or more) and sensitive species,where A* are mostly flat and encrusting forms and SB species bear symbionts (e.g. *Miniacina* spp. and *Peneroplis* spp., respectively). Opportunistic species with an upright aperture were categorized as D* (e.g. *Textularia* spp). Small, heterotrophic species were categorized as B or C, where B comprise species with wide apertures and pseudopodal networks, and B encompass species with multiple apertural openings, attached to the substrate via pseudopods.

Numbers of foraminifera were then related to the surface area of the substrate (*P. crispa*, respectively *P. oceanica* material as subsequently described). For *P. crispa* samples, all algae material was placed on top of laminated millimeter paper in a dissection dish with seawater and flattened with an acrylic glass pane. Using a tripod, pictures were taken from the top at a constant 90° angle. The surface area of the algae material was then calculated with ImageJ (version 1.52o) and multiplied by two to account for both sides of the phylloids. To calculate the *P. oceanica* shoot surface, a cylindrical shape was assumed and calculated after measuring the length and diameter of each sample. The straight leaves of *P. oceanica* were measured for length and width to calculate the surface area. This area was then doubled to account for both sides of each leaf.

Diversity descriptors

The diversity of the epiphytic foraminiferal community was assessed with five descriptors: The total number of taxa per site and habitat, the Shannon diversity index (Appendix, Formula A1)[56], the Pielou index for evenness (Appendix, Formula A2)[57], plus the FORAM index and the lifespan index[35,37]. Additionally, we assessed the total density of individual foraminifera per m² of seafloor. We calculated the FORAM index[46] and the lifespan index, as previously described for the Mediterranean Sea[35], based on the aforementioned morphotype categories. The FORAM index considers the proportions of small, heterotrophic taxa, larger, symbiont-bearing taxa, and stress-tolerant taxa. The index increases with higher abundances of symbiont-bearing taxa, and decreases with higher abundances of stress-tolerant taxa. The lifespan index emphasizes differences in the foraminiferal community regarding opportunistic vs. persistent taxa dominance. With lower values for high abundances of opportunistic taxa (D*), and higher values for longer living forms (A* and SB). A detailed figure of the morphotype classification and explanation of the calculations can be found in the original publication[35].

All results were plotted as boxplots and reported as means per site with the respective standard error. To further analyze the functional composition of the community, all found taxa were pooled according to their test material, and the relative composition of each site was plotted.

Statistical analysis

Differences in the structure of foraminiferal communities among sites and habitats were tested using multivariate permutational ANOVA (PERMANOVA)[58]. Differences in the diversity descriptors among sites and habitats at the site mix were tested using pairwise Wilcoxon-Mann-Whitney tests. Data was not transformed in order to minimize potential bias. The Foraminiferal assemblages were hierarchically clustered with Spearman ranked correlation (average linkage) using the software ’heatmapper’[59] to reveal differences in the taxonomic (Families) and ecological (test material) composition among sites and visually highlight variances in the assemblages among habitats. Analyses and boxplots were made with R (version 4.0.2)[60].

Results

Daily cycles of water parameters

Our measurements showed higher pH values inside the *P. crispa* mat (average 8.36 ± 0.01) than inside the *P. oceanica* meadow (average 8.21 ± 0.01) and on hardbottom reference habitat (average 8.32 ± 0.02; Figure 2A). Inside the *P. crispa* mat, the values fluctuated more (0.11 units) compared to *P. oceanica* meadow (0.05 units) and hardbottom (0.08 units).

The measured oxygen concentrations showed similar patterns for the *P. crispa* mat and hardbottom habitat (average 8.17 ± 0.10 mg l^{-1}, and average 8.20 ± 0.07 mg l^{-1}, respectively), compared to the *P. oceanica* meadow (average 7.90 ± 0.14 mg l^{-1}; Figure 2B). Here, the variation was highest inside the *P. oceanica* meadow (0.47 mg l^{-1}) compared to the *P. crispa* mat (0.33 mg l^{-1}) and the hardbottom habitat (0.24 mg l^{-1}).

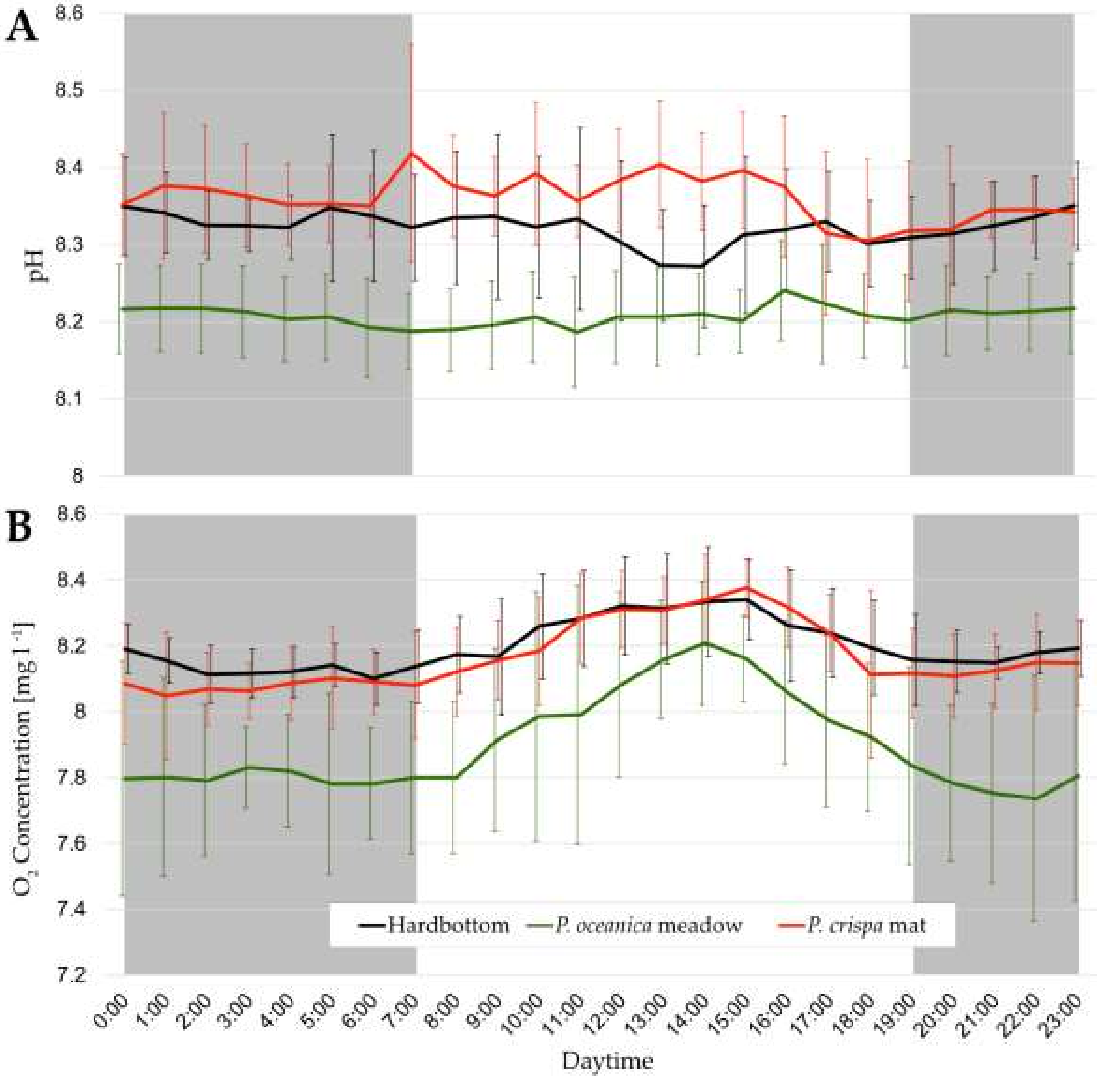

Figure 2: Daily cycles of water parameters measured on Site PC2 in Phyllophora crispa mat (red), Posidonia oceanica meadow (green), and bare rock bottom as reference (black). A) pH and B) oxygen concentration (mg l^{-1}), measured at the bottom of the respective habitat. Error bars indicate standard deviation, grey areas indicate dark phases.

Diversity of foraminiferan community

We examined a total surface of 0.215 m² of *P. crispa* material (average 0.054 m² per site). The examined surface of *P. oceanica* was 0.086 m² of shoots (average 0.043 m² per site) and 0.054 m² of leaves (average 0.053 m² per site). A total of 3.639 foraminiferal specimens were counted, and 104 taxa were examined, out of which 81 were found on *P. crispa* phylloids (46 exclusively on *P. crispa*). While the abundance of single taxa showed no significant differences among *P. crispa* sites, the communities differed significantly among *P. oceanica* sites and sub-habitats (shoots and leaves, Table 1). We thus consolidated the data of *P. crispa* mats for further assessment of differences in the diversity among the different habitats (Figure 3).

Table 1: Results of permutational ANOVA tests for differences among sites and habitats. Posidonia oceanica sub-habitats resemble leaves and shoots. Significant results (p > 0.05) are indicated in bold.

Foraminiferal Assemblages of *P. crispa* mats

Source	**Df**	**SS**	**R2**	**F**	***p***
site	3	0.3898	0.2161	1.1028	0.313
residual	12	1.4138	0.7839		
total	15	1.8036	1.0000		

Foraminiferal Assemblages of *P. oceanica* meadows

Source	**Df**	**SS**	**R2**	**F**	***p***
sub-habitat	1	4.5640	0.3886	25.7381	**0.001**
site	1	0.6696	0.0570	3.7759	**0.006**
sub-habitat:site	1	0.4833	0.0411	2.7252	**0.024**
residual	34	6.0291	0.5133		
total	37	11.7459	1.0000		

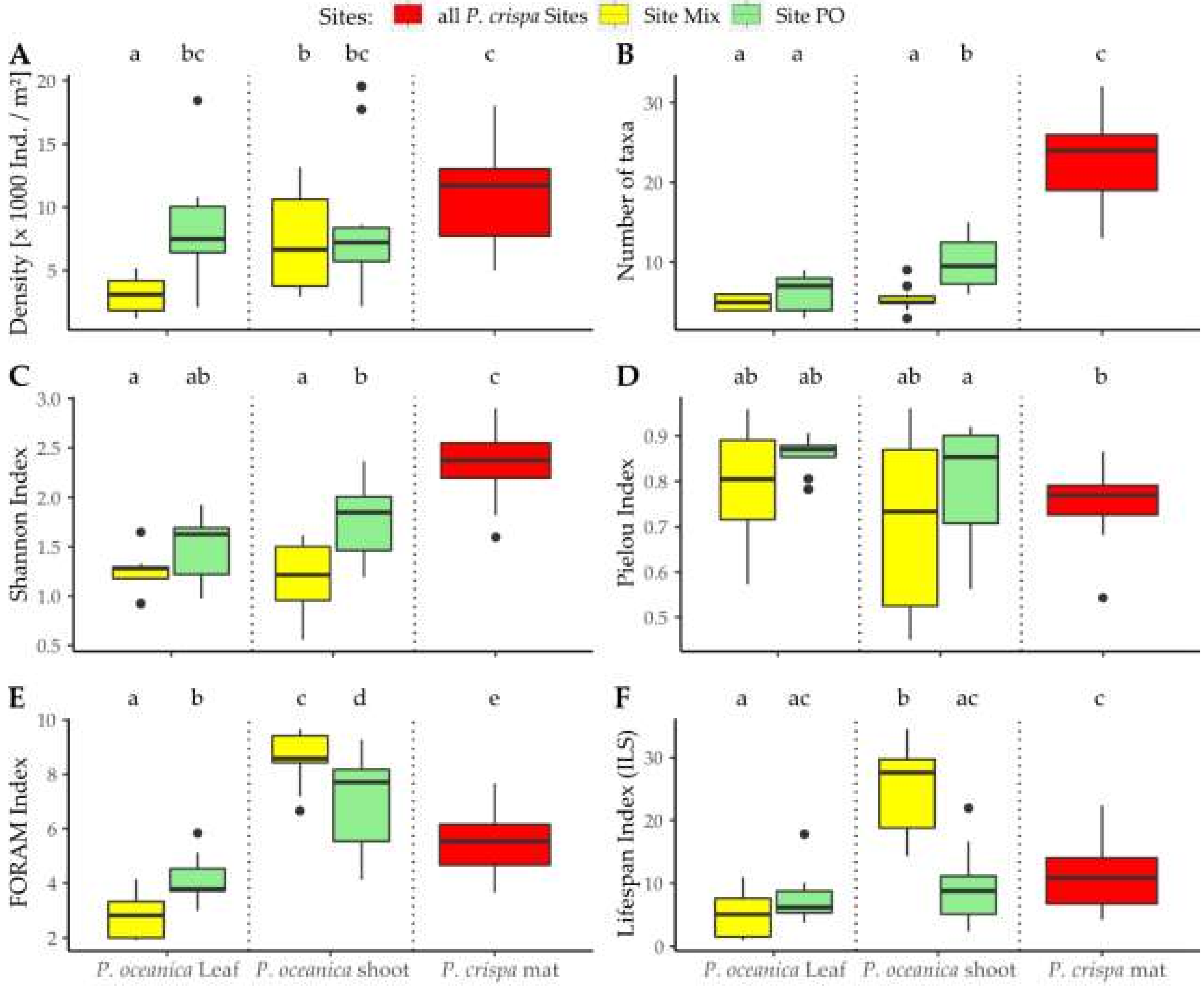

Figure 3: Abundance and diversity of foraminifera in P. crispa mats and P. oceanica meadows. A) mean densities of foraminifera per m² substrate per site, B) Total numbers of identified taxa, C) Shannon diversity indices, D) Pielou evenness indices, E) FORAM indices and F) Lifespan indices reported for the investigated Phyllophora crispa sites in comparison to Posidonia oceanica shoots and leaves. Compact letter displays show significance levelsresulting from Wilcoxon-Mann-Whitney pairwise comparison, boxes with different letters differed significantly ($p < 0.05$).

The most frequently found species across all samples was *Miniacina miniacea* (average 2,081 ± 2,848 individuals m^{-2} surface area), which was most abundant on the shoots (average 4,117 ± 3,602 individuals m^{-2} surface area; Table 2) and *P. crispa* mats (average 1,877 ± 1,366 individuals m^{-2} surface area), but not found on *P. oceanica* leaves. The most frequent species on both leaves (average 2,128 ± 1,783 individuals m^{-2} surface area) and *P. crispa* (average 3,147 ± 1,590 individuals m^{-2} surface area) was *Lobatula lobatula*. Both species develop calcareous perforate tests and resemble some of the larger species of Foriminiferans in this study. While *M. miniacea* and *L. lobatula* show the highest values across all *P. crispa* sites, site PC2 also showed a high amount of *Sejunctella* sp. (average 1,100 ± 303 individuals m^{-2} surface area). Total LBF (*Peneroplis* spp., *Sorites* sp., and *Vertebralina* sp.; Table 2) counts and diversity were highest in *P. crispa* samples (3 species, average 183 ± 239 individuals m^{-2} surface area) compared to *P. oceanica* shoots (2 species, average 119 ± 143 individuals m^{-2} surface area) and *P. oceanica* leaves (1 species, average 37 ± 123 individuals m^{-2} surface area). One LBF species (*Peneroplis planatus*) was only found on *P. crispa*.

Table 2: The five most abundant species per habitat (top) and average abundance of LBF species (bottom). Numbers are average abundance per m² substrate (AVG) ± standard deviation (STDEV); the most abundant species per habitat are indicated in bold. [1] according to ***Mateu-Vicens et al., 2014*** *and* ***Langer, 1993*** *[35,37]. No LBF were among the most abundant species.*

	Family	**Species**	**Ecotype[1]**	***P. crispa* mats**		***P. oceanica* leaves**		***P. oceanica* shoots**	
				AVG	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**
Most abundant	Cibicididae	*Lobatula lobatula*	B	**3147**	1590	**2127**	1783	129	218
	Homotrematidae	*Miniacina miniacea*	A*	**1877**	1366	0	0	**4117**	3602
	Hauerinidae	Unknown	A*	**1245**	855	716	1330	0	0
	Discorbinellidae	*Discorbinella bertheloti*	B	472	338	**931**	1076	249	362
	Spirillinidae	*Sejunctella* sp.	A*	465	505	15	62	**912**	899
	Planorbulinidae	*Planorbulina mediterranensis*	A*	365	266	353	484	132	252
	Hauerinidae	*Miliolinella subrotunda*	D*	169	285	25	67	**360**	506
	Hauerinidae	*Quinqueloculina seminula*	D*	163	201	0	0	281	388
	Ammoniidae	*Ammonia beccari*	B	86	275	**723**	605	119	248
	Trochamminidae	*Lepidodeuterammina ochracea*	A*	71	161	28	82	357	580
LBF	Peneroplidae	*Peneroplis pertusus*	SB	**153**	239	0	0	**66**	143
	Peneroplidae	*Peneroplis planatus*	SB	17	37	0	0	0	0
	Soritidae	*Sorites orbiculus*	SB	13	41	**37**	123	0	0
	Fischerinidae	*Vertebralina striata*	SB	0	0	0	0	53	122

The density of foraminifera was lowest at *P. oceanica* leaves on site mix (average 3,183 ± 671 individuals m^{-2} surface area), with a significant difference between the two leaf sites (site mix and site PO). The highest density was found on *P. crispa* site PC1 (average 12,647 ± 1017 individuals m^{-2} surface area). Comparing the density among habitats revealed significantly higher values for *P. crispa* compared to *P. oceanica* shoots and leaves on site Mix ($p = 0.0198$ and $p < 0.0001$, respectively; Figure 3A).

The number of taxa was lowest on the *P. oceanica* leaves at site mix (average 5.0 ± 0.3) and highest on *P. crispa* phylloids at site PC1 (average 28.0 ± 0.7). Significant differences were found among *P. oceanica* shoot sites and all habitats (Figure 3B). The number of taxa found on *P. crispa* was significantly higher compared to *P. oceanica* leaves and shoots on both *P. oceanica* sites (all values for $p < 0.0213$).

The lowest Shannon diversity index was found on *P. oceanica* shoots (average 1.2 ± 0.1) and highest on *P. crispa* phylloids (average 2.5 ± 0.2), both at site mix. Both *P. oceanica* sub-habitats differed significantly, with higher values for site PO (Figure 3C). Among all habitats, *P. crispa* showed significantly more diversity than *P. oceanica* leaves and shoots (all values for $p < 0.0039$).

The evenness was similar across all comparisons. The lowest average value was found on *P. oceanica* shoots at site mix (average 0.7 ± 0.06), and the highest on *P. oceanica* leaves at site PO (average 0.9 ± 0.01). The pairwise comparison between *P. crispa* and *P. oceanica* shoots on site PO showed the only significant effect ($p = 0.0002$; Figure 3D).

The FORAM index was highest for the *P. oceanica* shoots, with a significantly higher value for site mix (8.6 ± 1.0) and lowest for the leaves, with a significantly lower value for site mix (2.8 ± 0.8). The values for all *P. crispa* samples ranged between the two *P. oceanica* sub-habitats (Figure 3E).

The lifespan index (ILS) was highest in the *P. oceanica* shoot samples from site mix (25.4 ± 7.0) and lowest in the leaf samples from the same site (7.9 ± 4.0). The *P. crispa* community showed similar values compared to *P. oceanica* site PO, while it ranged between both sub-habitats on site mix (Figure 3F).

The relative abundances of foraminiferal taxa sorted by test material show differences among the two *P. oceanica* sites in the higher abundance of the porcellaneous Hauerinidae (mainly *Milionella* spp. and *Quinqueloculina* spp.) at site PO. In contrast, the composition across *P. crispa* sites is more homogenous (Figure 4). The phylloids of *P. crispa* host more porcelaneous foraminifera than both *P. oceanica* sub-habitats on the same site (site mix), mainly driven by an unidentified Hauerinidae species (Table 2). On the other hand, while agglutinated species were scarce in the whole study, they were relatively more abundant on *P. oceanica* sub-habitats (mainly *Trochammina inflata* and *Lepidodeuterammina ochracea*, Figure 4). The leaves of *P. oceanica* on the site mix did not host any porcelaneous foraminifera. The two *P. oceanica* sub-habitats mainly differ in the higher abundance of *Sejunctella* sp. (Spirillinidae; average 913 ± 196 individuals m^{-2} surface area; Figure 4).

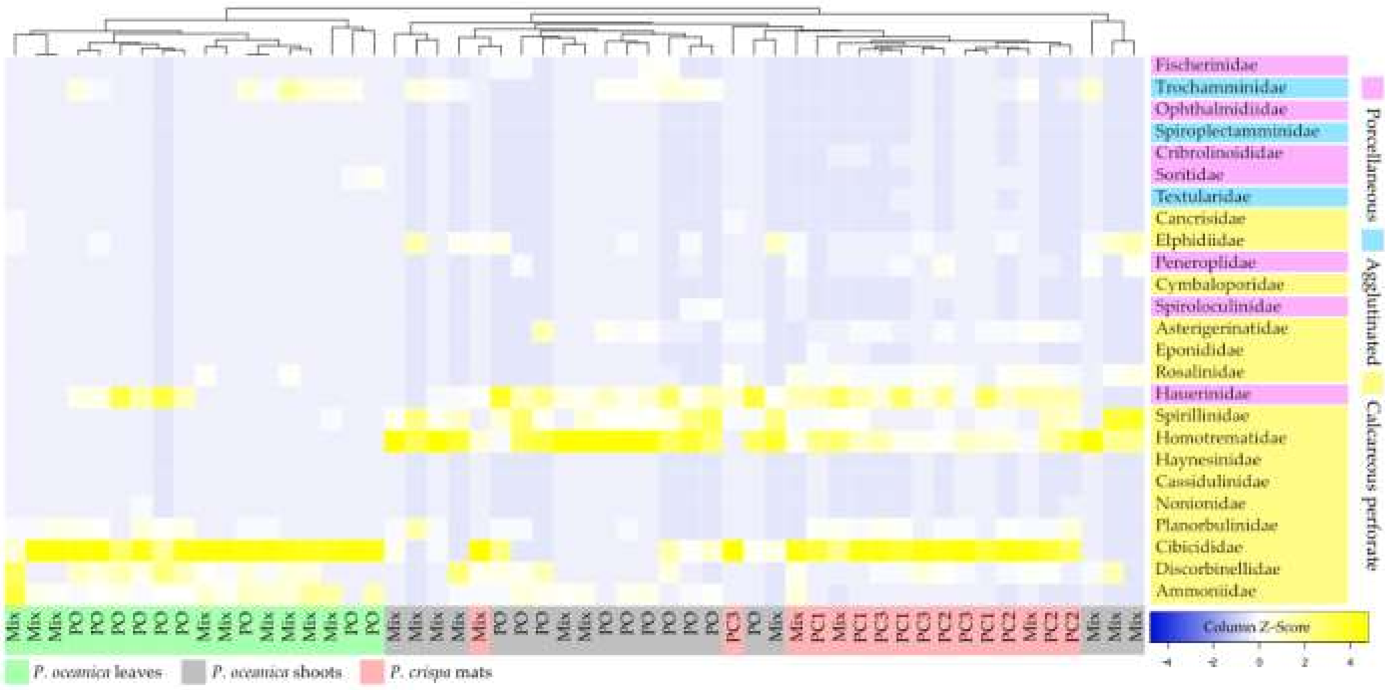

Figure 4: Hierarchical clustering (average linkage, Spearman ranked correlation) of family abundances among sites (individuals per m²) found in Phyllophora crispa (red) and Posidonia oceanica habitat sites (green = leaf; grey = shoot). Test types are indicated as purple (porcellaneaus), blue (agglutinated) and yellow (calcareous perforate). The Z-Score indicates the distance to the mean, which is indicated as "0".

Differences among sites for *P. crispa* were reflected in the lower abundances of Hauerinidae (unknown species) and Homotrematidae (i.e., *M. miniacea*) on site PC3 compared to all other sites (Figure 4). Among *P. oceanica* sites, differences were driven by the high abundance of an unknown Hauerinidae species on site PO (Figure 4).

Discussion

Environmental parameters inside *P. crispa* mats

The pH and oxygen concentrations inside the *P. crispa* mats differed from the environment inside *P. oceanica* meadows, mainly regarding the magnitude of their daily cycles (Figure 2). While the oxygen concentrations inside the *P. crispa* mat were similar to the bare rock reference habitat, the pH values showed more substantial fluctuations than the *P. oceanica* meadow. The lower oxygen concentrations inside the *P. oceanica* meadow were likely due to the measurements close to the rhizome layer, where more respiration occured[61]. This effect also explains the lower pH values measured inside the *P. oceanica* meadow. A reason could be the spatial proximity of the photosynthetically active thalli of the red algae with the epiphytic respirating community, in contrast to the spatially more detached situation in the *P. oceanica* meadow. The resulting small-scale gradients of environmental parameters inside *P. crispa* mats were also recently described for water movement, temperature, and light conditions[10].

Abundance and diversity of epiphytic foraminifera in *P. crispa* mats

Overall, the foraminiferal assemblages represent a typical composition for carbonated shelf environments[61]. Our results showed that the abundance of foraminifera was higher in *P. crispa* mats than in both *P. oceanica* sub-habitats. A similar, but more pronounced trend was confirmed for the species richness (Figure 3). This observation is strengthened by the fact that previous studies found lower values for foraminiferal diversity on seagrass[62]. Additionally, the analysis of species incidences showed that our sampling efforts might even underestimate the total diversity of foraminiferal communities in *P. crispa* mats (see Appendix, Figure A2).

Interestingly, the leaf sites differed in density, while the shoot sites differed in species richness. This observation supports that *P. oceanica* sub-habitats provide different ecological traits (e.g., water movement, particle supply, light availability), especially for foraminiferal species. The high individual count on site PO leaves could have resulted from a recent reproduction event in that area, which usually occurs during summer[62]. The relative abundance of foraminiferal orders shows that the lack of porcelaneous taxa and differences in the composition of calcareous perforate taxa were the main drivers for the observed differences. Additionally, the relatively short-lived *P. oceanica* leaves favor small species (ecotype B, mainly *L. lobatula*) with high turnover rates compared to the long-lived shoots and *P. crispa* mats, where LFB (e. g. *Peneroplis* spp.) occur in persistent habitats[30]. This observation is confirmed by the higher lifespan index values (ILS) for *P. oceanica* shoots and *P. crispa* mats. Shannon indices support the at least comparably high foraminiferal diversity in *P. crispa* mats and higher consistency among sites in this habitat. While no statistical differences in the evenness were confirmed, the data scattering in *P. oceanica* samples shows high dispersion across replicates (Figure 3), pointing towards small-scale variations in the resilience of foraminiferal communities across *P. oceanica* meadows. Although there is no significant effect, we observe a trend to lower values on the southernmost site (site PC3, Figure 3) while in contrast to *P. oceanica*, the sites within the north-western bay (bay of Campese) overall values are more similar. Our findings are consistent with previous studies on epiphytic foraminifera, where 55 living taxa were found on *Cystoseira* sp. (Phaeophyta) mats[25] and 62 taxa in shallow algae assemblages[24]. Adding to the results of Langer (1988)[38], who found lower diversities of foraminifera in green algae (*Udotea peticolata*) and brown algae (*Ectocarpus* sp.) compared to seagrass (*P. oceanica*), we confirm here, that red algae mats of *P. crispa* have the potential to host even higher diversities than *P. oceanica*.

Composition of epiphytic foraminifera morphotypes in *P. crispa* mats

The test type comparison shows a similar trend to a more homogenous community across sites in *P. crispa* mats, while foraminiferal groups of different test types are more abundant on few *P. oceanica* samples (e.g., Trochaminidae, Hauerinidae, Discorbinellidae; Figure 3). The higher abundance of porcelaneous LBF taxa reflects the long-term stability of *P. crispa* mats (similar to *P. oceanica* shoots), accompanied with higher light regimes (similar to *P. oceanica* leaves) as species of porcelaneous LBF are often multichambered species, bearing photosymbionts[61,63]. Because of their calcified test, porcelaneous species are more sensitive to ocean acidification processes [64]. This is further supported by the low number of agglutinated, typically opportunistic species, favoring more unstable, physically controlled environments[65]. High abundances of agglutinated species also indicate carbonate undersaturation[66]. The low values (average 38 ± 62 individuals m^{-2} surface area) in this study suggest a relatively high nutrient availability and water mixture inside the *P. crispa* habitat. These traits are generally provided by *P. crispa* mats, where we found the highest numbers of LBF. However, these high numbers were mainly driven by *Peneroplis pertusus*, which was also abundant on *P. oceanica* shoots. The lack of porcelaneous foraminifera on both *P. oceanica* sub-habitats on site mix compared to site PO (Appendix, Table A1) indicates local factors (i.e., disturbances or pollution). Another explanation could be a lack of larval supply, however the high amount of porcelaneous species in *P. crispa* mats on the same site (Appendix, Table A2) implicates local factors inside the *P. oceanica* meadow as the main drivers. This observation underlines the function of *P. crispa* mats as refuge habitat and potential mitigating mechanisms for environmental disturbances accompanied with the mats. A recent study suggests that diel pH fluctuations in *P. oceanica* meadows foster the resistance of epiphytic forams (*Rosalina* sp.) towards ocean acidification[67]. Our study found higher diel pH fluctuations within *P. crispa* mats (0.11 units) than in *P. oceanica* meadows (0.05 units, Figure 2). This observation conforms with a higher abundance and diversity of *Rosalina* sp. in *P. crispa* habitats (6 species, average 27 ± 53 individuals m^{-2} surface area) compared to *P. oceanica* habitats (1 species, average 3 ± 22 individuals m^{-2} surface area leaves and average 0 ± 4 individuals m^{-2} surface area shoots). This observation supports the hypothesis that *P. crispa* mats could also foster the resistance of epiphytic forams against acidification scenarios as observed in *P. oceanica*[67]. These higher pH regimes could result from an accumulation of organic particles and thus higher net respiration in combination with elevated water holding capacities of *P. crispa* mats[10] and lower rates of photosynthesis, as shown by lower oxygen concentrations inside the algae mats (Figure 2B).

While *P. crispa* was dominated by a combination of type B (*L. lobatula*) and type A* (*M. miniacea*, and unknown Hauerinidae), the leaves and shoots of *P. oceanica* were dominated by one very abundant type (B – *L. lobatula* and A* - *M. miniacea*, respectively, Table 2). This difference indicates that *P. crispa* mats offer various traits for long-lived, encrusting species (A*) and short-lived and temporary motile species (B), but on the other hand, offer suitable conditions for species from both *P. oceanica* sub-habitats. The high abundance and diversity of porcellaneous LBF species confirm the function of *P. crispa* mats as persistent habitats.

Notably, LBF species were overall scarce in this study and absent in some samples across habitats, resulting in high standard deviations (Table 2, Appendix Table A1 and A2). For example, *S. orbiculus* was only found in two leaf samples, with relatively high abundances. A reason for this aggregation of LBF species in some samples could be local asexual reproduction, previously described for LBF species[68]. However, while LBFs were present in 11 *P. crispa* samples across all sites, they were found in 2 samples of *P. oceanica* leaves from site PO, and 8 samples of *P. oceanica* shoots from both sites.

Conclusions

We conclude that the community of epiphytic foraminifera >300µm is of higher diversity in *P. crispa* mats (Appendix, Figure A2) and is more homogeneously distributed across sites and replicates than *P. oceanica* sub-habitats. The fact that *P. crispa* mats are a suitable habitat for many foraminiferal species of both *P. oceanica* sub-habitats leads to the hypothesis that *P. crispa* mats may act as refuge habitats harboring biodiversity in times of loss and degradation of neighboring diversity hotspots. It underlines the relevance of this habitat in the Mediterranean Sea and justifies further assessments of *P. crispa* mat distribution along the Mediterranean coastline to confirm our results on a regional scale. We suggest further investigating the supraregional distribution and diversity of this vital rhodophyte habitat across the Mediterranean coastline to prove further its role as a vital habitat of high biodiversity. The recently adopted EU Biodiversity strategy for 2030 aims to protect and restore habitats of significant biodiversity to mitigate effects of e.g., regional human impact and climate change in terms of biodiversity loss. Our results in line with previous studies on *P. crispa* mats[17–22], as well as the successful implementation of a marine protected area covering "Zernov´s *Phyllophora* field" in the Black Sea[23] suggest that this habitat hosts considerably high diversity of invertebrate taxa and should thus be considered in future conservation efforts.

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Chapter 5 – New Insights into a Mediterranean Sea Benthic Habitat: High Diversity of Epiphytic Bryozoan Assemblages on *Phyllophora crispa* (Rhodophyta) Mats

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Chapter 5 – New Insights into a Mediterranean Sea Benthic Habitat: High Diversity of Epiphytic Bryozoan Assemblages on Phyllophora crispa (Rhodophyta) Mats

Abstract

With its geographically isolated location and geological history, the Mediterranean Sea harbors well-known biodiversity hotspots, such as *Posidonia oceanica* seagrass meadows. Recently, long-living mats formed by the fleshy red alga *Phyllophora crispa* have been described to be associated with a high diversity of sessile invertebrates in the Tyrrhenian Sea. One of the key taxa among these sessile invertebrates are bryozoans: their abundance, diversity, and spatial distribution in *P. crispa* mats represent a gap in scientific knowledge. Thus, we conducted a pilot study on bryozoan assemblages associated with *P. crispa* mats around Giglio Island (Tuscan Archipelago, Italy) in 2018, followed by a comparative study on four sites distributed around the island in the subsequent year 2019. We compared these findings to bryozoan abundance and diversity on *P. oceanica* shoots and leaves during the second expedition. Findings revealed more than 46 families, with a significantly higher number of taxa identified in *P. crispa* mats (33) than in *P. oceanica* meadows (29). Shannon diversity index was similar between *P. crispa* and *P. oceanica* shoots, while Pielou's evenness index was lower in *P. crispa* mats. The most abundant families reported across all habitats were Crisiidae, Aetidae, and Lichenoporidae; but the most abundant family on *P. crispa* was Chlidoniidae (*Chlidonia pyriformis*). The assemblages associated with *P. crispa* differed among sites, with higher abundances but lower diversity on the exposed southernmost site. The total bryozoan abundance was significantly higher on *P. crispa* (average $2.83 \times 10^6 \pm 1.99 \times 10^6$ colonies per m^2 seafloor) compared to *P. oceanica* meadows (average $0.54 \times 10^6 \pm 0.34 \times 10^6$ colonies per m^2 seafloor). Our results show a high diversity of bryozoans on *P. crispa* thalli compared to *P. oceanica* meadows, consistent throughout the study. These findings confirm the value of the red alga-generated habitat for associated bryozoans and may have implications for future biodiversity assessments and conservation measures.

Keywords: phytal habitat, epiphytic communities, engineering species, sessile invertebrates

Introduction

Understanding the patterns of variability of benthic assemblages represents one of the main goals for ecologists and has a pivotal role in managing and conserving marine habitats[1]. This information might help scientists predict or understand organisms' responses to global environmental changes. Hotspots of biodiversity in the Mediterranean Sea are often created by key engineering species that provide structurally complex habitats for associated communities[2]. Some of the most-studied engineered habitats in the Mediterranean Sea are seagrass meadows of the endemic angiosperm *Posidonia oceanica* (L.) Delile, 1813 (Figure 1 C) and coralligenous reefs, mainly formed by the accumulation of crustose Rhodophytes thalli[3–6]. The rooted, flowering plants of *P. oceanica* build up dense meadows consisting of two sub habitats: a canopy of up to 50cm long leaves that is growing from a dense network of stems and roots[7]. The crustose structures of coralligenous reefs provide a rigid substrate, characterized by systems of canals and crevices[5]. Both of these ecosystems promote high biodiversities of sessile invertebrates, as they provide different environmental gradients (e.g., light intensity, water movement, food availability), as well as shelter and space for larval settlement[8–10] (Figure 1 D & E).

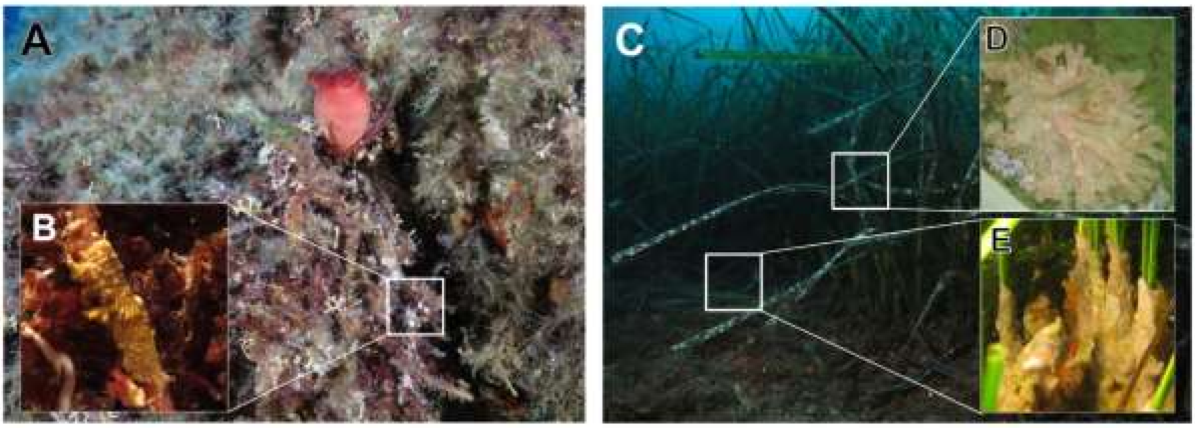

Figure 1: Edge of a Phyllophora crispa mat (A) with details on epiphytic fauna (B), including bryozoans, serpulids, and foraminiferans. Overview of Posidonia oceanica meadow (C), with details on the leaves (D) and shoots (E). Pictures: E.C. (A); F.R. (B, C, E); N.S. (D))

Bryozoans are filter feeders and form one of the most abundant and diverse groups of epiphytic invertebrates on hosting organisms like seagrass (i.e., *P. oceanica*) and macroalgae[10–15]. In particular, the richest bryozoan diversity in the Mediterranean Sea has been reported on coralligenous reefs and marine caves due to the availability of several microhabitats that enhance the presence of bryozoans characterized by different shapes and ecological traits[16–18]. Their typical colonial structures consist of often highly specialized zooids and may vary between thin crusts, erect and branched forms, or larger rigid structures[19,20]. Many species are considered bioindicators for environmental changes, as they often respond faster to environmental and human-mediated pressures[21–23]. Because of their calcium carbonate hulls, they are sensitive to ocean acidification[24–26] and hold an essential part in fossil records[27]. Some bryozoan species have been reported as habitat-forming organisms playing a pivotal role in promoting biodiversity[24], e.g., by overgrowing *P. oceanica* shoots and preventing the settlement of other species[28] (Figure 1 D). Furthermore, bryozoans play an essential role as primary consumers by transferring particulate organic matter from the water column into the benthic community[29,30].

The red macroalgae *Phyllophora crispa* ((Hudson) P.S.Dixon, 1964) is known for forming dense mats and hosting a diverse community of epiphytic invertebrates in the Black Sea[31]. In the north-western Mediterranean Sea, *P. crispa* forms dense mats[32–34] (Figure 1 A), which have recently been shown to host high diversities of invertebrate fauna. Especially epiphytic filter feeders (e.g., Bryozoa, Serpulidae), which benefit from the algal thalli as substrate and accumulated food particles from the water column, have been identified to contribute to the associated biodiversity[34–36] (Figure 1 B). However, little is known about the variations of bryozoan diversity inside *P. crispa* mats over space and time or their diversity compared to other Mediterranean habitats (e.g., *P. oceanica* meadows).

To address this knowledge gap, we carried out a comparative field study on the abundance and diversity of bryozoans at four locations of *P. crispa* mats around Giglio Island (Tuscan Archipelago, Italy, Tyrrhenian Sea) in two consecutive seasons (2018 and 2019) and on a *P. oceanica* meadow as a reference habitat (2019). We decided on *P. oceanica* as a reference habitat because its biodiversity has been well studied during the last decades, and the structure is more similar to the fleshy *P. crispa* thalli than, e.g., the calcareous substrate of coralligenous reefs. Additionally, extensive coralligenous reefs are generally found deeper, and other mat-forming macroalgae are not present at the same depth as *P. crispa* mats within the study area. With this work, we aim to answer the following research questions:

1. What are the abundances and diversity of bryozoans inside *P. crispa* mats compared to *P. oceanica* meadows?

2. Which are the most abundant families in the investigated habitats, and which families are unique to *P. crispa* mats?

3. What is the spatial variability of the bryozoan assemblages inside *P. crispa* mats?

Material and methods

Location and sampling procedure

The study area is located at the island of Giglio (42°21’19.4” N 10°54’06.1” E, Figure 2) and is characterized by steep granite slopes, alternating with sandy bottoms. The infralittoral seabeds are colonized by *Posidonia oceanica* meadows, *Phyllophora crispa* mats, and coralligenous reefs[35]. Scientific SCUBA divers collected all samples at water depths of 30 ± 4 m at four sites around the island. Sampling took place between May and June 2018 (21 *P. crispa* samples on Site Mix) and between May and July 2019 (4 *P. crispa* samples per site, resulting in 16 total samples; 9 *P. oceanica* leaves, and 10 *P. oceanica* shoots on Site Mix). The sampling sites were chosen for their similar topography and occurrences of *P. crispa* mats of at least 90% coverage (by visual census; Appendix Figure A1) at the target depth of 30 m. In 2018, we sampled *P. crispa* material from Site Mix. In 2019 all four sites (site PC1, PC2, PC3, and Site Mix) were sampled for *P. crispa* mats, and at Site Mix was additionally sampled for *P. oceanica* material. Site Mix was the only site with a continuous *P. oceanica* meadow present at the target depth.

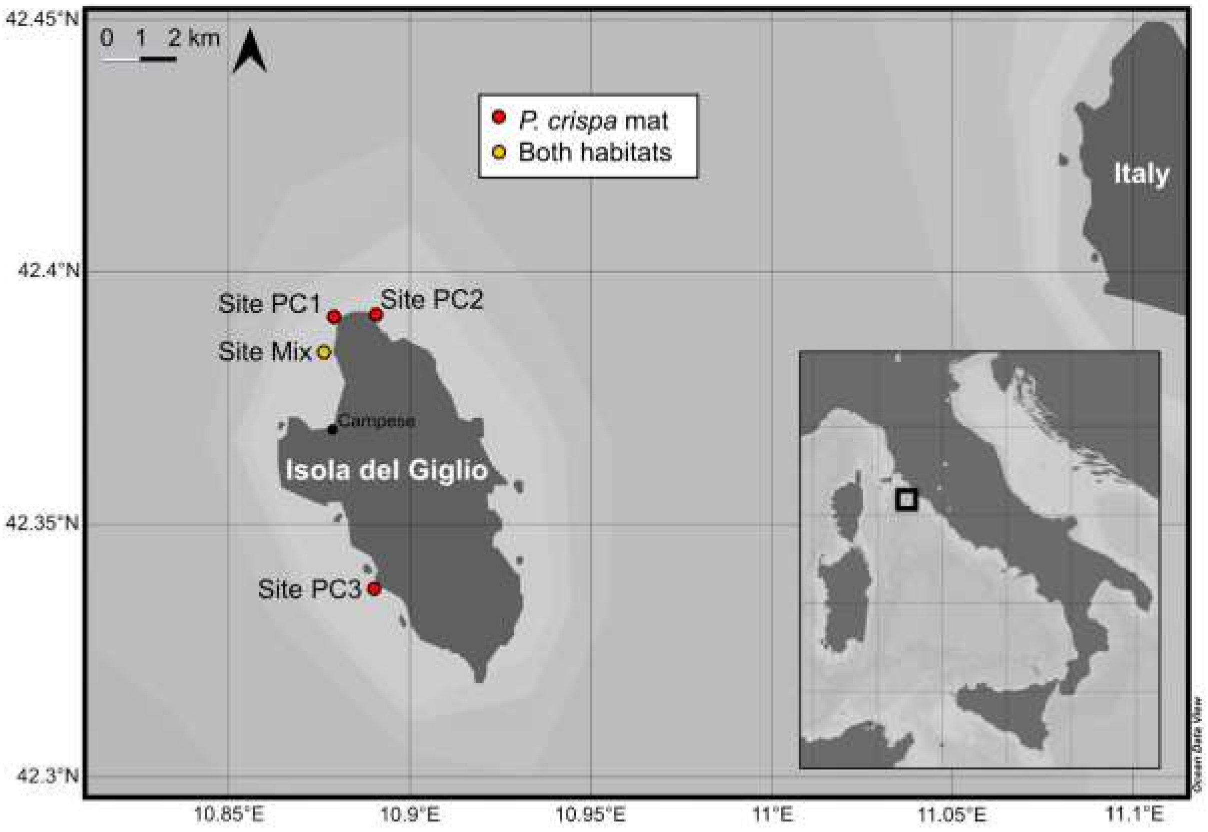

Figure 2: Locations of the sampling sites in the study area (Isola del Giglio, Italy). Red dots mark sampling sites of Phyllophora crispa in 2019, yellow marks the site of comparative sampling in both years (2018 and 2019), and Posidonia oceanica reference habitat (2019).

The *P. crispa* mats were sampled using a metal quadrat (size 30 × 30 cm), placed randomly inside a continuous mat of at least 5 cm thickness to define the exact sampling area. All material of these main samples was then carefully scraped off the rock surface with a spatula directly under the holdfast to avoid breaking or removing epiphytic organisms.

The *P. oceanica* leaves and shoots were cut with scissors, directly on the sheath or rhizome branching point, on meadows close to the sampling depth of *P. crispa* mats (30 ± 4 m). Additionally, we counted the number of *P. oceanica* shoots per m^2 ($n = 16$ counts) and the number of leaves per shoot ($n = 32$ counts) for later extrapolating bryozoan colonies per m^2 seafloor. These density measures were carried out by counting the number of shoots within a 40 cm × 40 cm frame. Shoots and leaves were treated as separate sub-habitats considering their different ecological traits, particularly regarding their longevity as a fundamental trait for larval settlement[37,38].

All main samples were carefully transferred into plastic jars immediately after sampling. Every jar contained approximately one-third of sampled material and two-thirds of seawater to avoid oxygen depletion during the transport to the holding facilities in the Institute for Marine Biology (IfMB, located in the near bay of Campese). The main samples were then kept in aerated seawater tanks at constant temperature (18° C; equivalent to *in situ* temperature) before they were analyzed within three days after sampling.

Species identification and abundance assessment

From the *P. crispa* main samples, subsamples between 20 and 100 g wet weight were extracted, roughly 20% of the respective main sample. The span of different wet weights resulted from the different amounts of main samples due to the randomly picked sampling area. The remaining material of the main samples was analyzed for other taxonomic groups that are not within the frame of this study. The shoots and leaves of *P. oceanica* were analyzed as a whole under stereo magnifiers (maximum 40× magnification). Bryozoan colonies were identified to the lowest possible taxonomic level using the relevant literature (Appendix, Table 2). Abundance was assessed by counting the number of colonies. In the case of branching, stolonal taxa – e.g., *Chlidonia pyriformis* (Bertoloni, 1810) – all parts connected by stolons were considered one colony. We opted to work with the family level for further analysis to reduce observer bias and increase work efficiency. Previous studies have shown that this method may not result in a significant loss of information[39]. Furthermore, the taxonomic sufficiency hypothesis applied to Mediterranean peculiar habitats revealed that surrogate taxonomic levels higher than species could be used to highlight the diversity pattern of benthic assemblages[40].

The surface area of all subsamples was assessed as follows. For *P. oceanica* shoots, the length and diameter were measured, and surface area calculations were based on an assumed cylindrical shape.

For *P. oceanica* leaves, the length and width were measured to calculate the rectangular surface (times two, to account for both sides of the leaf). The *P. crispa* subsamples were flattened with a glass pane on laminated graph paper before being photographed from above with a fixed tripod. The surface area was then determined using ImageJ (version 1.52o, https://imagej.nih.gov/ij/, accessed on the 23rd of April 2019) and multiplied by two to account for both sides of the thalli. The wet weight of all *P. crispa* main samples and subsamples was assessed after shaking off excess water to extrapolate the abundances from the subsamples to the main sample and finally to the surface of the seafloor. Bryozoan abundance was then calculated as the number of colonies per m² of seafloor ± standard deviation (SD) (Appendix, Formulas A1 – A3).

Diversity descriptors and statistical analysis

Bryozoan diversity was assessed using four descriptors: total numbers of families per site and habitat, Shannon diversity index[41], and Pielou's evenness index[42]. The descriptors were calculated as means per site and habitat and reported with the respective SD.

Differences in the composition of bryozoan assemblages in *P. crispa* mats among sites were tested using multivariate permutational analysis of variance (PERMANOVA[43]). The source of significant results ($p < 0.05$) was tested using Tukey's honestly significant difference (HSD) test. Based on the results of the multivariate analysis, the data of the northern sites were pooled for further comparison among habitats (Appendix, Table A1).

Statistical differences in the diversity descriptors among sites and (sub-) habitats were assessed with pairwise Wilcoxon-Mann-Whitney tests. The comparison among habitats on the northern sites was visualized using non-metric multidimensional scaling (NMDS). In addition, the bryozoan assemblages were clustered with a Spearman ranked correlation (average linkage) on a family level to visualize the composition of the bryozoan assemblages using the software' heatmapper'[42]. Analyses and plots were made with R (version 4.0.5)[44].

To avoid potential variability between observer and different sampling efforts in the two subsequent seasons, we decided against a direct statistical comparison on a temporal scale.

Results

Species richness and abundance

A total of 17,822 bryozoan colonies were found (2018 = 10,312; 2019 = 7,510) and 46 taxa identified to at least family level. The highest number of families was identified in *Phyllophora crispa* mats 2019 (33), while *Posidonia oceanica* meadows held 29 families (Figure 3). A similar amount was found in *P. crispa* mats during the pilot study in 2018 (28). In the comparative study of 2019, the two habitats shared 21 families, while this number was higher for *P. crispa* and *P. oceanica* shoots (20) than for *P. crispa* and *P. oceanica* leaves (13). A significantly higher number of families ($p < 0.05$) was reported in *P. crispa* compared to *P. oceanica* sub-habitats (Figure 4 F).

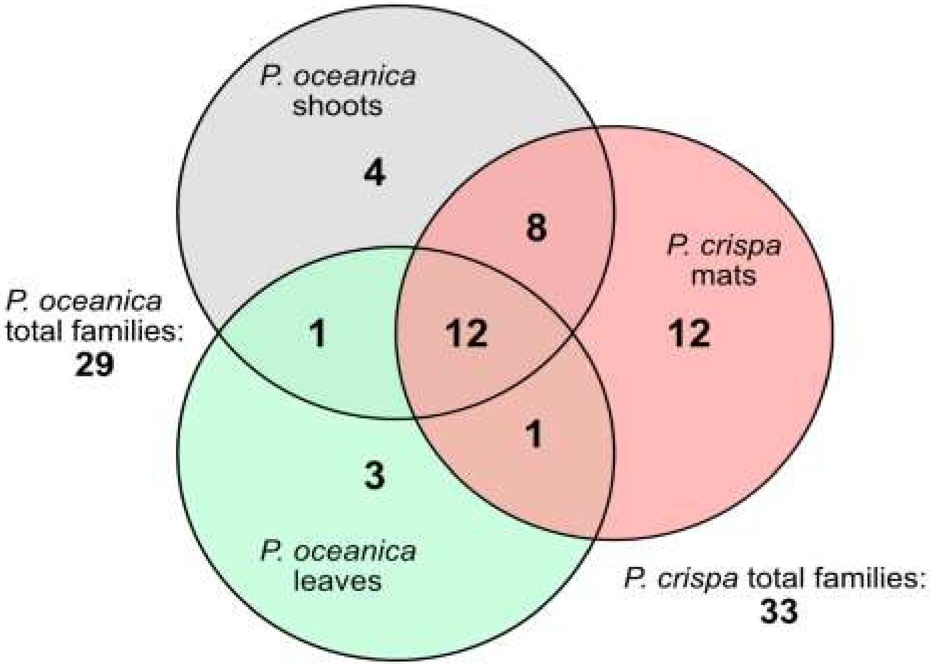

Figure 3: Number of families found per habitat during the second sampling season (2019), showing shared families between the respective habitats.

The most abundant families across all habitats were Crisiidae, Aetidae, and Lichenoporidae (Table 1). The highest density of bryozoan colonies was found in *P. crispa* mats in 2018 (average 2,827,762 ± 1,984,965 colonies per m² seafloor). Among *P. crispa* sites, the density was highest on the southernmost site (site PC3) and lowest on the northernmost site (site PC2, Figure 4 A). This observation was the only significant effect among sites in the diversity descriptors of *P. crispa* mats and confirmed the results of the multivariate analysis (Appendix, Table A1). Based on these results, the data of the northern *P. crispa* sites were pooled for further comparison among (sub-) habitats. The lowest abundance was recorded on *P. oceanica* leaves (average 177,912 ± 104,999 colonies per m² seafloor, Figure 4 E). The most abundant family contributing to the measured densities was

Chlidoniidae (*Chlidonia pyriformis*) in *P. crispa* mats, and *P. oceanica* shoots, with a higher abundance on *P. crispa*. Candidae were abundant on *P. crispa* mats and *P. oceanica* shoots as well. On the leaves of *P. oceanica*, Haplopomidae and Tubuliporidae were most abundant (Table 1). It is also notable that *P. crispa* mats host additional growth types such as petraliform (e.g., *Beania hirtissima* (Heller, 1867)), encrusting (e.g., *Watersipora* sp.), and creeping (e.g., *Aeta* sp.), while *P. oceanica* leaves mainly support encrusting forms (e.g., *Haplopoma* sp.).

Table 1: Most abundant families of every (sub-) habitat and both years of Phyllophora crispa sampling (top 5 indicated in bold for each habitat), and Posidonia oceanica sampling in 2019. Numbers show the mean number of colonies per m² seafloor ± standard deviation (SD).

	***P. crispa* 2018**		***P. crispa* 2019**		***P. oceanica* leaves 2019**		***P. oceanica* shoots 2019**	
Family	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Aetidae	15,976	5,887	**112,140**	13,312	**52,535**	12,360	**45,064**	5,863
Candidae	**268,368**	93,217	5,183	2,343	0	0	**37,916**	5,463
Chlidoniidae	**1,287,926**	276,738	**1,250,665**	445,419	0	0	**131,954**	46,711
Crisiidae	**192,089**	22,353	**347,948**	54,644	**48,945**	14,288	**64,743**	9,896
Haplopomidae	4,674	1,652	56,640	20,818	**155,149**	36,106	0	0
Tubuliporidae	**240,495**	33,541	**200,127**	31,551	**63,966**	19,210	**33,656**	7,157
Watersiporidae	**212,608**	37,342	24,788	5,333	0	0	0	0
Unknown	113,593	50,098	**130,742**	22,592	**183,536**	36,544	3,628	1,166

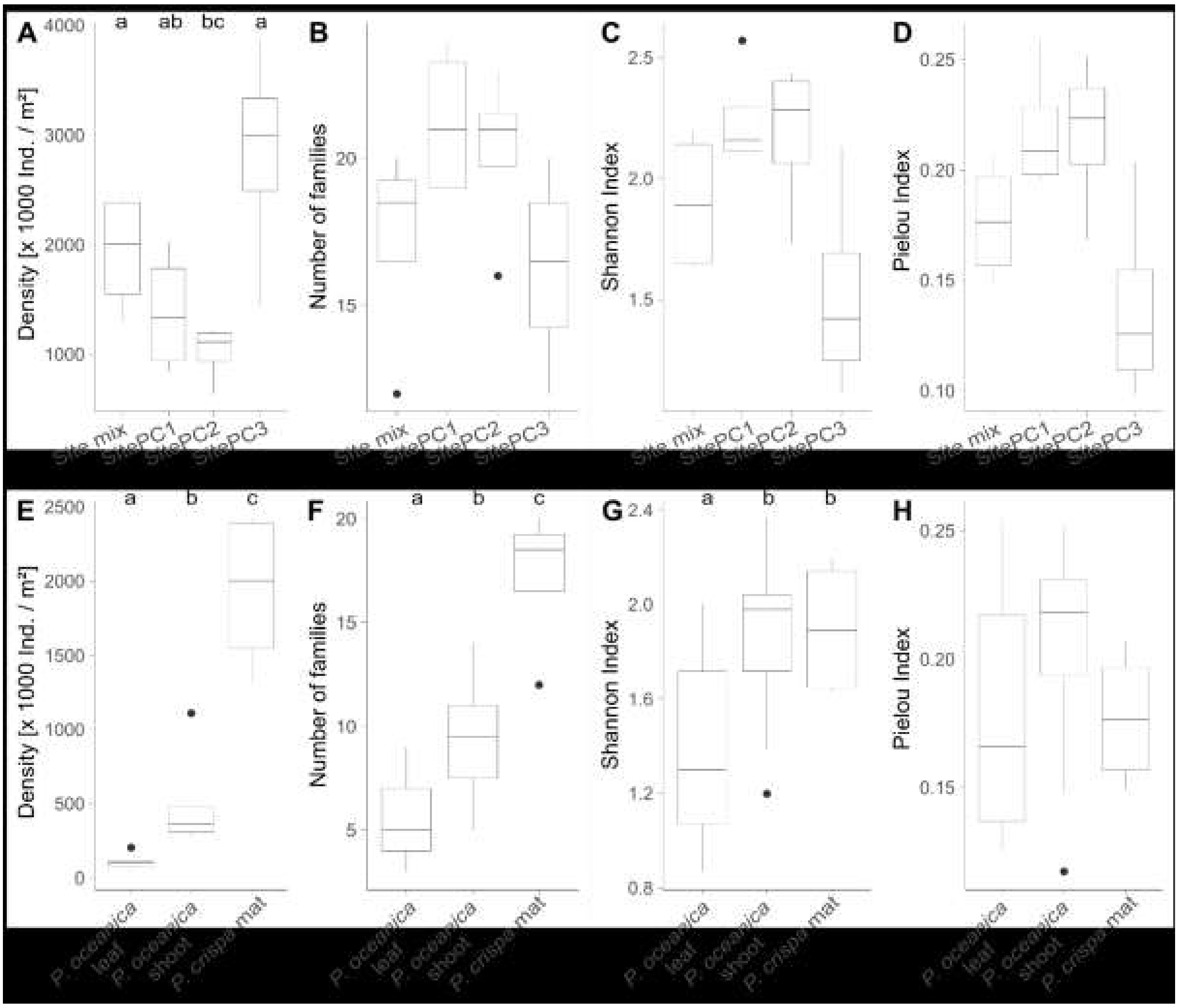

*Figure 4. Comparison of abundance and diversity descriptors among sites and habitats. First row (**A–D**): all four investigated Phyllophora crispa sites in 2019. Second row (**E–H**): all three (sub-) habitats on the northern sites (Site Mix, site PC1, site PC2) in 2019. Columns show the density of colonies per m² seafloor, number of identified taxa, Shannon index, and Pielou's index. Statistical differences ($p < 0.05$; Wilcoxon–Mann–Whitney test) are indicated by small letters (a–c) where significant results occurred. Black dots resemble outliers.*

Diversity indices

Shannon index was highest in *P. crispa* mats 2019 on sites PC1 and PC2 (mean 2.2 ± 0.1), Figure 4 C), while in the direct comparison of the northern *P. crispa* sites to *P. oceanica* on Site Mix, the *P. oceanica* shoots showed slightly higher values (mean 2.3 ± 0.4) than *P. crispa* (mean 2.1 ± 0.2) Figure 4 C).

Evenness (Pielou's index) was similar on *P. oceanica* leaves and shoots (mean 0.20 ± 0.01, and 0.18 ± 0.01, respectively), compared to *P. crispa* (mean 0.20 ± 0.01) on the northern sites. The northern sites showed slightly higher values, while the southern site – lower, without significant effects (Figure 4 D).

Structure of bryozoan assemblages

The diversity inside the *P. crispa* mats consisted of many taxa unique to this habitat. Out of the 46 families identified during this study, 18 were exclusively found on *P. crispa*. In the *P. oceanica* samples, we found 3 families not present on *P.crispa* (Figure 5). Most of the taxa solely found on *P. crispa* belonged to Watersiporidae (212.608 ± 37.342 and 24.788 ± 5.333 colonies per m^2 in 2018 and 2019, respectively).

The cluster analysis highlights the differences between *P. oceanica* sub-habitats and *P. crispa* and between sampling years (Figure 5). It also shows the dominance of *C. pyriformis* (the only species of Chlioniidae found) and Crisiidae (mainly *Filicrisia geniculate* and *Crisia* sp.) in *P. crispa* mats and on *P. oceanica* shoots. The family of Tubuliporidae was abundant across samples and habitats. Between the two years of *P. crispa* sampling, Watersiporidae and Candidae showed higher abundances in 2018, while Aetidae were more abundant in 2019 (Figure 5, Table 1).

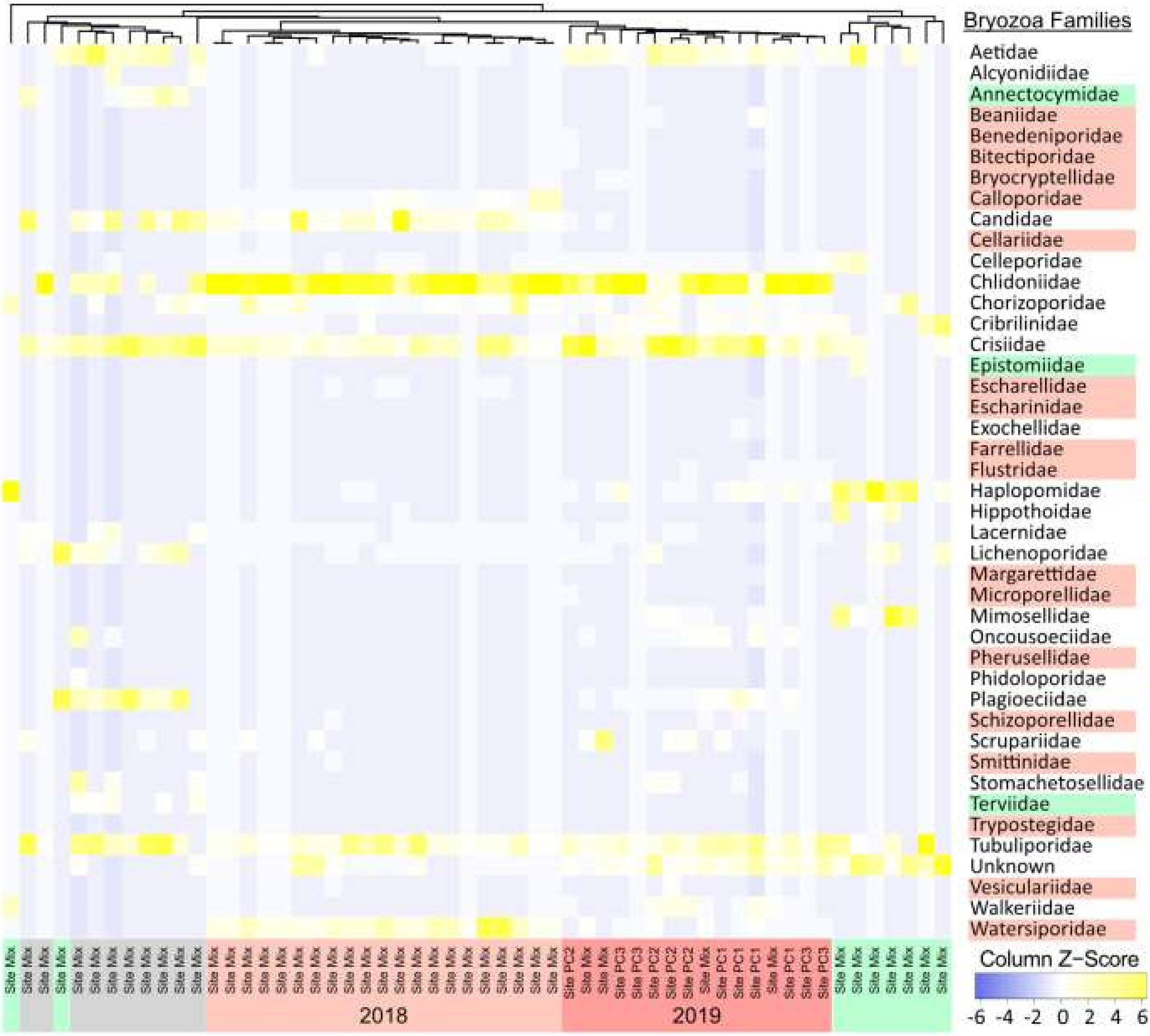

Figure 5. Visualization of Spearman ranked correlation of the abundances (colonies per m² seafloor) of bryozoan families. Samples of Posidonia oceanica leaves and shoots are indicated in green and grey, respectively. Phyllophora crispa samples are indicated in light red (2018) and dark red (2019). Bryozoa families highlighted in green were exclusive to Posidonia oceanica, highlighted in red were exclusive to Phyllophora crispa.

The non-metric multidimensional scaling (NMDS) further highlights the differences in the composition of bryozoan families among the (sub-) habitats in 2019 (only northern sites; Figure 6). While all three (sub-) habitats form distinct clusters, the *P. oceanica* sub-habitats samples show a higher degree of scattering than the *P. crispa* samples.

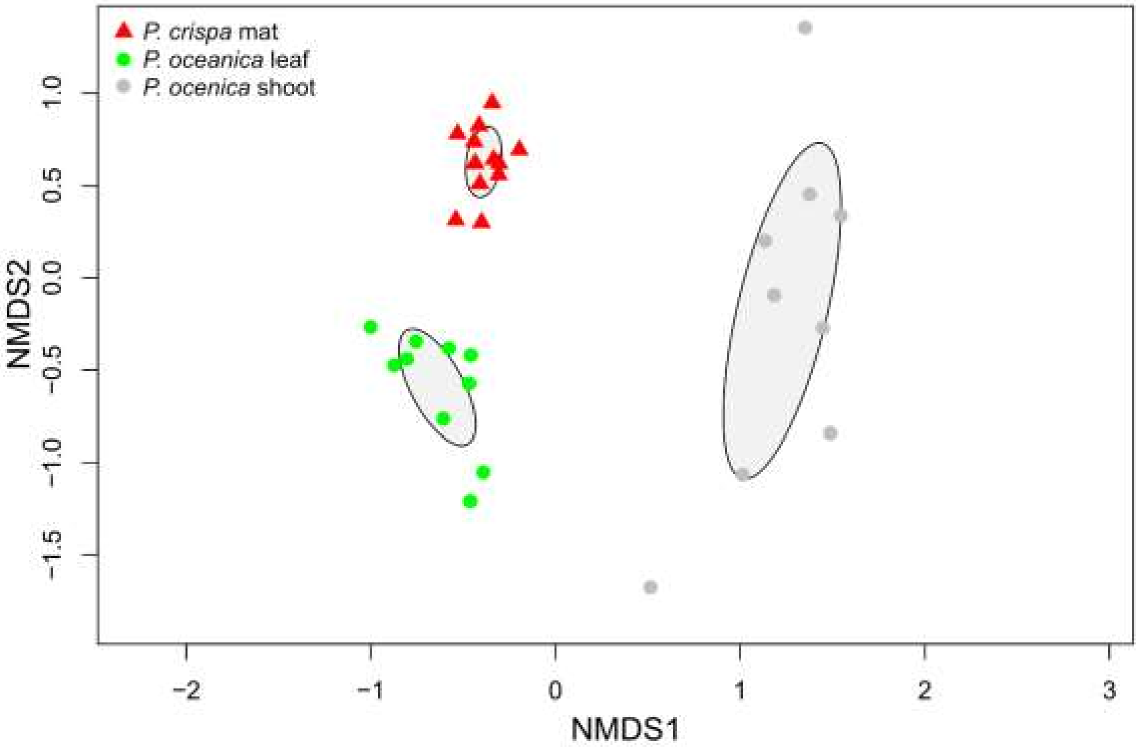

Figure 6. Non-metric multidimensional scaling (NMDS) plot comparing the bryozoan communities found on the three (sub-) habitats across the northern sites (Site Mix, site PC1, site PC2) during the second sampling season (2019).

Discussion

Differences in bryozoan abundances and diversity between *Phyllophora crispa* mats and *Posidonia oceanica* sub-habitats

Our results show differences among the three (sub-) habitats, with a clear trend to higher abundances and diversity of the bryozoan community inside the *P. crispa* mats (Figure 4 E-H). The results have shown that the bryozoan assemblages in *P. crispa* mats and on *P. oceanica* shoots are similar concerning the chosen diversity descriptors (Figure 4G, Figure 4H), whereas the distinct clusters in the NMDS analysis (Figure 6) highlight the differences according to the structure of the assemblages. While the quantitative diversity (abundance and number of taxa) was significantly higher in *P. crispa* mats, the diversity indices were similar to *P. oceanica* meadows. The relatively low indices for *P. crispa* compared to the indices on *P. oceanica* are likely due to the high abundance of one species (*Chlidonia pyriformis*, Chlioniidae), while the diversity on *P. oceanica* is more evenly distributed (Figure 4 H; Figure 5). The erect colonies of *C. pyriformis* are typical for calm water conditions and are often associated with macro-algae[45]. High abundances of erect and branching colonies (e.g., *C. pyriformis*, *Scrupocellaria* sp., *Crisia* sp.) on *P. crispa* and *P. oceanica* shoots are likely related to water current gradients inside *P. crispa* mats[46], as demonstrated before for *P. oceanica* meadows[47–49]. This is further supported by an experimental study, that has confirmed negative phototaxis for the larvae of some erect bryozoan species[50], since strong light gradients are present in both habitats[46].These traits, accompanied by the higher longevity of *P. crispa* and *P. oceanica* shoots compared to the relatively short-lived *P. oceanica* leaves, could be the main reason for the higher similarity of the two habitats in bryozoan families' composition (Table 1) and diversity (Figure 4 G). A richer diversity on *P. oceanica* shoots compared to the leaves has also been reported before in the Mediterranean Sea[51]. While the leaves of *P. oceanica* mainly host encrusting forms, *P. crispa* hosted additional growth types (petraliform and creeping), further underlining the structural diversity of the bryozoan community. As demonstrated above for *P. oceanica* meadows, this structural diversity is likely linked to gradients in water currents and light intensity which have recently been confirmed for *P. crispa* mats[46]. Further investigations are needed to identify the mechanisms of these gradients and their influence on the epiphytic community in this specific habitat.

The high amount of rare bryozoan taxa found exclusively on *P. crispa* during this study further emphasizes the relevance of *P. crispa* for the quantitative diversity and its role as an essential habitat besides classically known hotspots, such as *P. oceanica* meadows. Furthermore, a high diversity of filter feeders potentially supports a diverse and productive food web by transferring biomass from the water column into benthic communities[29]. Previous studies have shown that other sessile filter feeders are abundant inside *P. crispa* mats[34–36,39]. Bryozoans attract a wide range from invertebrates to fish and from incidental to specialized predators[52]. Some predators are highly specialized on bryozoans (e.g., nudibranchs[53,54] or pycnogonids[55]). We thus expect also to find high abundances and diversities of mobile benthic predators inside these mats in the future.

Some characteristic species for the *P. oceanica* leaf assemblages, like the endemic *Electra posidoniae* (Electridae; Gautier, 1954), were not found. This absence can be explained by the depth limitations and seasonality of these species[10].

Spatio-temporal variability of the bryozoan community inside Phyllophora crispa mats

The high diversity and abundance of bryozoans found in the pilot study of 2018 were confirmed in the comparative study of 2019 (Figure 5, Table 1). Because sampling took place within the same period (May – July) in both years, we can not evaluate seasonal changes during one year. The differences in the taxa composition (higher abundance of Watersiporidae and Candidae in 2018; higher abundance of Aetidae in 2019; Figure 5) are consistent with a previous study carried out on the eastern side of the island[34], where *Watersipora* sp. (Watersiporidae) was the most abundant species at depths between 25 and 35 m. Assemblages of cheilostome bryozoans (such as Candidae, Aeteidae, and Watersiporidae) have been shown to follow seasonal and depth-related variations[10]. Our study focused on the same depth range and season during both years to avoid impacts on the results. However, satellite data from both years show differences in the Mediterranean Sea surface temperature, with relatively high values for 2018, and lower values for the first half of 2019[56]. This effect has potentially shifted the natural seasonal variations and could have caused the observed effect.

Among the sampling sites of *P. crispa* during the 2019 campaign, our analysis showed higher diversity but lower abundance values for the northern sites (PC1 and PC2, Figure 4 –A-D). The high abundance accompanied by low diversity indices at Site PC3 was mainly driven by an exceptionally high density of *C. pyriformis*. The larvae of cheilostomate bryozoans (such as *C. pyriformis*) are often selective for suitable settling grounds[57]. The observed differences at Site PC3 could result from alterations in the environmental conditions due to the more exposed location towards the prevalent southern currents in the area[58], and thus an enhanced larval supply of this generalistic species. It remains unclear to what extent these differences could be explained by the relatively sheltered western site (Site Mix) from prevalent southern currents[58], resulting in different hydrodynamic patterns inside the bay. In addition, Campese bay is known for extensive touristic usage during the summer months, which might result in changes in the water quality. Physical disturbances and changes in water quality have been reported to affect bryozoan abundance and diversity[22,59,60]. Recent studies highlight the effects of local temperature and salinity changes in intertidal areas[61,62]. In the context of reoccurring temperature anomalies[63–65] and impacts on the thermohaline circulation[63], these effects are potentially becoming relevant also for sublittoral coastal habitats in the Mediterranean Sea. Further research is needed to describe hydrodynamic patterns and potential disturbances on these algal mats inside the bay.

Conclusion

We conclude that *P. crispa* mats provide an essential habitat for bryozoan diversity, harboring a high density of bryozoan colonies and a large number of families that were not present in the *P. oceanica* reference habitat. The Mediterranean "bryodiversity" (bryozoan diversity) has been recently estimated to 556 species, representing the 9.6% of global bryozoan diversity[17]. About 79% of the bryozoan species in the Mediterranean Sea colonize coralligenous and dark and semi-dark cave habitats (219 and 220 species, respectively). Nevertheless, as recently highlighted by Giampaoletti et al. (2020) for the mesophotic reefs in the Adriatic Sea, the understanding of bryozoans' diversity and ecological roles in Mediterranean habitats is still far from being thoroughly investigated. Our results further strengthen the significance of *P. crispa* mats as a habitat harboring an exceptional bryozoan diversity, along with previous studies on epiphytic epifauna[34–36,67]. Regional human impact and climate change threaten biodiversity in the Mediterranean Sea[68–70]. Therefore, identifying, protecting, and enhancing highly diverse habitats have become central parts of conservation strategies[71]. We suggest further investigations on the distribution of *P. crispa* mats along the Mediterranean coastline to confirm previous results on a larger scale and gain knowledge on the distribution of significant *P. crispa* aggregations. Furthermore, it is essential to understand how *P. crispa* mats are threatened by local and regional environmental impacts.

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Chapter 6 – Fleshy red algae mats act as temporary reservoirs for sessile invertebrate biodiversity

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Chapter 6 – Fleshy red algae mats act as temporary reservoirs for sessile invertebrate biodiversity

Abstract

Many coastal ecosystems, such as coral reefs and seagrass meadows, currently experience overgrowth by fleshy algae due to the interplay of local and global stressors. This is usually accompanied by strong decreases in habitat complexity and biodiversity. Recently, persistent, mat-forming fleshy red algae, previously described for the Black Sea and several Atlantic locations, have also been observed in the Mediterranean. These several centimetre high mats may displace seagrass meadows and invertebrate communities, potentially causing a substantial loss of associated biodiversity. We show that the sessile invertebrate biodiversity in these red algae mats is high and exceeds that of neighbouring seagrass meadows. Comparative biodiversity indices were similar to or higher than those recently described for calcifying green algae habitats and biodiversity hotspots like coral reefs or mangrove forests. Our findings suggest that fleshy red algae mats can act as alternative habitats and temporary sessile invertebrate biodiversity reservoirs in times of environmental change.

Keywords: *Phyllophora crispa*, *Posidonia oceanica*, seagrass meadows, habitat loss, climate change, Mediterranean Sea

Chapter 6 – Fleshy red algae mats act as temporary reservoirs for sessile invertebrate biodiversity

Introduction

Sessile plants and invertebrates play a central role in shaping biotic communities by increasing both the structural and habitat complexity, thus, promoting biodiversity[1–3]. In the marine environment, ecosystem engineers are responsible for forming biodiversity hotspots (i.e., areas rich in rare, threatened species)[4], such as seagrass meadows[5,6], tropical coral reefs[3], and mangrove forests[7]. Ecosystem engineers in these habitats change the abiotic and biotic components of the ecosystem, and in doing so, generate structurally complex environments that benefit both the engineers themselves and the associated biodiversity[1,8]. In the Anthropocene[9], human activity has negatively impacted almost all marine ecosystems. These threats have evoked ecosystem responses[10] leading them down a path of degradation[11]. Anthropogenic stressors occurring either singularly or in combination, such as ocean warming[6] and acidification[12] or nutrient pollution[6], can alter the community dynamics, shifting the system to alternative states dominated by more tolerant species[6,12]. These transitions, e.g., shifts from the reef or hard-bottom communities towards persistent, fleshy, non-calcifying (macro-) algal assemblages, are referred to as 'phase-shifts' to alternative states[13]. Phase shifts naturally entail a series of consequences on multiple levels, such as a loss of structural/ spatial complexity, a loss of ecosystem services and functioning[11,14], and consequently, a loss of biodiversity[3,6,15,16]. Identifying potential biodiversity refugia that are pivotal for rebuilding marine life[17] is therefore essential to appropriately adapt conservation strategies in times of increased biodiversity loss associated with anthropogenic global change[11,12,18] and direct local human impacts (e.g., pollution, coastal development)[5,6,19].

In the Mediterranean Sea, rocky hard-bottom communities and commonly identified biodiversity hotspots such as seagrass meadows are declining primarily due to environmental pressures[5,6,19,20]. Meadows formed by *Posidonia oceanica* seagrass rank amongst the most valuable coastal ecosystems worldwide as they provide a range of goods and ecosystem services[21,22], e.g., they exhibit high biodiversity, function as ecosystem engineers, and can act as natural coastal protection barriers[23]. *P. oceanica* meadows consist of the rhizome layer (often up to several m thick)[24] and the leaf canopy. The meadows occur from shallow waters down to depths of 40 m (depending on water turbidity). Due to anthropogenically induced environmental stressors[6], such as nutrient and sediment pollution, habitat loss and degradation[19], pollution[5,19], eutrophication[5,19] and/ or ocean warming[19], seagrass meadows are among the most threatened ecosystems worldwide[25].

In parallel, these stressors could have promoted the formation of persistent[26], turf- and mat-forming algal assemblages of the species *Phyllophora crispa* (formerly *P. nervosa*[27]) that have been observed across the Mediterranean[28,29], the Black Sea[30,31] and the Atlantic[29,32]. A growing number of publications addressing *P. crispa* suggests an increase of these algae in the Mediterranean[27,28,33,34]: *P. crispa* has been observed along the coast of Sardinia, Italy[28] and lately in the Tyrrhenian Sea, Italy, for the first time[27], where it has been found in dense mats of up to 15 cm thickness (Fig. 1a). *P. crispa* is a perennial rhodophyte of the order Gigartinales that typically produces branched thalli of up to 15 cm in length[26,30]. These red algae mats tolerate large variations in key environmental parameters and can proliferate under low water temperature (<10 °C) and salinity (18 PSU)[30]. *P. crispa* is sciaphilic[27,31], i.e., adapted to low-light conditions, and reaches large accumulations in water depths between 10 and 55 m[30,31]. The thalli of *P. crispa* can exhibit either an attached growth form covering hard substrates, an unattached form growing on sediments[31], or on reefs engineered by invertebrates, as recently observed in the Black Sea[31].

Algal assemblages can support high biodiversity, with several studies having found associations between high biodiversity and drifting algae in a lagoon off the west coast of the United States[35] and in the Baltic Sea[36–38]. Furthermore, the same has been found with calcifying green algae communities in coral reefs of the Great Barrier Reef, Australia[39], green algal blooms in the United States[40], Canada[41] and South Africa[42], as well as at further locations in the Atlantic[43,44]. In addition, kelp-forming brown algae in the United States[45] and United Kingdom[46] host a vast array of associated organisms. It remains unknown, however, whether the mat-forming red alga *P. crispa*, which is increasing in abundance and potentially replacing classical high biodiversity habitats also harbours high associated sessile biodiversity. Based on recent pilot studies that have identified non-colonial[27] and sessile polychaetes[34] to be associated with *P. crispa* mats, we here determined the role of *P. crispa* as habitat for overall sessile invertebrate biodiversity. The present study aims to answer the following research questions: (i) to what extent can *P. crispa* mats function as habitat for sessile invertebrates, and (ii) how does this biodiversity compare to neighboring *Posidonia oceanica* seagrass meadows? We focussed on the sessile biodiversity in *P. crispa* mats and adjacent *P. oceanica* meadows for several reasons. Firstly, previous pilot studies[27,34] lead to the hypothesis of a high associated sessile invertebrate diversity in *P. crispa* that is comparable in terms of community composition, invertebrate species richness and abundance to that of *P. oceanica*. This invertebrate diversity is likely linked to different habitat characteristics such as micro-niches caused by varying influences on key environmental parameters and ecosystem engineering functions[33]. Secondly, the presence of sessile invertebrates potentially reflects the stability and longevity of the red algae mats as habitats[47]. Hence, a particular sampling procedure was chosen to ensure the complete retrieval of sessile invertebrates.

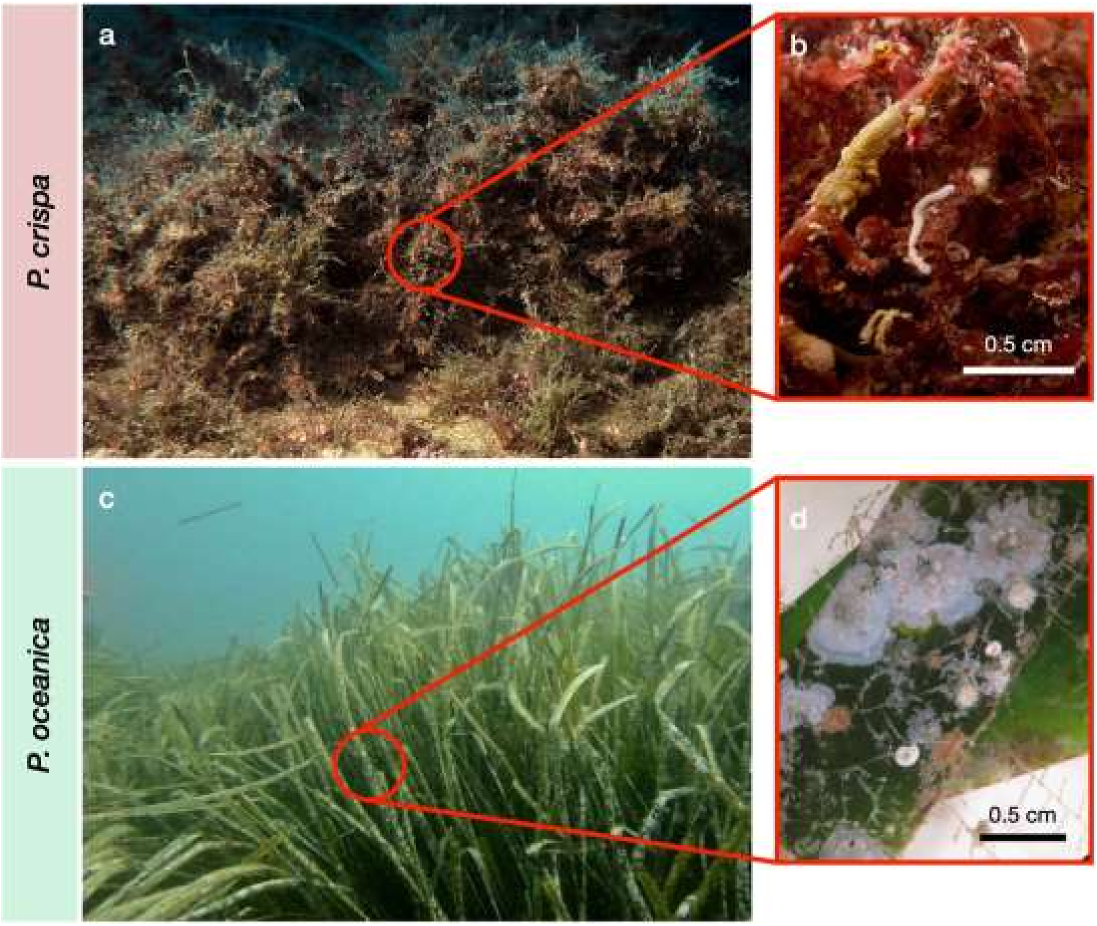

Figure 1: Phyllophora crispa mat and Posidonia oceanica seagrass meadow with associated sessile invertebrates. *P. crispa mat (**a**) and P. oceanica meadow (**c**) with Bryozoa, Polychaeta and Foraminifera on P. crispa thalli (**b**), Bryozoa, Polychaeta and crustose coralline algae (Corallinales) as epiphytes on P. oceanica leaves (**d**). Pictures taken by Felix I. Rossbach (**a, b, d**) and Friederike Peiffer (**c**).*

Results and discussion

Fleshy red alga mats as biodiversity hotspots for sessile invertebrates

We assessed the sessile invertebrate biodiversity in neighbouring *P. crispa* and *P. oceanica* habitats along the northeastern and north-western coasts of Giglio Island, within the Tuscan Archipelago National park, Tyrrhenian Sea, Italy (see Supplementary Fig. S1). *P. oceanica* community assessments included analysis of the holobiont (leaves + subsurface structures), as well as separate analyses of the leaves and rhizomes to account for potential differences[48] (see Methods). Briefly, invertebrates were determined to the lowest possible taxonomic level. However, in case no clear identification was possible, individuals were distinguished based on distinct visual characteristics, resulting in the identification of distinct phenotypes rather than species.

We recorded 312 distinct sessile invertebrate phenotypes (covering 9 higher taxa) for both *P. crispa* and *P. oceanica*, of which 223 occurred in *P. crispa* mats and 179 in *P. oceanica* holobionts, respectively (Fig. 2a). All (sub-) habitats accommodated distinct communities (Fig. 2b), with 133, 21 and 18 phenotypes uniquely found in *P. crispa* mats, *P. oceanica* leaves and *P. oceanica* rhizomes, respectively (Fig. 2a). Approximately 25% more phenotypes were found in *P. crispa* mats than in the neighbouring *P. oceanica* seagrass meadow holobionts. Calculations of classical diversity indices further endorsed *P. crispa* as a hotspot of sessile invertebrate diversity comparable to traditional biodiversity hotspots such as coral or Mediterranean coralligenous reefs (Table 1).

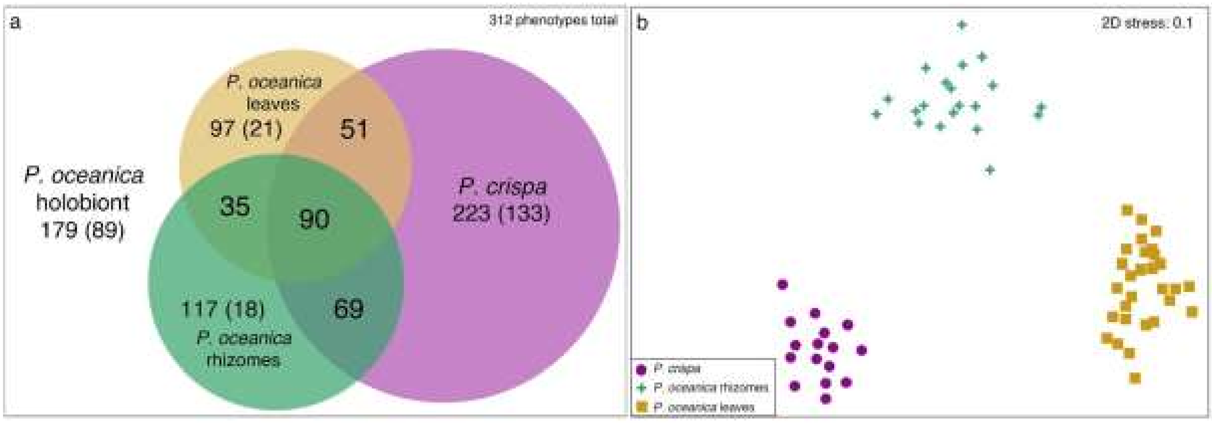

Figure 2: *(A)* ***Area-proportional Venn diagram*** *and ordination of biodiversity data by non-metric multidimensional scaling (nMDS). Area-proportional Venn diagram (**a**) displaying numbers of total (= present in the respective habitat), shared, and unique (in brackets) phenotypes found in investigated Phyllophora crispa (purple), Posidonia oceanica holobiont, P. oceanica leaves (gold) and P. oceanica rhizomes (green); area in proportion to number of phenotypes in P. crispa. Ordination of biodiversity (incidence) data by nMDS (**b**) based on Bray–Curtis similarities of P. crispa (purple dots), P. oceanica rhizomes (green crosses) and P. oceanica leaves (gold rectangles).*

The calculated abundances (mean number of individuals (ind) habitat m^{-2} ± standard error; note: colonies of colonial species are considered as individuals for readability hereafter) suggest that *P. crispa* mats provide a valuable habitat for sessile invertebrates that depend on a solid surface for attachment. Our data showed 64,008 ± 4609 ind m^{-2} associated with *P. crispa* mats, which was three times more than in *P. oceanica* holobionts (19,535 ± 1421; Dunn's test $p < 0.001$; Supplementary Table S2), four times more compared to *P. oceanica* leaves (15,857 ± 1654; Dunn's test $p < 0.001$; Supplementary Table S2) and two times the number observed in *P. oceanica* rhizomes (24,867 ± 1991; Dunn's test $p < 0.001$; Supplementary Table S2). Whereas *P. crispa* mats harboured an outstanding abundance of Bryozoa (44,222 ind habitat m^{-2}), both Bryozoa and Foraminifera were equally abundant in *P. oceanica* leaves and rhizomes (Supplementary Table S1).

***Table 1: Diversity indices** (richness = number of sessile phenotypes, H' = Shannon, D = Simpson) **and evenness accounting for sessile invertebrates** for investigated as well as reference biodiversity hotspots based on literature data. Note: indices and evenness were calculated based on classical formulas (see Methods), hence, discounting Hill numbers.*

Habitat	Location	Richness	Taxa	Evenness	*H'*	*D*	Reference
Phyllophora crispa	NW Mediterranean	223	9a,b,c,e,f,m,p,r,s	0.6969	2.209	0.269	Present study
Posidonia oceanica	NW Mediterranean	179	7a,b,c,f,m,p,s	0.7581	2.128	0.29	Present study
Posidonia oceanica	S Mediterranean	33	5a,b,f,p,s	0.8706	2.021	0.252	Mabrouk et al. (2014)[68]
Coralligenous reefs	NW Mediterranean	55	6a,b,c,f,p,s	0.807	2.086	0.254	Verdura et al. (2019)[69]
Coralligenous reefs	Mediterranean	786*	7a,b,c,f,m,p,s	0.9418	2.644	0.173	Ballestros (2006)[70]
Cystoseira zosteroides	NW Mediterranean	78	6a,b,c,f,p,s	0.7574	1.958	0.3	Ballestros et al. (2009)[71]
Coral reef	SW Indian Ocean	457	5a,c,f,m,s	0.8765	2.035	0.279	Cleary et al. (2016)[72]
Coral reef turf algae	W Indian Ocean	48[ɣ]	2p,m	0.995	0.995	0.493	Milne & Griffiths (2014)[73]
Coldwater coral reef	N Atlantic Ocean	213	7a,b,c,f,m,p,s	0.9523	2.673	0.165	Mortensen & Fossa (2006)[74]
Coldwater coral reef	N Atlantic Ocean	77	4a,b,c,s	0.8062	1.612	0.359	Henry et al. (2010)[75]
Mangrove forest	Caribbean Sea	54	6a,b,c,m,p,s	0.7494	1.937	0.297	Farnsworht & Ellison (1996)[76]
Kelp forest	NE Pacific Ocean	79[θ]	6a,b,c,m,p,s	0.9456	2.444	0.191	Graham (2004)[77]
Antarctic hard bottom	Weddell Sea	608[ƨ]	6a,b,c,f,m,s	0.85	2.197	0.28	Gutt et al. (2000)[78]
Halimeda bioherm	Coral Sea	474[ƨ]	5a,b,c,m,s	0.6965	1.617	0.42	McNeil et al. (2021)[27]

a Ascidiacea, b Bryozoa, c Cnidaria, e Entoprocta, f Foraminifera, m Mollusca (Bivalvia), p Polychaeta (Sedentaria), r Rotifera, s Porifera

* Data collated from multiple other publications

ɣ Excluded Cnidaria, Bryozoa and Ascidiacea from the analysis

θ Respective study included barnacles and phoronids that were not included in the current analysis

ƨ Excluded Polychaeta from the analysis

P. crispa harboured a similar number of phenotypes of Bryozoa and Foraminifera (76 and 81, respectively), whereas the number of bryozoan phenotypes exceeded that of Foraminifera in *P. oceanica* (78 and 52, respectively). In addition, we identified three distinct communities using non-metric multidimensional scaling (nMDS, Fig. 2b). The nMDS plot and appendant statistical analysis revealed that sessile invertebrate communities significantly varied among habitats (PERMANOVA with all $p < 0.001$; Supplementary Table S3), independent of the number of phenotypes and individuals of the investigated habitats.

To assess *P. crispa*'s role as a potential sessile invertebrate biodiversity hotspot compared to neighbouring *P. oceanica* meadows, we performed a diversity analysis based on the concept of Hill numbers. Hill numbers account for differences in sampling efforts, i.e., number of samples collected per habitat. The resulting metric represents the effective number of equally abundant species ${}^{q}D$[49,50], where q denotes the diversity order of a Hill number. The parameter q determines the sensitivity to species' frequencies and Hill numbers based on increasing values of q place more emphasis on frequently occurring species. In our analysis, ${}^{q}D$ of orders q = 0, q = 1, and q = 2 were calculated, representing phenotype richness (i.e., phenotypes quantified equally disregarding frequency, ${}^{0}D$), Shannon diversity (i.e., effective number of frequent phenotypes, ${}^{1}D$) and Simpson diversity (i.e., effective number of highly frequent phenotypes, ${}^{2}D$), respectively [51] (see Methods for further details).

The estimated sample completeness (i.e., diversity detected) profiles implied that there was undetected diversity within the habitats (Fig. 3a). Sample completeness profiles revealed that between 73.0% (*P. oceanica* holobiont) and 85.7% (*P. crispa*) of phenotype richness (q = 0) was detected with no significant differences among (sub-) habitats (i.e., respective 95% confidence intervals overlapped). The diversity detected in the (sub-) habitats rose with order q (i.e., diversity detected increased for more frequently occurring species) and increasingly aligned in all habitats for Shannon (q = 1) and Simpson (q = 2) diversity, with the majority of frequent and highly frequent phenotypes being detected (Fig. 3a and Supplementary Table S6). To test if we could estimate diversity based on our data reliably, sample-size-based rarefaction and extrapolation curves were computed to check for asymptoted values of q. An estimation of true Simpson diversity based on our data for all (sub-) habitats was indeed reliable (i.e., size-based rarefaction and extrapolation curves asymptoted for q = 2; Fig. 3b). Hence, we could confirm that *P. crispa* harboured assemblages with significantly higher Simpson diversity (~132; Fig. 3c and Supplementary Table S6; no overlap of 95% confidence intervals[52]) compared to all other (sub-) habitats, which underlines its role as a biodiversity hotspot for sessile invertebrates. For phenotype richness and Shannon diversity, only conservative minimum estimates could be obtained, as sizebased rarefaction and extrapolation curves did not asymptote for q = 0,1 (Fig. 3b). In this case, a statistically reliable comparison between habitats' phenotype richness and Shannon diversity may only be performed based on standardised data. For this purpose, we compared diversities based on standardised data at a sample coverage level of C_{max}= 96.9% (Fig. 3d and Supplementary Table S6). C_{max} is the lowest sample completeness at q = 1 of any (sub-) habitat when samples are extrapolated to double the respective number of samples per (sub-) habitat. Consequently, we showed that *P. crispa* exhibited significantly (i.e., no overlap of respective 95% confidence intervals[52]; Fig. 3d and Supplementary Table S6) higher phenotype richness compared to neighbouring *P. oceanica*: phenotype richness of *P. crispa* mats (~234 phenotypes) exceeded those of *P. oceanica* rhizomes (~142) and leaves (~102) at a fixed sample coverage of C_{max}= 96.9% (Fig. 3d and Supplementary Table S6[52]), whereas the difference compared to the *P. oceanica* holobiont (~207) was marginal. For Shannon diversity, *P. crispa* showed a significantly higher index value (~159) compared to the *P. oceanica* holobiont (~111), leaves (~64) and rhizomes (~84; see Fig. 3d and Supplementary Table S6[52]). Phenotype evenness (i.e., Pielou's J at C_{max}; an evenness measure based on phenotype occurrences) was high for all habitats, being lowest for the *P. oceanica* holobiont (0.88) and highest for *P. crispa* (0.93; Supplementary Table S6). Furthermore, *P. crispa* harboured the most evenly diverse biotic communities among all (sub-) habitats for all orders of q > 0 at C_{max} (i.e., for orders of increasing sensitivity to phenotype frequencies; Fig. 3e and Supplementary Table S6). The difference in estimated phenotype Simpson diversity between *P. crispa* and the *P. oceanica* holobiont at C_{max} was larger than the difference in phenotype richness (i.e., ~50 and ~27, respectively; Supplementary Table S6). When comparing the empirical richness values (i.e., values for q = 0) with the values estimated asymptotically and non-asymptotically (the latter standardised for C_{max}), the number of undetected phenotypes was larger for (sub-) habitats of *P. oceanica* (holobiont and rhizomes) than for *P. crispa* (Supplementary Table S6). These findings indicate that the higher overall diversity in *P. crispa* may be driven by the higher abundance of frequently occurring rather than rare phenotypes. However, even though the estimated number of undetected phenotypes was higher for *P. oceanica* compared to *P. crispa*, the overall estimated diversity for all orders of q in the red algae habitats still remained higher relative to the seagrass meadows (Fig. 2c). Taken together, our data have identified *P. crispa* as a habitat that harbours more even and diverse sessile invertebrate communities compared to neighbouring *P. oceanica* meadows.

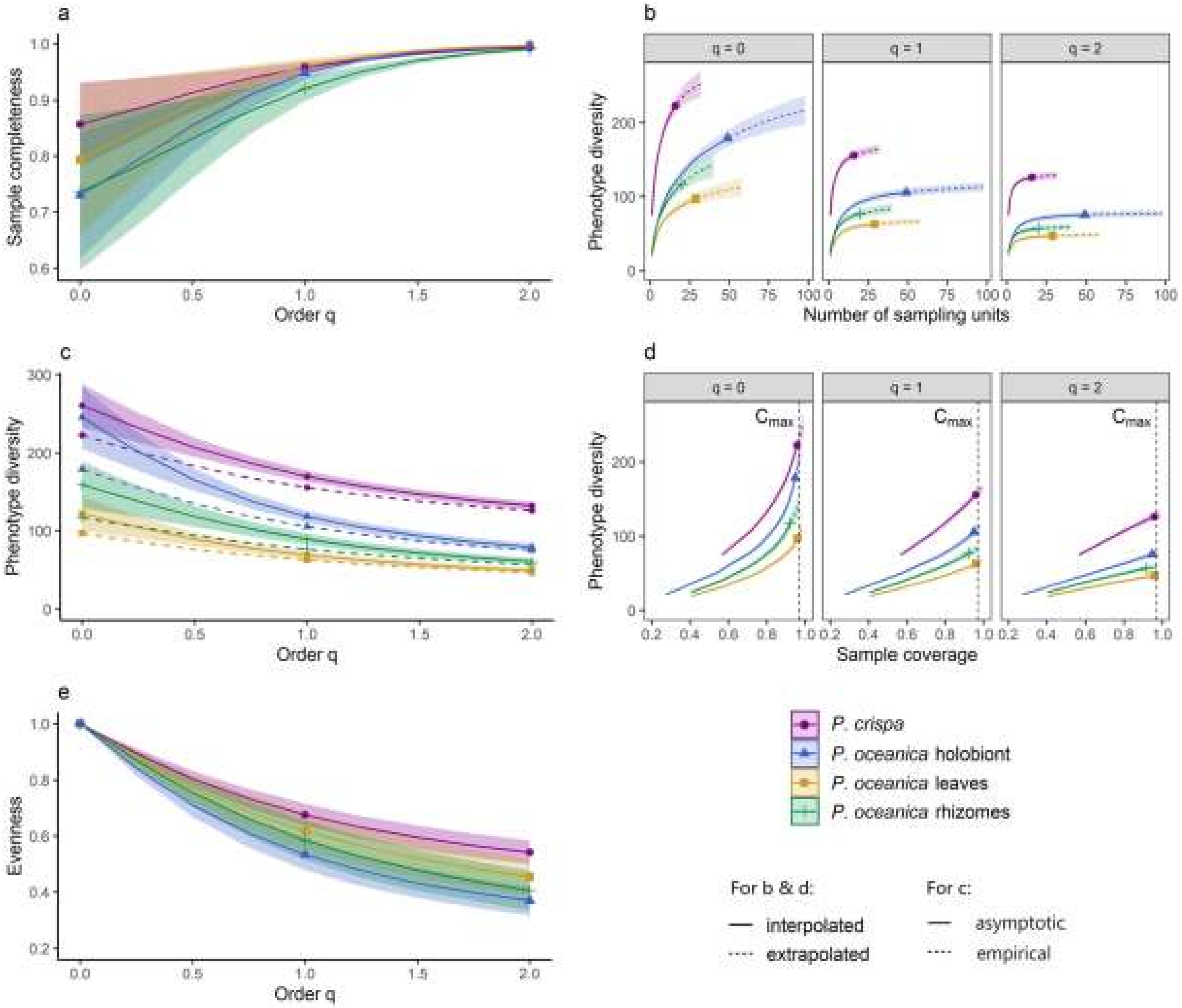

Figure 3: Overview of biodiversity analysis based on Hill numbers: *(**a**) Estimated sample completeness curves as a function of order q between 0 and 2. (**b**) Size-based rarefaction (solid lines) and extrapolation (dashed lines) curves up to double the respective sample size. (**c**) Asymptotic estimates of diversity profiles (solid lines) and empirical diversity profiles (dashed lines). (**d**) Coverage-based rarefaction (solid lines) and extrapolation (dashed lines) curves up to double the reference sample size. Vertical dashed lines show the standardised sample coverage* C_{max} *= 96.6 % (**e**) Evenness profiles as a function of order q, 0 < q ≤ 2, based on the normalised slope of Hill numbers. Dots (P. crispa), triangles (P. oceanica holobiont), rectangles (P. oceanica leaves) and crosses (P. oceanica rhizomes) denote observed data points. All shaded areas in a-e denote 95 % confidence intervals obtained from a bootstrap method with 500 replications. Note: some bands are invisible due to narrow width.*

Red algae mats fulfill ecosystem engineer functions

We measured key environmental parameters (i.e., oxygen concentrations, light availability, pH, temperature, chlorophyll α concentration, and water movement) in neighbouring *P. crispa* and *P. oceanica* to assess *P. crispa*'s functioning as an ecosystem engineer. Our results suggest that *P. crispa* shapes key environmental parameters similarly to neighbouring *P. oceanica* seagrass meadows (Fig. 4). In particular, water movement and light intensity within the red algae mats and in the seagrass meadows were lower than for the neighbouring bare substrate (Fig. 4b, f). This extends the findings of a parallel study that has identified *P. crispa* as an ecosystem engineer modifying its environment[33]. This functioning as an ecosystem engineer seems to apply to further environmental parameters: daily oxygen concentration fluctuations of *P. crispa* (7.73–8.14 mg l^{-1}) were similar to those of *P. oceanica* (7.59–8.04 mg l^{-1}), with the daily mean of oxygen concentrations being slightly higher in *P. crispa* (7.99 mg l^{-1}) compared to those of *P. oceanica* (7.75 mg l^{-1}). This contradicts previous findings stating that shallow, macroalgae-covered environments undergo wider oxygen concentration fluctuations compared to seagrass meadows[53,54]. Our findings indicate that this may not necessarily be the case in deeper environments (Fig. 4a). In addition, the average pH within *P. crispa* mats was lower (8.44) compared to *P. oceanica* meadows (8.64), which resembled the observed differences in O_2 concentrations (Fig. 4a, c). Photosynthesis by algae and plants requires hydrogen ions, which results in increased pH levels while respiration lowers pH levels[55,56]. Furthermore, our data suggest higher light availability in *P. crispa* (538 lux) compared to *P. oceanica* meadows (315 lux; Fig. 4b) at the same depth. These findings corroborate with previous studies that identified strong light attenuations in seagrass macrophyte habitats due to self-shading effects[57,58]. A lessened self-shading effect in the red algae habitat compared to the *P. oceanica* seagrass meadows could be explained by morphological differences between the two habitats. The latter forms meadows of higher thickness relative to the mats formed by *P. crispa*, with *P. oceanica* leaves being wider than thalli of *P. crispa*. Finally, the reduced water movement (Fig. 4f) in both habitats and higher O_2 availability in *P. crispa* compared to *P. oceanica* (Fig. 4a) may benefit the settlement of specific bryozoans (e.g., Bugula sp., Schizoporella sp.)[59–63], bivalves[64] and polychaetes (Hydroides sp.)[63]. This may explain the findings of the present study, in which we identified moror individuals associat/or individuals associated with *P. crispa* compared to *P. oceanica* of bryozoans (76 vs. 78 phenotypes, 44,222 vs. 7655 ind habitat m^{-2}), molluscs (bivalves; 4 vs. 4 phenotypes, 112 vs. 38 ind habitat m^{-2}) and polychaetes (23 vs. 13 phenotypes, 5950 vs. 3734 ind habitat m^{-2} [65]; see Supplementary Table S1). Potentially, lower pH in *P. crispa* mats (Fig. 4c) may have limited the presence of organisms such as bivalves[66] or benefitted comparatively resilient organisms such as specific bryozoans[67]. Hence, the extent to which lower pH conditions in *P. crispa* compared to *P. oceanica* may have counteracted potential benefits such as higher O_2 availability (Fig. 4a) remains speculative.

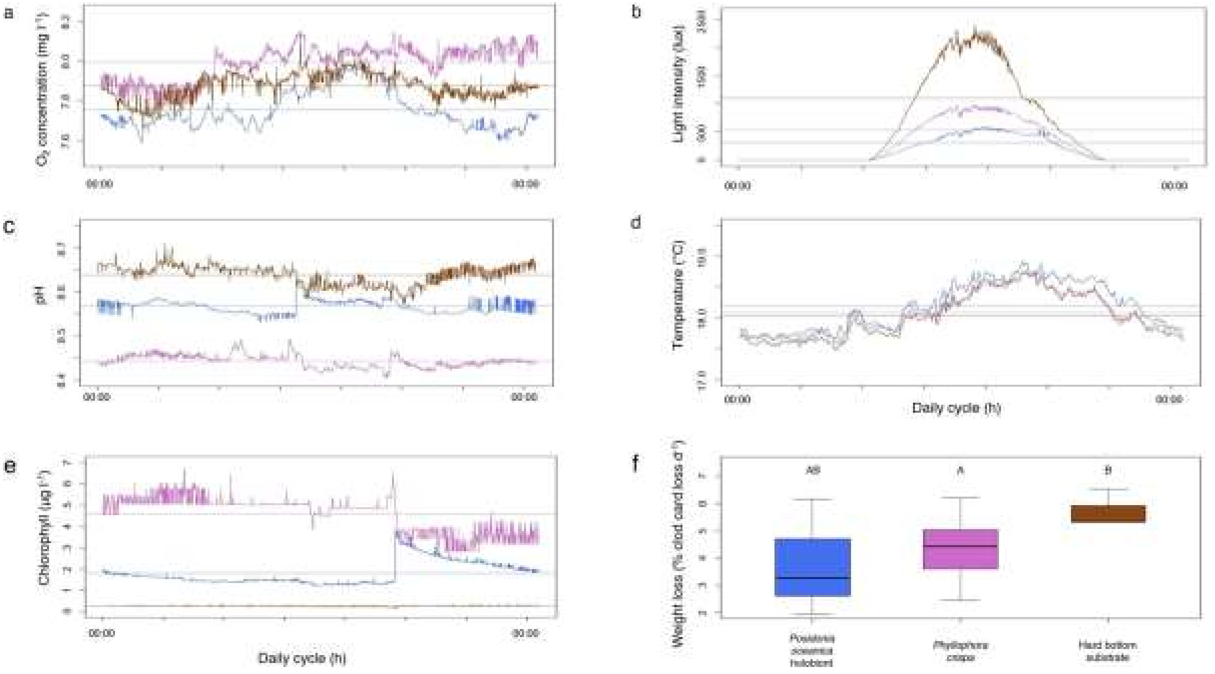

Figure 4: Environmental parameters measured in Phyllophora crispa and Posidonia oceanica. *Environmental data consisting of oxygen (O_2) concentration (**a**), light intensity (**b**), pH (**c**), temperature (**d**), chlorophyll α concentration (**e**) and water movement (estimated via weight loss of clod cards; **f**) in Phyllophora crispa (purple), Posidonia oceanica (blue) and neighbouring hard-bottom substrate serving as a reference habitat (brown). Horizontal lines within panels a–e display daily mean of respective deployment (with n = 13 for P. crispa, n = 7 for P. oceanica, n = 6 for reference habitat for O_2 concentration, pH and chlorophyll α concentration, and with n = 10 for P. crispa, n = 6 in P. oceanica, n = 4 in reference habitat for light intensity and temperature) of respective parameters in each habitat. Note for panel f: different letters above box plots indicate significant differences between habitats (ANOVA and subsequent Tukey HSD test), with n = 9 for P. crispa, n = 4 for P. oceanica, n = 2 for reference habitat.*

The higher number of phenotypes and individuals in *P. crispa* relative to *P. oceanica* may be partly explained by the specific surface area that potentially offers substrate, and thus microhabitats for mobile and sessile invertebrates[27,30]. The complex morphology of *P. crispa* mats is reflected in the 2D to 3D surface area enlargement factor. Here, a high surface area provided by complex thalli relative to a small volume (mats of several cm thickness) resulted in an enlargement factor of 4.9 ± 0.2 (mean ± standard error; Supplementary Table S5) for *P. crispa*, which was lower than for *P. oceanica* (both leaves (7.3 ± 0.5) and the *P. oceanica* holobiont (8.3 ± 0.5) but higher than for *P. oceanica* rhizomes (2.0 ± 0.1)). This structural complexity may also explain the observed reduced water movement within *P. crispa* mats (Fig. 4f) that could favour sediment trapping. The extent to which further functions such as sediment trapping, similar to the reduced water movements induced by *P. oceanica* meadows[68–70], apply to *P. crispa* mats needs to be determined in future studies. Trapped sediment and particulate matter could provide (1) a heterogeneous habitat for infaunal species[71] and (2) (in-) organic matter for tube-building species such as sessile polychaetes[71]. Growth form, enlargement factor and persistence[30] of *P. crispa* contradict the common notion that structural complexity is reduced when spatially complex and long-living habitats, such as seagrass meadows, decline[2,6]. We further estimated the number of individuals per area m^2 of seafloor by multiplying the calculated numbers of individuals per habitat m^2 with the respective enlargement factor (Supplementary Table S5). *P. crispa* supported

313,635 ± 27,486 ind seafloor m^{-2}, which was approximately twice that of the *P. oceanica* holobiont (162,139 ± 11,794 ind seafloor m^{-2}; Dunn's test $p < 0.001$; Supplementary Table S4).

We conclude that *P. crispa* mats facilitate the colonisation of sessile organisms [27,72] by providing (micro-) habitats for associated alpha diversity (Table 1 and Fig. 3), thus, allowing us to propose *P. crispa* as an ecosystem engineer[1,73]. Together with the considerable surface area enlargement (Supplementary Table S5), environmental parameters shaped by *P. crispa* (Fig. 4), its wide distribution[27,29,31,32] and the comparative biodiversity analysis (Table 1 and Figs. 2 and 3), red algae mats may function as overlooked ecosystem engineers and harbour high sessile invertebrate biodiversity.

Fleshy red algae as refuge habitat

Like many other marine ecosystems, *P. oceanica* seagrass meadows experience a range of anthropogenic threats, which have caused a drastic decline in the spatial distribution throughout the Mediterranean[6]. The loss of biodiversity is only one among many consequences of declining *P. oceanica* meadows[5,6,19]. The high biodiversity associated with red algae *P. crispa* mats may positively impact sessile invertebrate communities in bordering *P. oceanica* seagrass meadows[74], which is reflected by a total of 90 shared phenotypes that occurred in all investigated habitats (Fig. 2a).

Even though *P. crispa* mats harboured sessile invertebrates in numbers that exceeded those of neighbouring *P. oceanica* meadows[65] and other ecosystems (Table 1), these mats substantially differed from seagrass meadows in their longevity. In the Mediterranean, *P. oceanica* meadows form dense rhizome layers that can be of several metres of thickness when admixed with trapped sediment[24]. Similar to coral reefs or mangrove forests, seagrass meadows can persist for several millennia[75], which exceeds the currently estimated lifespan of *P. crispa* formations (i.e., decades)[30]. The evolved size and physical structure of seagrass meadows can result in a dissipation of wave energy on multiple levels (reviewed in ref.[23]) and reduce coastal damage and erosion. Wave energy is a key limiting factor defining the upper physical boundary that shapes the bathymetric spatial distribution for *P. oceanica* meadows[76]. The properties of *P. oceanica* allow it to withstand these physical impacts and grow at depths as shallow as 0.5 m[77]. In contrast to *P. oceanica* meadows, *P. crispa* mats can be dislodged and translocated by waves[30], particularly those with an unattached growth form on sediments[31]. Although dislodged *P. crispa* may not offer a stable environment over longer time scales, mobile algal thalli may function as an effective dispersal mechanism. Drifting algae parts may offer substrate to diverse sessile invertebrate communities[35,36] and function as a transport vector over large distances[37]. The extent to which the associated phenotypes identified in this study tolerated this drifting behaviour remains speculative[38]. The translocation of *P. crispa* mats may have consequences for associated biodiversity through two pathways: (i) translocated *P. crispa*[30], which can colonise and spread vegetatively, may still provide habitat for associated sessile invertebrates; or (ii) *P. crispa* mats are severely damaged, losing their function as ecosystem engineers, and, hence, biodiversity hotspots. We conclude that in both cases, *P. crispa* mats serve as temporary ecosystem engineers forming temporary refuge habitats, and subsequently as transitory biodiversity hotspots. Potentially, more tolerant sessile species could reach more favourable areas such as healthy seagrass beds that are possibly beyond the reach of planktonic larval stages. *P. crispa* formations in the Atlantic and Black Sea provide a relatively stable habitat over several decades[30], which underlines the general functioning as a biodiversity substratum. The extent to which this function applies to *P. crispa* mats of the Mediterranean as well needs to be determined in future studies.

We postulate that sessile invertebrates can re-colonise recovering *P. oceanica* meadows, if appropriate conservation measures are implemented[18,78]. Seagrass meadows can recover from anthropogenic or natural threats on a decadal timescale[79], which corresponds with the lifespans of *P. crispa* mats[30]. Hence, red algae mats may function as overlooked biodiversity refuge habitats supporting the recovery of classical habitats such as seagrass meadows, particularly due to their proliferation across the Mediterranean[27–29], the Black Sea[30,31] and the Atlantic[29,32]. Likewise, similar patterns (i.e., the supported recovery of a habitat by neighbouring habitats) were reported from the Great Barrier Reef, where the recovery of a bleached reef was facilitated by larval inflows originating from non-bleached reefs[80]. In the Mediterranean, we hypothesise that *P. crispa* can support *P. oceanica* meadows (and other habitats, see Table 1) by maintaining their sessile invertebrate biodiversity[74,81], particularly due to an overlap of shared phenotypes, i.e., sessile invertebrates that occurred in both *P. crispa* and *P. oceanica* (Fig. 2a), even though both habitats harbour a range of unique phenotypes. It remains to be determined (i) to what extent the community composition in re-colonised *P. oceanica* meadows differs from their initial sessile invertebrate community composition, considering the clear distinction of associated sessile invertebrate communities in *P. crispa* mats and *P. oceanica* meadows (Fig. 2b), and (ii) whether this function applies to all shared phenotypes and potentially further taxa. Our findings suggest that *P. crispa* mats and their associated sessile invertebrate communities potentially aid in reviving classical marine (sessile invertebrate) biodiversity hotspots such as invaluable seagrass meadows in the Mediterranean Sea once threats are reduced or removed[17,79,82].

Methods

Study Site and Sampling

Study site and sampling. All data were generated by SCUBA diving between May and July 2019 along the north-eastern and north-western coasts of Giglio Island, within the Tuscan Archipelago National Park, Tyrrhenian Sea, Italy (Supplementary Fig. S1). Samples for biodiversity assessments were taken at six sites (two each for *P. crispa* mats of >5 cm thickness and *P. oceanica*, and two for co-occurring habitats, resulting in four sampling sites for *P. crispa* and *P. oceanica* each, see Supplementary Fig. S1) according to accessibility and occurrence of target habitats at water depths between 28 and 30 m.

To sample *P. crispa* mats for the present study, a sampling frame (30 × 30 cm) was randomly placed in the target area four times (i.e., each time 50 cm apart), and all algal material within the frame was carefully removed using a spatula and subsequently placed into 1 L PP-bottles (each holding a ratio of algae:water = 1:3). A total of 16 replicates for *P. crispa* were sampled. *P. oceanica* rhizome and leaf specimens were sampled separately into 1 L Kautex jars to avoid oxygen depletion or physical damage during transport. An attached growth form of *P. crispa* was chosen for the present study. *P. oceanica* root-rhizomes were cut including the sheaths, both vertical and horizontal rhizome as well as the upper layers of the roots (Supplementary Fig. S2, hereafter referred to as '*P. oceanica* rhizome'). Leaves were cut with scissors directly at the sheath of the shoot. A total of 20 *P. oceanica* rhizome specimens and 29 single leaves were collected from the four sampling sites to minimise the impact on threatened *P. oceanica* meadows. The number of sampled specimens at the respective sampling locations was 4× 'Corvo', 4× 'Fenaio', 4× 'Punta del Morto', and 4× 'Secca 2' for *P. crispa*; 10× '3 Fratelli', 5× 'Fenaio', 5× 'Cala Calbugina', and 9× 'Secca 2' for *P. oceanica* leaves; 10× '3 Fratelli' and 10× 'Secca 2' for *P. oceanica* rhizomes (see Supplementary Fig. S1).

All samples (*P. crispa*, *P. oceanica* leaves and rhizomes) were transferred immediately to the seawater husbandry tanks of the Institute for Marine Biology (IfMB, located on the island of Giglio, Italy) upon return from sea under stable physical conditions (18 °C, 12:12 h dark/light cycle, light similar to in situ conditions) until further analysis. For biodiversity assessments, four subsamples of *P. crispa* were taken from each of these main samples.

Biodiversity Assessment

All samples were analysed within three days after collection. For *P. crispa*, subsamples (sensu Bianchi (2004)[83]) were transferred to plastic bowls, where *P. crispa* mats were cut into single thalli, and subsequently placed in single Petri dishes. Thalli were then analysed using stereo magnifiers (max. ×40 magnification) to determine invertebrates that were assigned to one of the following taxa: Ascidiacea, Bryozoa, Cnidaria, Entoprocta, Foraminifera, Mollusca (Bivalvia), Polychaeta (Sedentaria), Rotifera, and Porifera. Foraminifera were determined using a microscope (max. 400x magnification). Seagrasses such as *P. oceanica* are typically divided into two sub-habitats: the leaf canopy-forming part and a dense root-rhizome layer[48,84], both varying in their habitat characteristics and associated biotic assemblages[85,86]. Thus, we investigated the sessile invertebrate diversity in both sub-habitats in our analysis by assessing invertebrate phenotype abundances separately for *P. oceanica* leaves and rhizomes to account for potential differences. *P. oceanica* rhizomes were analysed as a whole using a stereo microscope, whereas *P. oceanica* leaves were cut into pieces of ~8 cm length for handling and to avoid double counting. All *P. oceanica* samples were analysed for the aforementioned taxa as well. All specimens were identified according to relevant literature (Supplementary Table S7) and crosschecked online with the World Register of Marine Species (marinespecies.org). Individual specimens or colonies in case of colonial species (i.e., Bryozoa) were then counted for further analysis. In case no clear identification was possible, individuals were distinguished based on distinct visual characteristics, resulting in a dataset consisting of distinct phenotypes rather than species. We refer to Supplementary Table S8, which consists of a subset exemplarily showing the applicability via a clear correlation of the number of species and phenotypes, respectively. Finally, all numbers were normalised to their respective habitats' surface area using the corresponding enlargement factor (see next section), resulting in a total number of individuals per habitat m^2.

To test for statistical differences between the number of individuals among habitats, a Shapiro–Wilk test for normality, Kruskal-Wallis-test and a subsequent post-hoc Dunn's test were performed in R (version 4.0.4)[87] with the interface RStudio (version 1.0.153)[88] using the 'shapiro.test', 'kruskal.test' and 'dunnTest' functions from the 'stats'[87] and 'FSA'[89] packages. We expected numbers in *P. oceanica* leaves and rhizomes to exceed those of *P. crispa* given higher sampling efforts for the former (n = 29 and n = 20, respectively vs. n = 16). To allow for comparisons among habitats—despite differences in sampling efforts—we applied a combination of asymptotic and non-asymptotic diversity estimations based on rarefaction and extrapolation analysis tools, and Hill numbers (see below). We used phenotype incidence instead of abundance data, as diversity estimations based on Hill numbers rely on species (or phenotypes in the present study) occurring as singletons (i.e., occurring in one sample or with abundances of one individual). Given that we normalised phenotype abundance counts to habitat and seafloor area (m^2) to enable comparison between habitats, the assemblages sampled by us are devoid of singleton occurrences, ultimately leading to samples appearing complete in terms of capturing true diversity. This is highly unlikely with a non-exhaustive sampling effort and we, thus, opted to use phenotype incidence data for diversity and sample completeness estimation, as this has been shown to not be statistically inferior for the use of count abundances (e.g., ref.[90] and ref.[49]).

A statistical biodiversity assessment was performed using a combination of the iNext4steps online tool (https://chao.shinyapps.io/iNEXT4steps/) and the 'iNext' package [91] in R (version 4.0.4)[87] with the interface RStudio (version 1.0.153)[88]. Given that the official online tool was not yet available at this time, Chao et al. [51] provided a hyperlink to a trial version that we used in this study. Plots were created using 'iNext's ggiNext' function and the 'ggplot2' package[92]. We refer to Daraghmeh and El-Khaled[93] for a detailed workflow and scripts. Briefly, to assess and compare sample completeness and alpha diversity of the respective habitats, we followed the protocol proposed by Chao et al.[51]. It is based on their extensive earlier works (e.g., Chao et al.)[49], which use the now widely accepted concept of Hill numbers, also known as the effective number of equally abundant species ${}^{q}D$[49,50]. Here, q denotes the diversity order of a Hill number and determines its sensitivity to species' relative abundances or frequencies (in case of incidence data, i.e., species presence/absence). Hill numbers based on higher values of q put more emphasis on more commonly occurring species. The most widely used members of the family of Hill numbers are the ones of orders q = 0, q = 1 and q = 2. For sampling-unit-based phenotype incidence data as used in the present analysis (see below), ${}^{0}D$ indicates the measure of phenotype richness (i.e., all phenotypes are quantified equally without regard to their actual frequencies) and ${}^{1}D$ and ${}^{2}D$ represent Shannon (i.e., exponential of Shannon entropy) and Simpson (i.e., inverse of Simpson concentration index) diversity, i.e., the effective number of frequent and highly frequent phenotypes, respectively[51]. Here, we used phenotype incidence data as described above.

The calculation of Hill numbers based on sample data (i.e., empirical or observed Hill numbers) is biased regarding sample completeness and size[49]. We followed the workflow and steps listed below to achieve meaningful comparisons of the investigated biotic communities (see ref.[51] and Supplementary Table S6):

(I) Estimation of sample-completeness profiles from sample data via a bootstrap method (n = 500) to obtain confidence intervals: this enabled comparison of sample completeness (i.e., diversity detected) of our various habitat datasets. Profiles that increase with order q indicate incomplete sampling and therefore undetected diversity.

(II) Empirical and asymptotic estimation of true diversities based onhypothetical large sample sizes[94]: sufficient data are a prerequisite for the latter, however. To investigate if our data fulfilled this requirement, we computed samplesize-based rarefaction and extrapolation (R/E) sampling curves for Hill numbers of different orders[49,90]. Extrapolation was performed to double the actual number of samples per habitat, as further extrapolation is unreliable in the case of phenotype richness[90]. Levelling out of R/E curves indicates that asymptotic estimates are accurately representing true diversities. In this case, asymptotic and empirical Hill numbers may be compared to assess undetected diversity and the comparison of asymptotic diversity profiles allows the assessment of differences in diversity between habitats. If R/E curves do not level out, asymptotic diversity estimates represent true diversity only up to a certain level of sample coverage (i.e., C_{max}, see below) and, therefore, have to be considered as minimum estimates of true diversity.

(III) Comparing diversity for a non-asymptotically standardised sample coverage (i.e., sample completeness for q = 1) in the case where asymptotic estimation of true diversity is unreliable: diversity may then be compared between equally complete samples. Here, coverage-based R/E curves were computed to the maximum coverage C_{max}. This value represents the sample coverage of the habitat exhibiting the lowest coverage when samples are extrapolated to double the respective number of samples per habitat.

(IV) Estimation of evenness profiles for q > 0 at C_{max} based on ref.[95]: to compare evenness profiles of assemblages with varying levels of richness, the slopes of Hill-number diversity profiles connecting two points at q = 0 and any q > 0 are being analysed, whereby steeper slopes represent higher unevenness of phenotype incidences. The slopes were normalised and converted to an evenness value. This was possible for orders of q > 0, but not for q = 0, as all phenotypes are accounted for equally in the latter. In addition, Pielou's J' was calculated as a phenotype evenness measure based on Hill numbers of q = 0 and 1[95,96]. Both evenness profiles and Pielou's J' are based on the richness and Hill-number diversity and were therefore estimated at a standard level of C_{max}.

Biodiversity indices for study comparison and community composition analysis

Due to missing original data of studies investigating sessile invertebrate biodiversity hotspots in the Mediterranean and elsewhere (see Table 1), but to ensure comparability with the present study, classical alpha biodiversity (Shannon, Simpson) indices, as well as Evenness index not based on Hill numbers were calculated as followed[50]:

1) Shannon index $-\sum i\left[\frac{n_i}{N}\times log_2\left(\frac{n_i}{N}\right)\right]$

2) Simpson index $\frac{\sum i\, n_i\,(n_i-1)}{N(N-1)}$

3) Evenness index $-\frac{\sum i\left(\frac{n_i}{N}\times \ln\left(\frac{n_i}{N}\right)\right)}{\ln N}$

where n_i is the number of phenotypes/species in a taxon, and N is the total number of taxa, with a maximum of 9 as previously defined.

Non-parametric permutational multivariate analysis of variance (PERMANOVA[97]; based on species abundance data using Primer-E v6[98] with the PERMANOVA+ extension)[99] was used to check for significant differences (i.e., $p \leq 0.05$) in the sessile invertebrate community composition among (sub-) habitats. For this, raw count data (related to habitat m^2) were square-root transformed to generate Bray–Curtis similarity matrices for PERMANOVAs with habitats as a factor. Pair-wise PERMANOVA tests were then performed with the unrestricted permutation of raw data (999 permutations), Type III (partial) sum of squares and Monte Carlo tests. In case pair-wise comparisons exhibited significant differences, we checked if these differences may partially or fully be driven by the heterogeneity of multivariate dispersion. In addition, differences in the sessile invertebrate community composition were visualised by applying nMDS based on Bray–Curtis similarities. To exclude the parameter 'sampling location' as a major driver shaping biodiversity patterns, an nMDS plot based on Bray–Curtis similarities was performed (see Supplementary Fig. S3). A PERMANOVA was performed based on the similarity calculations and on Bray–Curtis similarities (incidence data), in order to test for differences between (sub-) habitats. Lastly, an area-proportional Venn diagram was constructed to describe the shared and unique phenotypes among (sub-) habitats, i.e., *P. crispa* mats, *P. oceanica* leaves and *P. oceanica* rhizomes.

Surface Area Quantification

For *P. crispa*, the wet weight of sub- and main samples (see above) was measured after taking algal material of approximately 10 g and shaking off excess water three to five times with one hand. The subsamples were then placed in a bowl on a laminated grid paper and flattened with a glass pane without overlaying thalli parts. Then, pictures were taken from the top with a 90° angle using a Canon G12 digital camera and a monopod stand (KAISER RS1) to ensure a constant distance and angle to the respective thallus. The surface area of the algae in the subsamples was then calculated from the picture using ImageJ (version 1.52)[100] and multiplied by two to consider both sides of the thalli. The surface area and enlargement factor of the main sample were then calculated as followed:

$$SA_M = \frac{WW_{MS} \times SA_{SS}}{WW_{SS}}$$

$$EF_{PC} = \frac{SA_{MS} + 0.09\ m^2}{0.09\ m^2}$$

Where WW = wet weight, SA = surface area, MS = main sample, SS = sub sample, EF = enlargement factor (i.e., 0.09 m^2 corresponds to the area of the sampling frame).

For *P. oceanica*, the commonly applied Leaf Area Index[101] was extended to include *P. oceanica* rhizomes in the surface area calculation. The surface area of *P. oceanica* was modelled using advanced geometry (sensu ref.[102]), as a cylindrical shape was assumed for the shoots and a rectangular shape for the leaves. Both length and width of the leaves were measured with a ruler. Subsequently, the number of leaves was determined at the sheath of each shoot. During additional sampling dives, shoot density was counted 16 times using a 40 x 40 (= 0.16 m^2) sampling frame. Following this, the enlargement factor was calculated according to:

$$EF_{PO} = \frac{[SA_{shoot} + (SA_{leaves} \times leaves\ /\ shoot)\,] \times shoots\ /\ m^2 + 0.16\ m^2}{0.16\ m^2}$$

For reference purposes, the surface area enlargement factor of neighbouring bare granite/hard-bottom substrates was calculated as well using a 20 cm × 20 cm × 2.9 cm PVC-frame (RA = 0.04 m^2) with ball chains (metal ball diameter = 2.4 mm) attached to at least three of the four corners of the frame. The chains served to trace the actual dimensions (diagonals and edges) of the underlying substrate enclosed by the projection of the frame's planar dimensions onto the sample surface. Metal chains were laid out from corner to corner of the frame whilst being aligned to the uneven sample surface. The ball chain link numbers up to the intersection point with the corners of the frame were counted and converted into the equivalent distance. Using these values, an estimation of the actual surface area could be calculated using Heron's formula[103] (Supplementary Method 1). This was done by calculating two triangular partial surfaces for one diagonal each. The surface area of the underlying substrate was calculated twice (1× for each diagonal), to generate a mean value as an estimate of the actual surface area. A total of 15 frames were sampled to form a mean value for the inorganic surface magnification factor of the granite substrate.

Environmental Parameters

Environmental parameters were assessed in situ at a depth of 28–30 m close to the Punta del Morto dive site (42°23'22.2"N 10°53'24.3"E; Supplementary Fig. S1) of Giglio Island in September and October 2019, where all target habitats (i.e., *P. crispa* mats of >5 cm thickness, *P. oceanica* seagrass meadows of >20 cm height, hard-bottom substrate serving as a reference habitat for environmental parameter assessments) were found less than 10 m apart from each other. Thus, all habitats likely experienced similar environmental conditions allowing a direct comparison of environmental parameters between the habitats. Oxygen concentration, pH, and in situ Chlorophyll (Chl) α-like fluorescence were obtained from Eureka Manta logger (GEO Scientific Ltd.) that recorded data at 1-min intervals. Chl α-like fluorescence was measured with an optical sensor with a light-emitting diode at an excitation wavelength of 460 nm and emission wavelength of 685 nm (resolution of 0.01 μgL^{-1} and accuracy of ±3%). Manta loggers were deployed multiple times (13× in *P. crispa* mats, 7× in *P. oceanica* meadows, 6× in reference habitat) for 2–3 days.

Water movement within the habitats was measured using clod cards[104,105]. Gypsum (Quick-mix gips, toom #3050388 $CaSO_4$) clod cards (hereafter GCC) were produced and constant dry-weighted before deployment. The GCCs were placed 1 cm above the seafloor (i.e., within the mat) and 20 cm above the mat. They were attached to a metal stick with a 90° offset (Supplementary Fig. S4) to address water movement within and above *P. crispa* mats. An identical GCC setup was used on hard-bottom substrates as a reference. Due to differences in height, the setup was adjusted for *P. oceanica* meadows, i.e., GCCs were placed 20 cm above the seafloor within the meadows, as well as 20 cm above the meadows. All setups were assembled prior to deployment and were positioned multiple times (9× in *P. crispa* mats, 4× in *P. oceanica* meadows, 2× in reference habitat) for 6–7 days in the respective habitat. Afterwards, GCCs were cautiously transported, rinsed with freshwater, and dried at 60° until they reached a final constant weight. The difference in weight prior to and post deployment was related to deployment time, resulting in weight loss d^{-1} as an indicator for the strength of the relative water movement. This allowed for relative comparisons within and among *P. crispa*, *P. oceanica* and hard-bottom substrates. To check for statistically significant differences in the water movement in *P. crispa* compared to *P. oceanica* and hardbottom substrate, an analysis of variance and subsequent post-hoc test (Tukey HSD) was performed.

Similar setups were prepared for light intensity and temperature assessments. Instead of GCC, multiple Onset HOBO Pendant Data Loggers (part #UA-002-64) were placed (10× in *P. crispa* mats, 6× in *P. oceanica* meadows, 4× in reference habitat) accordingly, recording data at 15-sec intervals for 5 consecutive days. A total of 10–18 days and respective data points covering every 15 s (in case of Onset HOBO Pendant Data Loggers) or every minute (in case of Eureka Manta Loggers) of a daily cycle were collected.

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Chapter 7 – General discussion

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Key findings

- For the first time, we show that *P. crispa* takes a role as an ecosystem engineer and has the potential to influence environmental factors and create habitat for associated biodiversity (Chapter 2)[1].
- *P. crispa* mats harbor diverse assemblages of serpulid polychaetes of similar or higher magnitude than neighboring *P. oceanica* meadows (Chapter 3)[2].
- The community of epiphytic foraminifera (>300µm) is of higher diversity and more homogenously distributed across sites on *P. crispa* mats compared to *P. oceanica* meadows in the investigated area (Chapter 4)[3].
- *P. crispa* mats provide essential habitats for bryozoan diversity, harboring a high density of bryozoan colonies and a large number of families that were not present in the *P. oceanica* reference habitat (Chapter 5)[4].
- *P. crispa* mats harbor sessile organisms across nine taxa in numbers that exceed those of neighboring *P. oceanica* meadows and other ecosystems (Chapter 6)[5].
- *P. crispa* mats are ecosystem engineers that serve as temporary refuge habitats and transitory biodiversity hotspots for sessile organisms (Chapter 6)[5].

Overview

Large and long-living sessile organisms like the seagrass *Posidonia oceanica* play a vital role in building biodiversity hotspots by providing habitat and shaping complex ecosystems[6–8]. Many hotspots in the Mediterranean Sea are declining due to environmental pressure and human impacts on the key species[9–13]. The resulting habitat losses may result in phase shifts to other habitat-forming species[9,10,14,15]. Besides protecting established hotspots, it is thus of central importance to identify and understand developing biodiversity hotspots, such as *P. crispa* mats, which have been described in some areas across the northern Mediterranean Sea[16–19] and are reported to be associated with different sessile invertebrate taxa[19–21]. Knowledge of the magnitude of associated sessile biodiversity and how these mats function as potential biodiversity hotspots remain limited. This thesis contributes to the in-depth understanding of the biodiversity associated with *P. crispa* mats and provides new insights into the influence of this red macroalga on key environmental factors.

The study area is located in the northern Tyrrhenian Sea, around the island of Giglio (Italy, N42°21'23.1", E10°54'05.5"). This location allowed us to sample and observe extensive *P. crispa* mats in close proximity to neighboring *P. oceanica* meadows as a reference habitat. The fieldwork was conducted in cooperation with our local partner institute ("Institut für Marine Biologie"; IfMB), allowing daily excursions and analysis of samples within a few hours. The investigations included extensive surveys of all sessile invertebrate taxa and foraminifera on *P. crispa* and *P. oceanica* reference material. Furthermore, we assessed and compared the effects of both habitats on key environmental parameters (water movement, temperature, light intensity, oxygen concentration, pH, and chlorophyll α). Following this outline, the presented thesis builds up knowledge on:

I) the holistic overview of sessile organisms on *P. crispa* mats across nine taxa (ascidiacea, bryozoa, cnidaria, entoprocta, foraminifera, mollusca (bivalvia), polychaeta (sedentaria), rotifera, porifera),

II) direct comparison to an established biodiversity hotspot (i.e., *P. oceanica* meadow) to evaluate the magnitude of associated biodiversity,

III) first insights into the role of *P. crispa* as ecosystem engineer and key species for associated sessile biodiversity, and

IV) the potential of *P. crispa* mat characteristics to alter environmental factors and create a refuge for diversity. In conclusion, we demonstrate that *P. crispa* mats provide a valuable habitat of similar diversity to *P. oceanica* and a potential refuge for taxa threatened by habitat loss in the neighboring seagrass meadows.

Biodiversity in Mediterranean *P. crispa* mats across phyla

Assemblages of macroalgae support high biodiversities from temperate regions[22–25] to coral reef environments[26]. While this general trait has been shown for established *P. crispa* mats in the Black Sea[27,28], recent pilot studies have confirmed high diversities of benthic polychaetes[20], molluscs[21], bryozoa[19], and other epiphytic algae[19], associated with *P. crispa* mats spreading in the Mediterranean Sea. However, a comprehensive overview of the biodiversity associated with Mediterranean *P. crispa* mats and their relevance compared to established hotspots has been missing. Especially benthic suspension feeders play a paramount role in marine food webs by transferring particulate organic matter from the water column into benthic communities[29,30]. Therefore, a central part of this thesis was an extensive sampling campaign to provide a comparative biodiversity assessment of sessile organisms in *P. crispa* mats and neighboring *P. oceanica* meadows (Chapter 6), including spotlights on essential taxa: I) serpulid polychaetes (Chapter3), II) epiphytic foraminifera (Chapter 4), and III) bryozoa (Chapter 5).

The serpulid polychaetes' overall abundance, species richness, diversity, and evenness were significantly higher in *P. crispa* samples compared to *P. oceanica*. Exceptions were *P. oceanica* shoots, where similar densities of individuals were found compared to *P. crispa* mats. The species composition of these two (sub-) habitats was also similar, with the same dominant species (*Josephalla marenzelleri*) and lower numbers of the photophilic Spirorbinae (e.g., *Janua* spp.)[31] compared to the *P. oceanica* leaf stratum. The distribution of these pioneer species[32,33] underlines that *P. crispa* mats composed of prostrate and erect thalli (heterotrichous species)[19] constitute a sheltered and long-lived habitat rather than oscillating structures of shallow algae or *P. oceanica* leaves. Overall, *P. crispa* mats harbor a rich serpulid assemblage comparable to or exceeding other neighboring biodiversity hotspots (i.e., *P. oceanica* meadows). Furthermore, two taxa (*Vermiliopsis* spp. and *Bathyvermilia* sp.) were solely found on *P. crispa* during this study (Chapter 3).

The foraminiferal assemblages represented a typical composition for carbonated shelf environments[34]. Our results showed that the abundance of foraminifera was higher in *P. crispa* mats than in both *P. oceanica* sub-habitats (i.e., shoots and leaves). A similar but more pronounced trend was confirmed for the species richness. This observation is strengthened by previous studies finding lower values for foraminiferal diversity on seagrass[35]. Additionally, the analysis of species incidences showed that our sampling efforts might underestimate the total diversity of foraminiferal communities in *P. crispa* mats. We conclude that the community of epiphytic foraminifera is of higher diversity in *P. crispa* mats and is more homogeneously distributed across sites and replicates than in *P. oceanica* sub-habitats (Chapter 4).

The diversity of the bryozoan community show differences among the three (sub-) habitats (i.e., *P. crispa* mats, *P. oceanica* leaves, and – shoots), with a clear trend of higher abundances inside the *P. crispa mats*. The results have shown that the bryozoan assemblages in *P. crispa* mats and on *P. oceanica* shoots are similar concerning the chosen diversity descriptors (Density of individuals per m^2, Number of families, Shannon index, Pielou Index). In contrast, our further analysis highlights the differences according to the composition of the assemblages. Overall, *P. crispa* mats showed high bryozoan diversity by harboring high densities of bryozoan colonies and a large number of families that were not present in the *P. oceanica* reference habitat (Chapter 5).

We recorded 312 distinct sessile organism phenotypes across nine higher taxa for *P. crispa* and *P. oceanica*; 223 occurred in *P. crispa* mats and 179 in *P. oceanica* holobionts, respectively. All (sub-) habitats accommodated distinct communities, with 133, 21, and 18 phenotypes uniquely found in *P. crispa* mats, *P. oceanica* leaves, and *P. oceanica* rhizomes, respectively[5]. Approximately 25% more phenotypes were found in *P. crispa* mats than in the neighboring *P. oceanica* seagrass meadows. Thus, our data have identified *P. crispa* as a habitat that harbors more even and diverse sessile invertebrate communities than neighboring *P. oceanica* meadows. Calculations of diversity indices further endorsed *P. crispa* as a hotspot of sessile invertebrate diversity comparable to traditional biodiversity hotspots such as Mediterranean coralligenous reefs (Chapter 6).

P. crispa serving as an ecosystem engineer and potential refuge for threatened biodiversity

The term "ecosystem engineer" is often associated with large-scale bioengineers, such as corals, mangroves, and seagrasses, which create extensive habitats[36–38]. However, scale is not the appropriate defining parameter[39,40]. Bivalves, such as mussels or oysters, are well-recognized structural bioengineers that form dense beds or reefs[41,42], which create small-sized habitats and act mainly on a local scale[43]. Their shells alter the local water flow regime[44], local sediment dynamics[45] and provide a substrate for colonization[45]. Our data shows that *P. crispa* influences its environment similarly, i.e., by reducing water movement[1,5] and creating habitat[5]. Despite reaching only about 15 cm in height[1], this red alga acts as a small-scale ecosystem engineer, forming dense but thin mats that cover up to 10,000 km^2 [28] (Chapter 2). These dense mats consist of complex thalli that provide a high surface area relative to the small volume[5], and thus offer substrate and microhabitats for a diverse benthic community[19,28]. For the first time, we also confirmed gradients in physio- chemical parameters (i.e., oxygen concentrations, light availability, pH, temperature, and chlorophyll α concentration) in *P. crispa* mats similar to neighboring *P. oceanica* meadows which confirms *P. crispa*'s functioning as small-scale ecosystem engineer[5] (Chapter 6).

The high biodiversity associated with the *P. crispa* mats may positively impact sessile benthic communities in neighboring *P. oceanica* seagrass meadows[46], which is reflected by a total of 90 shared phenotypes in the investigated habitats[5]. While *P. crispa* is relatively short-lived compared to *P. oceanica*[28,47], it is reported that *P. crispa* aggregations may be dislodged, translocated, and in some cases, thrive in an unattached growth form on sediments[27,28]. Drifting algae parts may offer substrate to diverse sessile communities[24,48] and function as a transport vector over large distances[23]. The translocation of algae material may have consequences for associated biodiversity through two pathways: (I) translocated *P. crispa* may still provide habitat for associated sessile invertebrates; or (II) *P. crispa* mats are severely damaged, losing their function as biodiversity hotspots. In both cases, *P. crispa* mats serve as ecosystem engineers forming temporary refuge habitats and transitory biodiversity hotspots. In case I), *P. crispa* mats may function as a temporary biodiversity refuge, supporting the recolonization of recovered seagrass meadows[49,50] by maintaining parts of their sessile biodiversity[46,51] (Chapter 6).

Synoptic answers to the specific research questions

Research question 1: What are the abundances and phenotype richness of sessile organisms inside *P. crispa* mats compared to *P. oceanica* meadows, and can *P. crispa* mats be considered biodiversity hotspots?

The calculated abundances (mean number of individuals (ind) per m^2; colonies of colonial species were considered individuals) suggest that *P. crispa* mats provide valuable habitat for sessile organisms. Our data showed ~64,008 ind m^{-2} associated with *P. crispa* mats, which was three times more than on *P. oceanica* holobionts (~19,535 ind m^{-2}). The most abundant taxa were bryozoa, foraminifera, and serpulid polychaetes (Chapters 3 and 6). While *P. crispa* mats harbored a great abundance of bryozoa (~44,222 ind m^{-2}), both bryozoa and foraminifera were equally abundant on *P. oceanica*. *P. crispa* harbored a similar number of phenotypes of bryozoa and foraminifera (76 and 81), whereas the number of bryozoan phenotypes exceeded that of Foraminifera in *P. oceanica* (78 and 52; Chapter 4 and 5). The abundance of serpulid polychaetes was similar in *P. crispa* mats (~5665 ind m^{-2}) compared to neighboring *P. oceanica* meadows (~2304 ind m^{-2} leaves and ~5890 ind m^{-2} shoots). The number of serpulid polychaete phenotypes was significantly higher in *P. crispa* mats (~7) compared to *P. oceanica* beds (~2; Chapter 3). Total phenotype richness found on *P. crispa* mats (~234 phenotypes) exceeded those on *P. oceanica* rhizomes (~142) and leaves (~102). In addition, *P. crispa* mats harbored more even and diverse sessile invertebrate communities than neighboring *P. oceanica* meadows (Chapter 6). These findings, in combination with a relatively short lifespan and missing data on the distribution of *P. crispa* in the Mediterranean Sea, lead to the conclusion that *P. crispa* mats may temporarily act as transitory biodiversity hotspots[5] (Chapter 6).

Research question 2: What are the effects of *P. crispa* mats on water parameters compared to the overlaying water body, and does *P. crispa* fulfill ecosystem engineer functions?

Our findings revealed that *P. crispa* significantly reduced water movement by 41 % compared to the overlying water column, while water movement was reduced by 25 % in *P. oceanica* meadows. Surprisingly, *P. crispa* increased the water temperature by 0.3 °C compared to the water column, while the water temperature in *P. oceanica* and on bare rocky substrates was reduced by 0.5 °C (Chapter 2). Light intensity inside the red algae mats was decreased significantly by 69 % compared to the water column. This reduction was similar in *P. oceanica* (77 %; Chapters 2 and 6). Daily dissolved oxygen fluctuations inside *P. crispa* mats (7.73 – 8.14 mg l^{-1}) were similar to those of *P. oceanica* (7.59 – 8.04 mg l^{-1}), with the daily mean of oxygen concentrations being slightly higher in *P. crispa* (7.99 mg l−1) compared to those of *P. oceanica* (7.75 mg l^{-1}). The average pH within *P. crispa* mats was lower (8.44) compared to *P. oceanica* meadows (8.64), which resembled the observed differences in O_2 concentrations. A high surface area provided by complex thalli of *P. crispa* relative to a small volume (mats of < 15 cm thickness) resulted in an enlargement factor of 4.9, which was lower than for *P. oceanica* (both leaves (7.3) and whole *P. oceanica* plants (8.3) but higher than for *P. oceanica* rhizomes (2.0); Chapter 6).

These findings highlight the strong influence of *P. crispa* red algae mats on key environmental factors. Together with the significant surface area enlargement[1,5] (Chapters 2 and 6), environmental parameters shaped by *P. crispa*, its wide distribution[19,27,52,53], and the comparative biodiversity analysis[2–5] (Chapters 3 – 6), algae mats formed by *P. crispa* may function as overlooked ecosystem engineers.

Research question 3: Can *P. crispa* mats potentially serve as a biodiversity refuge in times of biodiversity loss and degradation of established hotspots in the Mediterranean Sea?

We show that the biodiversity of sessile communities in *P. crispa* mats is high and exceeds that of neighboring seagrass meadows[2–5]. Comparative analysis of biodiversity indices in calcifying green algae habitats and biodiversity hotspots like coral reefs or mangrove forests showed similar or higher values compared to our results[5]. A potential interaction between *P. oceanica* meadows and *P. crispa* mats is indicated by a total of 90 shared phenotypes across major taxa found in all habitats during this study[2-5] (Chapters 3 – 6).

Our findings further highlight the strong influence of *P. crispa* mats on key environmental factors, with an impact similar to or even higher than for the well-known ecosystem engineer *P. oceanica*[1,5] (Chapters 2 and 6). These environmental gradients may be a factor that facilitates associated biodiversity, similarly as described for *P. oceanica*[54–56]. These arguments suggest that *P. crispa* mats and their associated sessile communities potentially aid in reviving classical marine benthic biodiversity hotspots in the Mediterranean Sea once threats are reduced or removed[57–59] (Chapter 6).

Perspective and future research directions

The present thesis summarizes the value of *P. crispa* as a mat-forming macroalga, temporary bioengineering species, and transitory biodiversity hotspot. The recently adopted EU Biodiversity strategy for 2030 aims to protect and restore habitats of significant biodiversity to mitigate effects of, e.g., regional human impact and climate change in terms of biodiversity loss[60]. Our results in Chapters 3 – 6, in line with previous studies on Mediterranean *P. crispa* mats[16,19,21,27,61], as well as the successful implementation of a marine protected area covering “Zernov’s Phyllophora field” in the Black Sea[62] suggest that this habitat hosts a considerably high diversity of sessile organisms and may act as a transitory refuge for threatened biodiversity of neighboring habitats. The sessile filtrating organisms we focused on represent an important group by transferring food energy from pelagic to benthic habitats[29,30]. Bryozoans – the most abundant phylum in this study (Chapter 5) – attract a wide range of predators, from invertebrates to fish and from incidental to specialized species[63–66]. Thus, further research is needed to assess the role of *P. crispa* as a potential biodiversity hotspot and refuge habitat for higher trophic levels, from mobile benthic invertebrates to demersal fish (i.e., groundfish).

We showed that drifting *P. crispa* material is one possible pathway to recolonizing other recovered biodiversity hotspots (e.g., *P. oceanica* meadows; Chapter 6). It remains unclear which of the associated organisms tolerate drifting periods and, thus, what the limitations are in terms of the transferable biodiversity of this pathway. The differences in the community composition of recolonized *P. oceanica* meadows compared to their initial state need to be further investigated, and whether this function applies to more taxa beyond the scope of this study.

While this thesis substantially adds to the previous observations of *P. crispa* mats in the Mediterranean Sea[16,19,21,27,61], comprehensive information on the supraregional distribution is still missing. Future research about mat occurrences along the coastline and their local extension is therefore needed to gain more insights into the connectivity of *P. crispa* mats and their relevance across the Mediterranean Sea.

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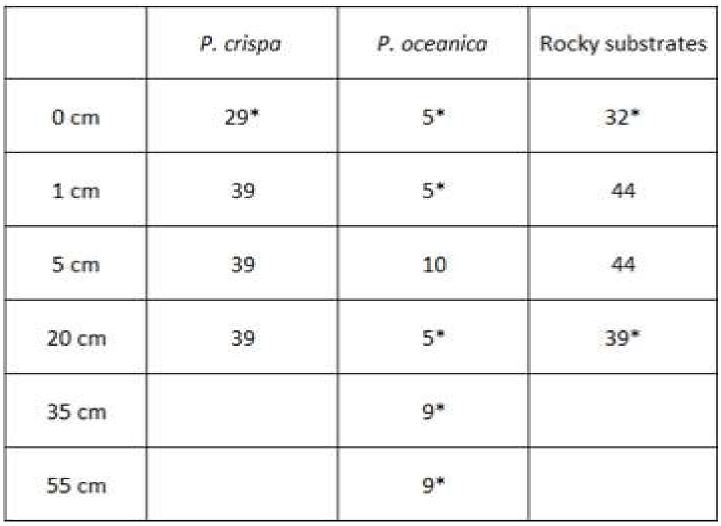
Appendix – Supplementary material

Chapter 2

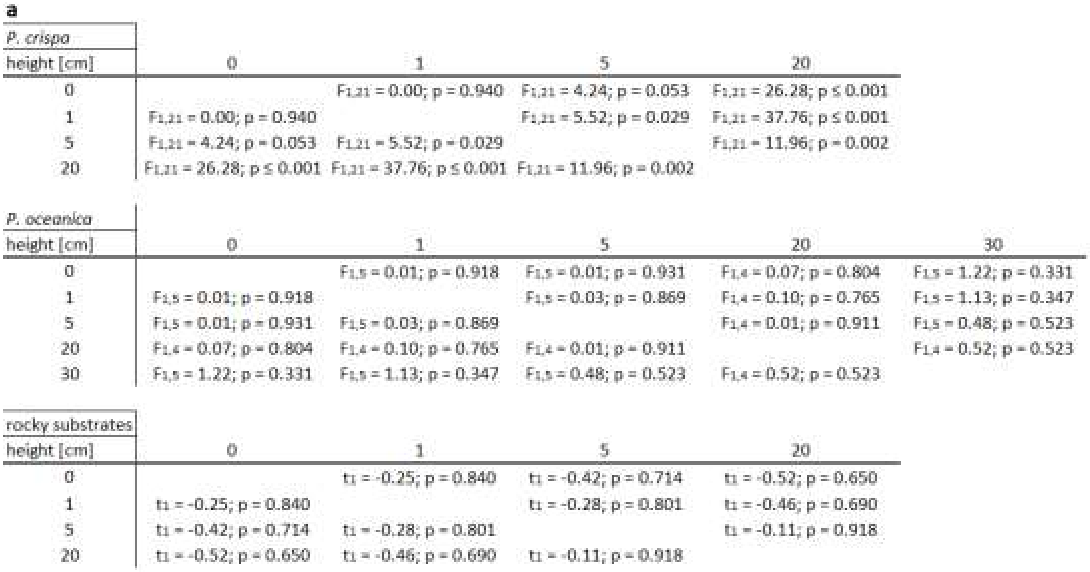
SM 1 - Supplementary Tables

Table S1: *Replication of daily temperature and light cycles sampled at different positions above the seafloor in Phyllophora crispa, Posidonia oceanica and on rocky substrates. * = less cycles sampled due to technical issues.*



	P. crispa	*P. oceanica*	Rocky substrates
0 cm	29*	5*	32*
1 cm	39	5*	44
5 cm	39	10	44
20 cm	39	5*	39*
35 cm		9*	
55 cm		9*	

Table S2: *Detailed statistical test results for (a) water movement data, (b) temperature data, and (c) light intensity data observed in Phyllophora crispa mats, Posidonia oceanica meadows, and on rocky substrates recorded at different heights above the seafloor.*

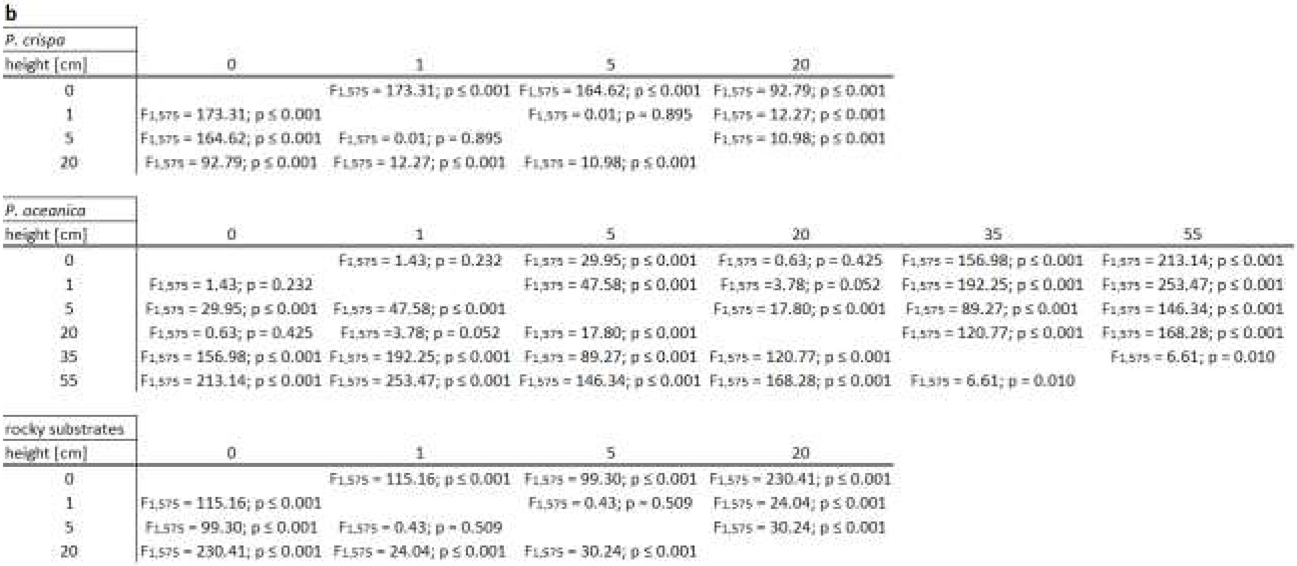


a

P. crispa				
height [cm]	0	1	5	20
0		$F_{1,21} = 0.00; p = 0.940$	$F_{1,21} = 4.24; p = 0.053$	$F_{1,21} = 26.28; p \leq 0.001$
1	$F_{1,21} = 0.00; p = 0.940$		$F_{1,21} = 5.52; p = 0.029$	$F_{1,21} = 37.76; p \leq 0.001$
5	$F_{1,21} = 4.24; p = 0.053$	$F_{1,21} = 5.52; p = 0.029$		$F_{1,21} = 11.96; p = 0.002$
20	$F_{1,21} = 26.28; p \leq 0.001$	$F_{1,21} = 37.76; p \leq 0.001$	$F_{1,21} = 11.96; p = 0.002$	

P. oceanica					
height [cm]	0	1	5	20	30
0		$F_{1,5} = 0.01; p = 0.918$	$F_{1,5} = 0.01; p = 0.931$	$F_{1,4} = 0.07; p = 0.804$	$F_{1,5} = 1.22; p = 0.331$
1	$F_{1,5} = 0.01; p = 0.918$		$F_{1,5} = 0.03; p = 0.869$	$F_{1,4} = 0.10; p = 0.765$	$F_{1,5} = 1.13; p = 0.347$
5	$F_{1,5} = 0.01; p = 0.931$	$F_{1,5} = 0.03; p = 0.869$		$F_{1,4} = 0.01; p = 0.911$	$F_{1,5} = 0.48; p = 0.523$
20	$F_{1,4} = 0.07; p = 0.804$	$F_{1,4} = 0.10; p = 0.765$	$F_{1,4} = 0.01; p = 0.911$		$F_{1,4} = 0.52; p = 0.523$
30	$F_{1,5} = 1.22; p = 0.331$	$F_{1,5} = 1.13; p = 0.347$	$F_{1,5} = 0.48; p = 0.523$	$F_{1,4} = 0.52; p = 0.523$	

rocky substrates				
height [cm]	0	1	5	20
0		$t_1 = -0.25; p = 0.840$	$t_1 = -0.42; p = 0.714$	$t_1 = -0.52; p = 0.650$
1	$t_1 = -0.25; p = 0.840$		$t_1 = -0.28; p = 0.801$	$t_1 = -0.46; p = 0.690$
5	$t_1 = -0.42; p = 0.714$	$t_1 = -0.28; p = 0.801$		$t_1 = -0.11; p = 0.918$
20	$t_1 = -0.52; p = 0.650$	$t_1 = -0.46; p = 0.690$	$t_1 = -0.11; p = 0.918$	



b

P. crispa				
height [cm]	0	1	5	20
0		$F_{1,575} = 173.31; p \leq 0.001$	$F_{1,575} = 164.62; p \leq 0.001$	$F_{1,575} = 92.79; p \leq 0.001$
1	$F_{1,575} = 173.31; p \leq 0.001$		$F_{1,575} = 0.01; p = 0.895$	$F_{1,575} = 12.27; p \leq 0.001$
5	$F_{1,575} = 164.62; p \leq 0.001$	$F_{1,575} = 0.01; p = 0.895$		$F_{1,575} = 10.98; p \leq 0.001$
20	$F_{1,575} = 92.79; p \leq 0.001$	$F_{1,575} = 12.27; p \leq 0.001$	$F_{1,575} = 10.98; p \leq 0.001$	

P. aceanica						
height [cm]	0	1	5	20	35	55
0		$F_{1,575} = 1.43; p = 0.232$	$F_{1,575} = 29.95; p \leq 0.001$	$F_{1,575} = 0.63; p = 0.425$	$F_{1,575} = 156.98; p \leq 0.001$	$F_{1,575} = 213.14; p \leq 0.001$
1	$F_{1,575} = 1.43; p = 0.232$		$F_{1,575} = 47.58; p \leq 0.001$	$F_{1,575} =3.78; p = 0.052$	$F_{1,575} = 192.25; p \leq 0.001$	$F_{1,575} = 253.47; p \leq 0.001$
5	$F_{1,575} = 29.95; p \leq 0.001$	$F_{1,575} = 47.58; p \leq 0.001$		$F_{1,575} = 17.80; p \leq 0.001$	$F_{1,575} = 89.27; p \leq 0.001$	$F_{1,575} = 146.34; p \leq 0.001$
20	$F_{1,575} = 0.63; p = 0.425$	$F_{1,575} =3.78; p = 0.052$	$F_{1,575} = 17.80; p \leq 0.001$		$F_{1,575} = 120.77; p \leq 0.001$	$F_{1,575} = 168.28; p \leq 0.001$
35	$F_{1,575} = 156.98; p \leq 0.001$	$F_{1,575} = 192.25; p \leq 0.001$	$F_{1,575} = 89.27; p \leq 0.001$	$F_{1,575} = 120.77; p \leq 0.001$		$F_{1,575} = 6.61; p = 0.010$
55	$F_{1,575} = 213.14; p \leq 0.001$	$F_{1,575} = 253.47; p \leq 0.001$	$F_{1,575} = 146.34; p \leq 0.001$	$F_{1,575} = 168.28; p \leq 0.001$	$F_{1,575} = 6.61; p = 0.010$	

rocky substrates				
height [cm]	0	1	5	20
0		$F_{1,575} = 115.16; p \leq 0.001$	$F_{1,575} = 99.30; p \leq 0.001$	$F_{1,575} = 230.41; p \leq 0.001$
1	$F_{1,575} = 115.16; p \leq 0.001$		$F_{1,575} = 0.43; p = 0.509$	$F_{1,575} = 24.04; p \leq 0.001$
5	$F_{1,575} = 99.30; p \leq 0.001$	$F_{1,575} = 0.43; p = 0.509$		$F_{1,575} = 30.24; p \leq 0.001$
20	$F_{1,575} = 230.41; p \leq 0.001$	$F_{1,575} = 24.04; p \leq 0.001$	$F_{1,575} = 30.24; p \leq 0.001$	

c

P. crispa				
height [cm]	0	1	5	20
0		$F_{1,241} = 136.39; p \le 0.001$	$F_{1,241} = 6775.93; p \le 0.001$	$F_{1,241} = 8814.44; p \le 0.001$
1	$F_{1,241} = 136.39; p \le 0.001$		$F_{1,241} = 19754.14; p \le 0.001$	$F_{1,241} = 30849.27; p \le 0.001$
5	$F_{1,241} = 6775.93; p \le 0.001$	$F_{1,241} = 19754.14; p \le 0.001$		$F_{1,241} = 2158.29; p \le 0.001$
20	$F_{1,241} = 8814.44; p \le 0.001$	$F_{1,241} = 30849.27; p \le 0.001$	$F_{1,241} = 2158.29; p \le 0.001$	

P. oceanica						
height [cm]	0	1	5	20	35	55
0		$F_{1,241} = 0.09; p = 0.754$	$F_{1,241} = 60.09; p \le 0.001$	$F_{1,241} = 529.79; p \le 0.001$	$F_{1,241} = 3199.98; p \le 0.001$	$F_{1,241} = 12286.68; p \le 0.001$
1	$F_{1,241} = 0.09; p = 0.754$		$F_{1,241} = 106.83; p \le 0.001$	$F_{1,241} = 572.09; p \le 0.001$	$F_{1,241} = 3978.46; p \le 0.001$	$F_{1,241} = 22022.70; p \le 0.001$
5	$F_{1,241} = 60.09; p \le 0.001$	$F_{1,241} = 106.83; p \le 0.001$		$F_{1,241} = 855.33; p \le 0.001$	$F_{1,241} = 6257.48; p \le 0.001$	$F_{1,241} = 87562.47; p \le 0.001$
20	$F_{1,241} = 529.79; p \le 0.001$	$F_{1,241} = 572.09; p \le 0.001$	$F_{1,241} = 855.33; p \le 0.001$		$F_{1,241} = 193.47; p \le 0.001$	$F_{1,241} = 852.49; p \le 0.001$
35	$F_{1,241} = 3199.98; p \le 0.001$	$F_{1,241} = 3978.46; p \le 0.001$	$F_{1,241} = 6257.48; p \le 0.001$	$F_{1,241} = 193.47; p \le 0.001$		$F_{1,241} = 610.87; p \le 0.001$
55	$F_{1,241} = 12286.68; p \le 0.001$	$F_{1,241} = 22022.70; p \le 0.001$	$F_{1,241} = 87562.47; p \le 0.001$	$F_{1,241} = 852.49; p \le 0.001$	$F_{1,241} = 610.87; p \le 0.001$	

rocky substrates				
height [cm]	0	1	5	20
0		$F_{1,241} = 725.74; p \le 0.001$	$F_{1,241} = 8165.84; p \le 0.001$	$F_{1,241} = 21853.20; p \le 0.001$
1	$F_{1,241} = 725.74; p \le 0.001$		$F_{1,241} = 1491.29; p \le 0.001$	$F_{1,241} = 4476.24; p \le 0.001$
5	$F_{1,241} = 8165.84; p \le 0.001$	$F_{1,241} = 1491.29; p \le 0.001$		$F_{1,241} = 2274.83; p \le 0.001$
20	$F_{1,241} = 21853.20; p \le 0.001$	$F_{1,241} = 4476.24; p \le 0.001$	$F_{1,241} = 2274.83; p \le 0.001$	

SM 2 - Supplementary Figures

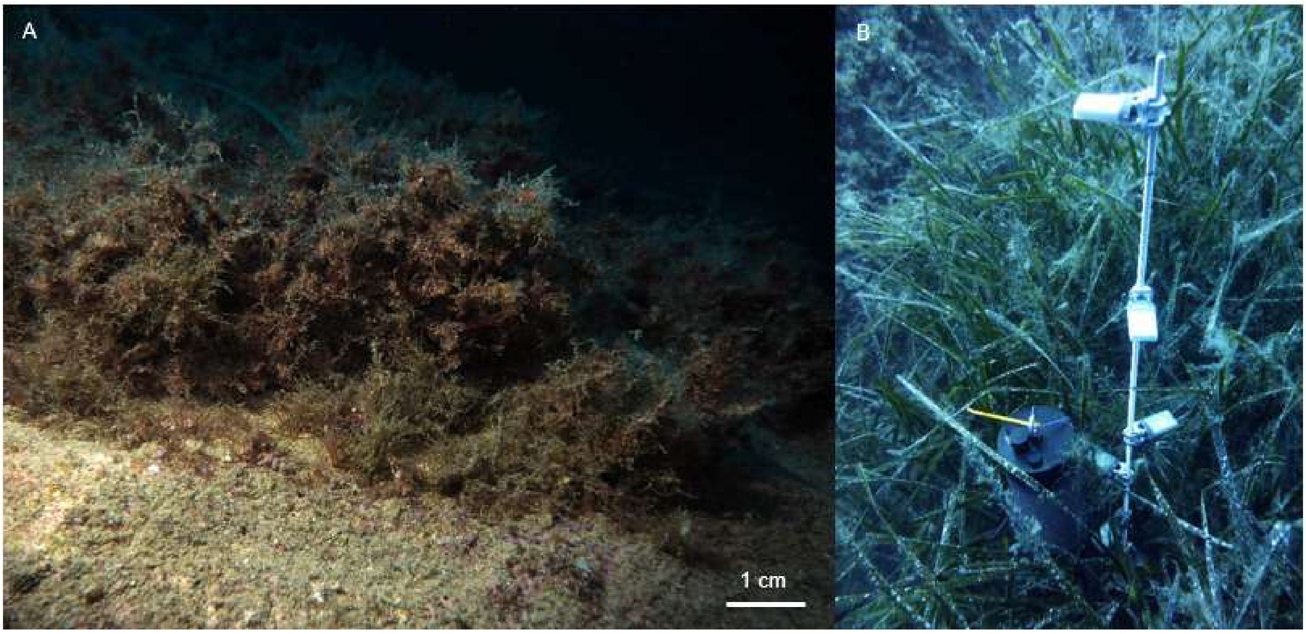


Figure S1: *The three investigated habitats at the study site (A) Phyllophora crispa mat and rocky substrate, (B) Posidonia oceanica meadow.*

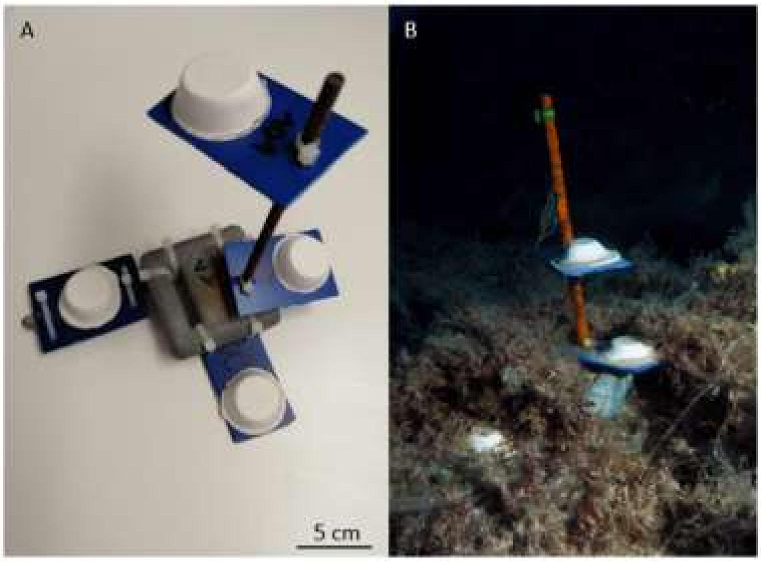


Figure S2: *Clod-card set-up (A) ex situ and (B) in situ in Phyllophora crispa first day of water exposure.*

Chapter 3

SM 1 – Calculation of sample completeness

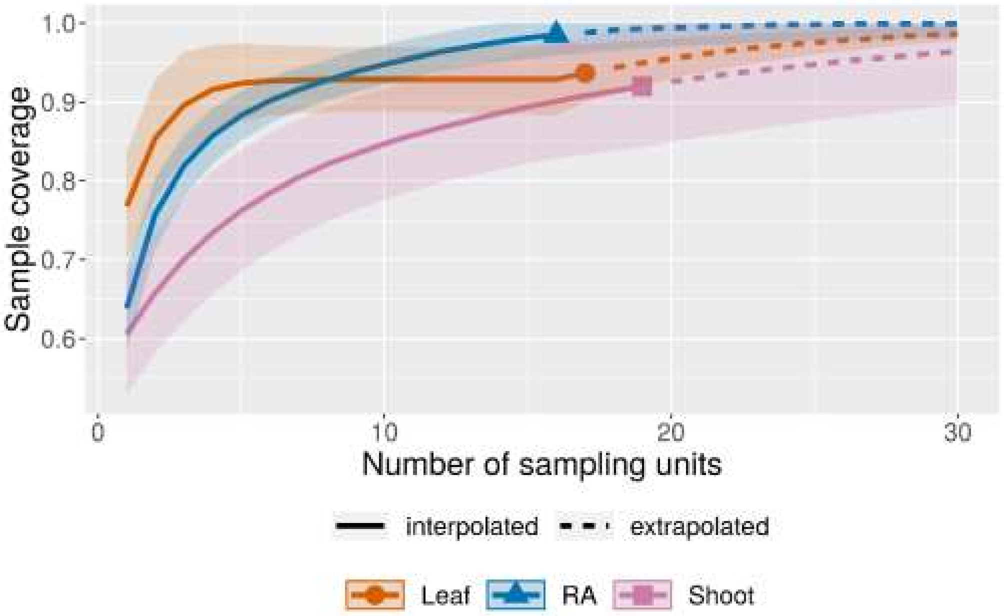


Figure S1: *Sample completeness curve based on incidence data according to Chao et al. (2016)* [1]*. Number of bootstraps used: 500; level of confidence: 0.95. RA = P. crispa mats, Leaf = P. oceanica leaves, Shoot = P. oceanica shoots with sample coverages (C.hat) of 0.98, 0.93 and 0.92, respectively.*

SM 2 – Identification literature

Table S1: *Literature used for identification of species.*

Author(s)	Year	Title
Riedl, R.	2011	Fauna und Flora des Mittelmeeres
Stresemann, E.	1992	Wirbellose
Hayward, P. J. and Ryland, J. S.	1999	Handbook of the Marine Fauna of North-West Europe
Bianchi et al.	1981	Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane
Ten Hove et al.	2009	Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs
Zibrowius H.	1968	Etude morphologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille
Zibrowius H.	1972	Mise au point sur les especes mediterraneennes de Serpulidae (Annelida Polychaeta) déecrites par Stefano delle Chiaje (1822–1829, 1841–1844) et Oronzio Gabriele Costa (1861)

SM 3 – Results of univariate statistics

Table S2: *Results of univariate tests (Wilcoxon–Mann–Whitney) among P. crispa sites (ns = p > 0.05; * = 0.01 < p < 0.05).*

	Group 1	Group 2	*p*-Value	*p*-Signif
Density	Site Mix	Site PC1	0.34	ns
	Site Mix	Site PC2	0.34	ns
	Site Mix	Site PC3	0.69	ns
	Site PC1	Site PC2	0.69	ns
	Site PC1	Site PC3	0.34	ns
	Site PC2	Site PC3	0.20	ns
No of taxa	Site Mix	Site PC1	0.278	ns
	Site Mix	Site PC2	0.069	ns
	Site Mix	Site PC3	1.000	ns
	Site PC1	Site PC2	0.766	ns
	Site PC1	Site PC3	0.454	ns
	Site PC2	Site PC3	0.306	ns
Shannon index	Site Mix	Site PC1	0.486	ns
	Site Mix	Site PC2	0.686	ns
	Site Mix	Site PC3	0.029	*
	Site PC1	Site PC2	0.200	ns
	Site PC1	Site PC3	0.029	*
	Site PC2	Site PC3	0.029	*
Pielou index	Site Mix	Site PC1	0.057	ns
	Site Mix	Site PC2	0.886	ns
	Site Mix	Site PC3	0.029	*
	Site PC1	Site PC2	0.200	ns
	Site PC1	Site PC3	0.029	*
	Site PC2	Site PC3	0.029	*

SM 4 – Formulas

Formula (S4.1): Calculation of serpulid individuals on *P. crispa* per m² seafloor (Ind_{SF}) from individuals per m² substrate (Ind_{SS}), using wet weights (WW) and surface area of the sub sample (SA_{SS}) (0.09 m² corresponds to the size of the sampling frame:

$$Ind_{SF} = \frac{\frac{WW_{MS} \times SA_{SS}}{WW_{SS}}}{SA_{SS}} * Ind_{SS} * \frac{1\ m^2}{0.09\ m^2} \qquad \text{(S4.1)}$$

Formula (S4.2): Calculation of serpulid individuals on *P. oceanica* leaves per m² seafloor using the average leaf surface area (SA_{leafAVG}), average number of leaves per m² (162), surface area of investigated leaf sample (SA_{leafSS}) and individual count per m² substrate (Ind_{SS}):

$$Ind_{SF} = \frac{162 \times SA_{leafAVG}}{SA_{leafSS}} * Ind_{SS} \tag{S4.2}$$

Formula (S4.3): Calculation of serpulid individuals on *P. oceanica* shoots per m² seafloor using the shoot surface area (SA_{shootSS}), average number of shoots per m² (40.5) and individual count per m² substrate (Ind_{SS}):

$$Ind_{SF} = \frac{40.5 \times SA_{shootSS}}{SA_{shootSS}} * Ind_{SS} \tag{S4.3}$$

SM 5 – Data availability

The dataset generated for this study is publicly available in the PANGEA repository (www.pangaea.de): https://doi.org/10.1594/PANGAEA.936571

SM 6 – References

1. Chao A, Chiu C-H. Nonparametric Estimation and Comparison of Species Richness. *eLS John Wiley Sons, Ltd Chichester*. Published online 2016. doi:10.1002/9780470015902.a0026329

Chapter 4

SM 1 – Formulas

Formula A1: Calculation of the Shannon index (*H’*), using the proportion of each individual taxon $p_i = n/N$, where *n* = the number of individuals of a given taxon and *N* = the total number of individuals per sample.

$$H' = \sum_{i=0}^{R} p_i \ln p_i \qquad \text{(A1)}$$

Formula A2: Calculation of the Pielou index (*J*), using the Shannon index (*H’*), and the total number of taxa (*S*).

$$J = H'/\ln S \qquad \text{(A2)}$$

SM 2 – Abundance Data

Table A1: Abundance of LBF species in P. oceanica sites and sub-habitats. Numbers are average abundance per m² substrate (AVG) ± standard deviation (STDEV).

	Leaves Site Mix		**Leaves Site PO**		**Shoots Site Mix**		**Shoots Site PO**	
Species	**AVG**	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**
Vertebralina striata	0	0	0	0	0	0	105	151
Peneroplis pertusus	0	0	0	0	92	177	41	82
Sorites orbiculus	0	0	73	160	0	0	0	0

Table A2: Abundance of LBF species in P. crispa sites. Numbers are average abundance per m² substrate (AVG) ± standard deviation (STDEV).

	Site PC1		**Site PC2**		**Site PC3**		**Site Mix**	
Species	**AVG**	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**
Peneroplis pertusus	92	132	272	272	49	49	200	324
Peneroplis planatus	0	0	17	30	31	54	20	34
Sorites orbiculus	0	0	41	72	0	0	10	17

SM 3 – Calculation of sample completeness

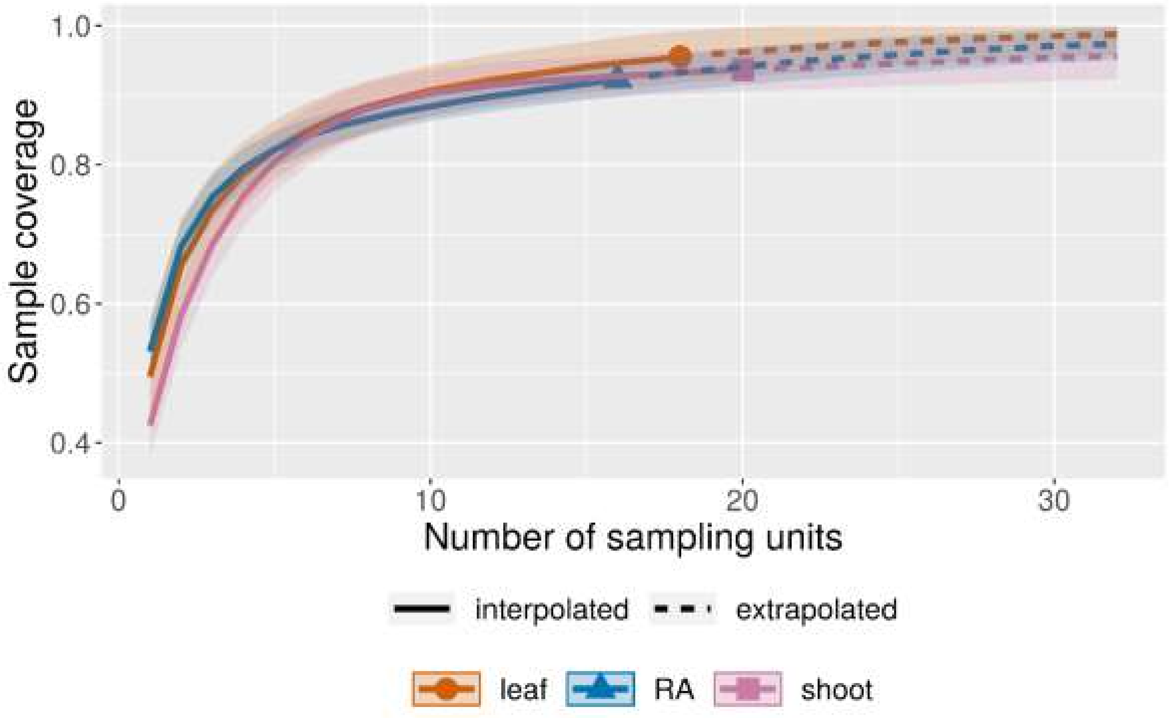


Figure A1. Sample completeness based on species incidence data according to Chao et al. (2016) [1]. Number of bootstraps used: 500; level of confidence: 0.95. Habitats with sample coverage (C.hat): "leaf" = Posidonia oceanica leaves (0.95), "RA" = Phyllophora crispa mats (0.92), "shoot" = Posidonia oceanica shoots (0.94).

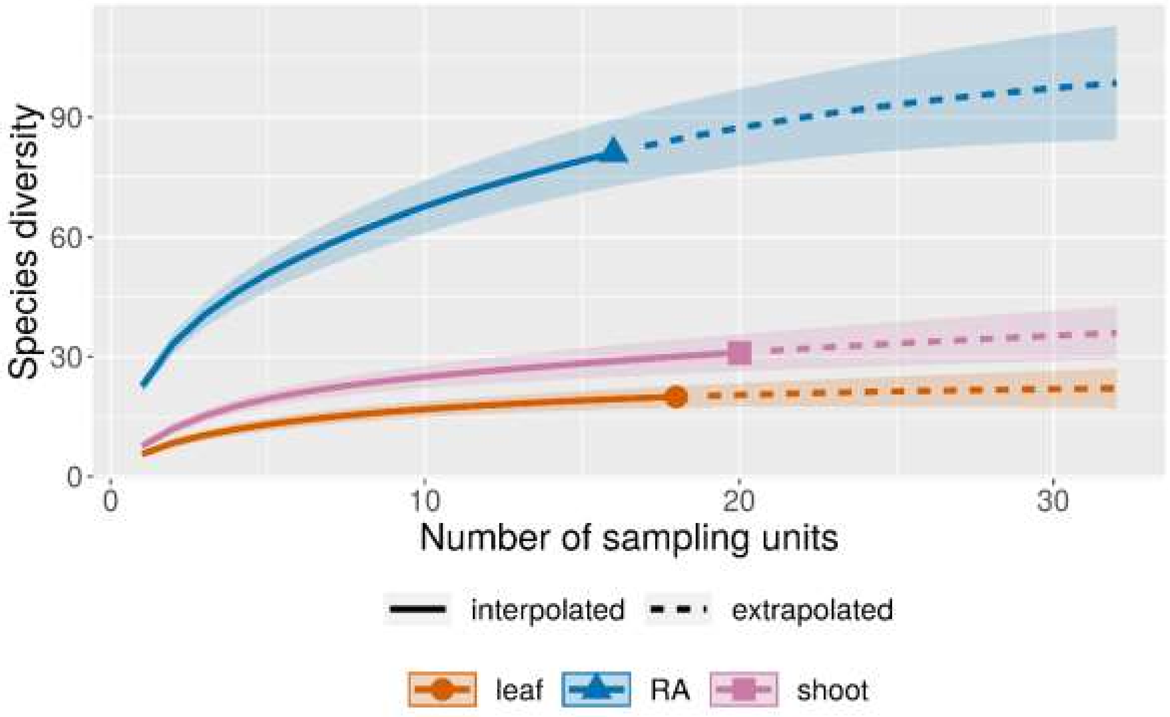


Figure A2. Species diversity extrpolated on number of sampling units, following the method of Chao et al. (2016) [1]. Number of bootstraps used: 500; level of confidence: 0.95. Habitats: "leaf" = Posidonia oceanica leaves, "RA" = Phyllophora crispa mats, "shoot" = Posidonia oceanica shoots.

SM 4 – Data availability

The Dataset, including a complete species list, supporting the conclusions of this study is available in the "PANGAEA" online repository: https://doi.org/10.1594/PANGAEA.936751

SM 5 – References

2. Chao A, Chiu C-H. Nonparametric Estimation and Comparison of Species Richness. *eLS John Wiley Sons, Ltd Chichester*. Published online 2016. doi:10.1002/9780470015902.a0026329

Chapter 5

SM 1 – Supplementary Figures

Figure A1: *Diver assessing Phyllophora crispa mat on site PC2. The picture shows the typical closed coverage of Phyllophora crispa across the rocky surface of all sampling sites (Picture: F. I. Rossbach).*

SM 2 – Results of statistical tests

Table A1: *Results of permutational multivariate analysis of variance (PERMANOVA) of bryozoan communities among P. crispa sites, and pairwise comparison (Tukey's honestly significant difference (HSD) test) of sites and (sub-) habitats. Significant results (p < 0.05) are indicated in bold.*

PERMANOVA Broyzoan Assemblages *P. crispa* All Sites

source	Df	SS	R2	F	*p*
Site	3	0.9441	0.40007	2.6674	0.018
Residual	12	1.4158	0.59993		
Total	15	2.3599	1		

PERMANOVA Broyzoan assemblages *P. crispa*, northern sites

source	Df	SS	R2	F	*p*
Site	2	0.35393	0.2497	1.4976	0.192
Residual	9	1.06348	0.7503		
Total	11	1.41741	1		

Pairwise comparison all sites

pairs		Df	SS	F	R2	*p*	*p* adj
SiteMix	SitePC3	1	0.258842	2.391555	0.284995	0.097	0.582
SiteMix	SitePC2	1	0.295435	2.6252	0.304364	0.109	0.654
SiteMix	SitePC1	1	0.127757	1.11843	0.157118	0.359	1
SitePC3	SitePC2	1	0.685668	5.632692	0.484212	0.029	0.174
SitePC3	SitePC1	1	0.412804	3.344677	0.357923	0.09	0.54
SitePC2	SitePC1	1	0.107701	0.843212	0.123219	0.444	1

Pairwise comparison (sub-) habitats, northern sites

pairs		Df	SS	F	R2	*p*	*p* adj
P. oceanica shoot	*P. oceanica* leaf	1	3.10581	14.89658	0.287044	0.001	0.003
P. oceanica shoot	*P. crispa* mat	1	1.654007	9.524627	0.24098	0.001	0.003
P. oceanica leaf	*P. crispa* mat	1	1.923498	10.44726	0.264841	0.001	0.003

SM 3 – Identification Literature

Table A2. Literature used for species identification.

Authors	Year	Title
Ryland, J. S. & Hayward, P. J.	1977	British Anascan Bryozoans
Hayward, P. J. & Ryland, J. S.	1979	British Ascophoran Bryozoans
Hayward, P. J. & Ryland, J. S.	1985	Cyclostome Bryozoans
Hayward, P. J.	1985	Ctenostome Bryozoans
Zabala, M. & Maluquer, P.	1988	Treballs del museu de zoologia – illustrated keys for the classification of Mediterranean Bryozoa
Hayward, P. J. & Ryland, J. S.	1995	Handbook of the Marine Fauna of North-West Europe
Hayward, P. J. & Ryland, J. S.	1998	Cheilostomatous Bryozoa: Part 1 Aeteoidea - Cribrilinoidea
Hayward, P. J. & Ryland, J. S.	1999	Cheilostomatous Bryozoa: Part 2 Hippothooidae - Celleporoidae
Bedini, R.	2003	Gli animali delle praterie a Poseidonia oceanica: dai macroinvertebrati ai pesci

SM 4 – Formulas

Formula (A1): Calculation of bryozoan colonies on *P. crispa* per m² seafloor (Col_{SF}) from colonies per m² substrate (Col_{SS}), using wet weights of the main sample (WW_{MS}) and subsample (WW_{SS}), and surface area of the subsample (SA_{SS}) (0.09 m² corresponds to the size of the sampling frame):

$$Col_{SF} = \frac{\frac{WW_{MS} \times SA_{SS}}{WW_{SS}}}{SA_{SS}} \times Col_{SS} \times \frac{1\ m^2}{0.09\ m^2} \qquad \text{(A1)}$$

Formula (A2): Calculation of bryozoan colonies on *P. oceanica* leaves (Col_{SF}) per m² seafloor using the mean leaf surface area ($SA_{leafAVG}$), the mean number of leaves per m² (162), the surface area of investigated leaf sample (SA_{leafSS}), and colony count per m² substrate (Col_{SS}):

$$Col_{SF} = \frac{162 \times SA_{leafAVG}}{SA_{leafSS}} \times Col_{SS} \qquad \text{(A2)}$$

Formula (A3): Calculation of bryozoan colonies on *P. oceanica* shoots per m² seafloor (Col_{SF}) using the shoot surface area ($SA_{shootSS}$), the mean number of shoots per m² (40.5), and colony count per m² substrate (Col_{SS}):

$$Col_{SF} = \frac{40.5 \times SA_{shootSS}}{SA_{shootSS}} \times Col_{SS} \qquad \text{(A3)}$$

SM 5 – Data availability

The dataset supporting the conclusions of this study is available in the “PANGAEA” online repository: https://doi.org/10.1594/PANGAEA.942472

Chapter 6

SM 1 – Detailed table highlighting the number of phenotypes and individuals per taxon in total and per m² habitat (*P. crispa*, *P. oceanica* leaves, rhizome and holobiont)

Table S1: *Mean numbers of individuals/colonies (Ind.) and phenotypes (PT) per taxon and per m² habitat identified in Phyllophora crispa, Posidonia oceanica holobiont, P. oceanica leaves and P. oceanica rhizome.*

	P. crispa		*P. oceanica* holobiont		*P. oceanica* leaves		*P. oceanica* rhizome	
	Ind.	PT	Ind.	PT	Ind.	PT	Ind.	PT
Ascidiacea	223.03 ± 54.00	9	178.82 ± 45.04	7	197.16 ± 68.54	4	152.24 ± 47.32	4
Bryozoa	44222.25 ± 5474.96	76	7654.80 ± 681.14	78	6013.22 ± 650.41	48	10035.09 ± 1190.28	50
Cnidaria	24.93±10.59	4	122.91 ± 42.38	6	156.17 ± 67.43	4	74.67 ± 31.98	2
Entoprocta	425.65 ± 353.20	1	0	0	0	0	0	0
Foraminifera	11145.08 ± 900.71	81	7610.81 ± 775.65	52	7316.31 ± 1101.70	33	8037.82 ± 1021.76	31
Mollusca (Bivalvia)	112.07 ± 34.50	4	37.58 ± 26.72	4	12.35 ± 9.58	2	74.15 ± 63.08	2
Polychaeta (Sedentaria)	5949.83 ± 617.91	23	3734.15 ± 578.82	13	2145.46 ± 454.98	4	6037.74 ± 1061.84	11
Rotifera	255.07 ± 45.49	1	0	0	0	0	0	0
Porifera	1649.17 ± 151.50	24	195.81 ± 45.64	13	16.99 ± 9.99	2	455.10 ± 81.31	17
Total	64007.06 ± 5609.48	223	19535.88 ± 1420.98	179	15857.67 ± 1654.42	97	24866.81 ± 1990.69	117

SM 2 - Detailed information on enlargement factor calculations

Table S 2: *Mean Enlargement Factors (EL), respective replication, standard deviations (SD) and standard errors (SE) for Phyllophora crispa, Posidonia oceanica leaves, P. oceanica rhizomes and P. oceanica holobiont.*

	P. crispa	*P. oceanica* holobiont	*P. oceanica* leaves	*P. oceanica* rhizome
Mean EL	4.87	8.33	7.33	1.99
Replication	64	32	32	32
SD	1.52	2.94	2.89	0.43
SE	0.19	0.52	0.51	0.08

SM 3 - Environmental Data measured for *P. crispa*, *P. oceanica* and neighbouring granite hard-bottom

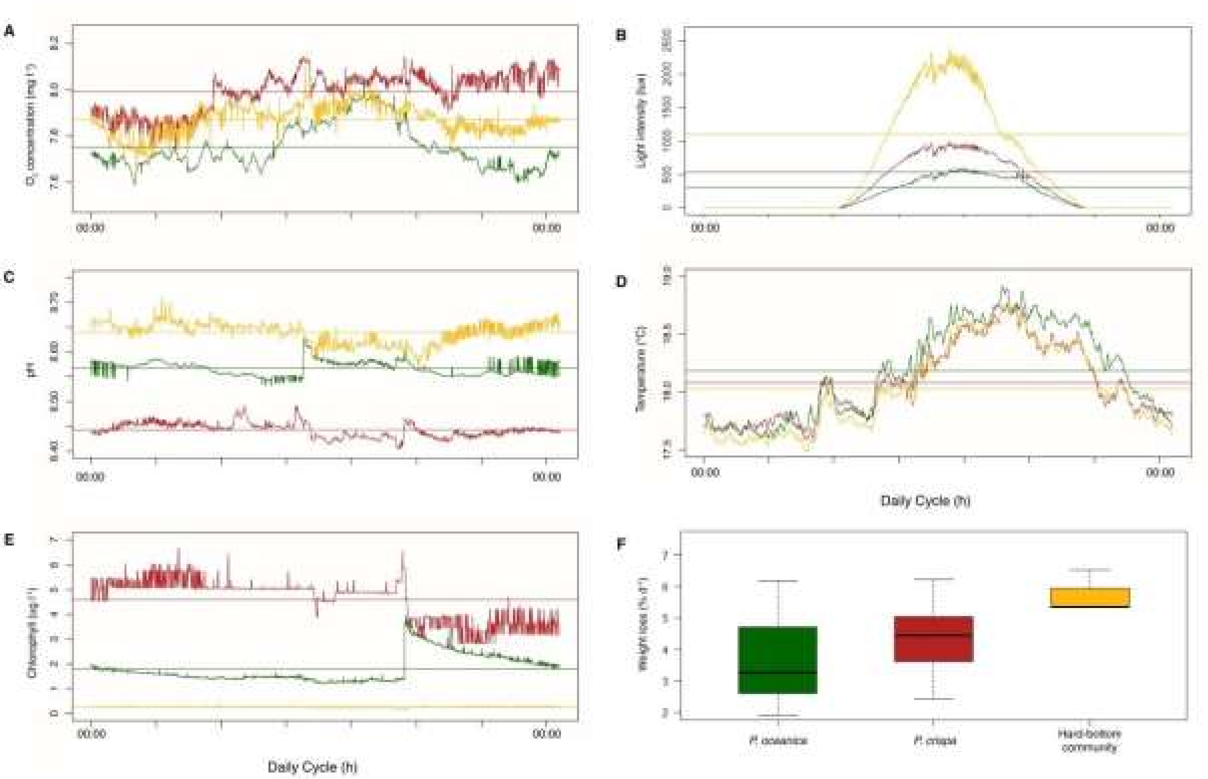


Figure S 1: *Environmental data consisting of oxygen (O_2) concentration (A), light intensity (B), pH (C), temperature (D), chlorophyll α concentration (E) and water movement (estimated via weight loss; F) in Phyllophora crispa (red), Posidonia oceanica (green) and neighbouring hard bottom substrate serving as a reference habitat (yellow). Horizontal lines display daily mean of respective parameter in respective habitat.*

SM 4 Details of biodiversity assessment calculations

Table S 3: *Sample completeness profiles, asymptotic and empirical estimates and resulting undetected diversity, non-asymptotic rarefaction and extrapolation as well as evenness analysis for phenotype richness (q = 0), Shannon diversity (q = 1) and Simpson diversity (q = 2), and Pilou's J' for evenness calculated for Phyllophora crispa, Posidonia oceanica holobiont, P. oceanica leaves and P. oceanica rhizome.*

	q = 0	*q* = 1	*q* = 2
Step I: Sample completeness			
P. crispa	85.67 %	95.80 %	99.37 %
P. oceanica holobiont	72.97 %	94.77 %	99.67 %
P. oceanica leaves	79.33 %	95.99 %	99.69 %
P. oceanica rhizome	73.57 %	92.04 %	99.09 %
Step II: Asymptotic and empirical di	versity estimate	and resulting u	detected diversity
P. crispa			
Asymptotic	260.31	170.04	132.15
Empirical	223.00	155.58	126.12
Undetected	37.31	14.46	6.03
P. oceanica holobiont			
Asymptotic	245.31	118.27	79.33
Empirical	179.00	105.44	75.29
Undetected	66.31	12.83	4.04
P. oceanica leaves			
Asymptotic	122.28	68.94	49.11
Empirical	97.00	62.53	46.76
Undetected	25.28	6.41	2.35
P. oceanica rhizome			
Asymptotic	159.03	89.53	60.59
Empirical	117.00	76.69	56.45
Undetected	42.03	12.84	4.14

Step III: Non-asymptotic coverage-based rarefaction and extrapolation: diversity estimates at C_{max}

	Maximum standardised coverage C_{max} = 96.9 %		
P. crispa	234.08	158.65	127.28
P. oceanica holobiont	206.63	110.83	76.81
P. oceanica leaves	102.74	63.93	47.25
P. oceanica rhizome	142.81	84.04	58.44

Step IV: Evenness

	Maximum standardised coverage C_{max} = 96.9 %		
P. crispa	0.93*	0.68	0.54
P. oceanica holobiont	0.88*	0.593	0.37
P. oceanica leaves	0.90*	0.62	0.45
P. oceanica rhizome	0.89*	0.59	0.41

*for Pilou's *J'* instead evenness at $q = 0$

SM 5 - Map of Giglio Island and Sampling Points

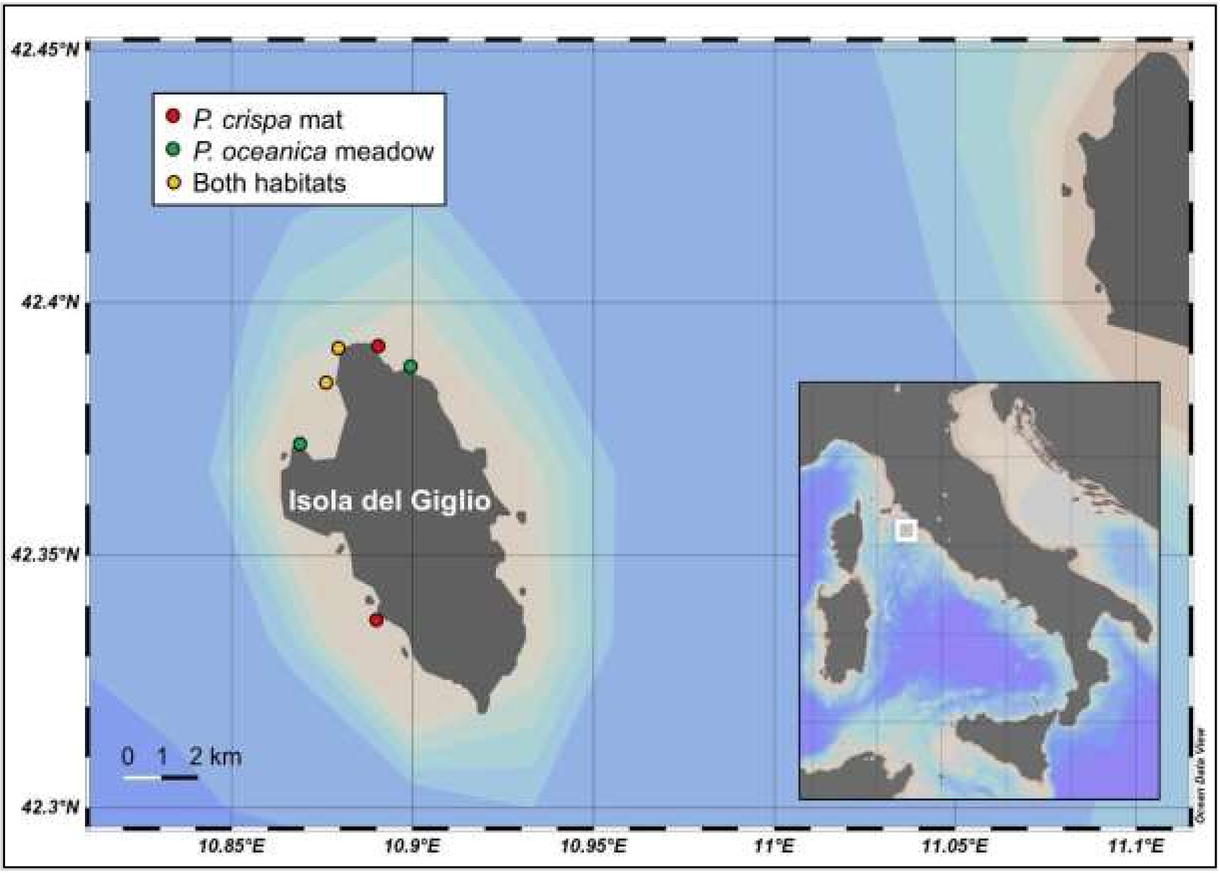


Figure S 2: Location of Giglio Island and sampling sites in the northern part of the Tyrrhenian Sea. Locations (clockwise, starting with the southernmost): "Corvo": 42°20'17.74"N 10°53'22.48"E; "3 Fratelli": 42°22'5.77"N 10°52'14.80"E; „Secca 2": 42°23'1.85"N 10°52'42.12"E; „Fenaio"_ 42°23'19.98"N 10°52'47.92"E; „Punta del morto": 42°23'22.20"N 10°53'21.07"E; "Cala Calbugina": 42°23'6.09"N 10°53'51.24"E; Red dots indicate P. crispa sampling sites, green dots indicate P. oceanica sampling sites and yellow dots indicate sampling sites where both P. crispa and P. oceanica were sampled. Made with Ocean Data View according to Schlitzer (2016)

SM 6 - Schematic representation of *P. oceanica*

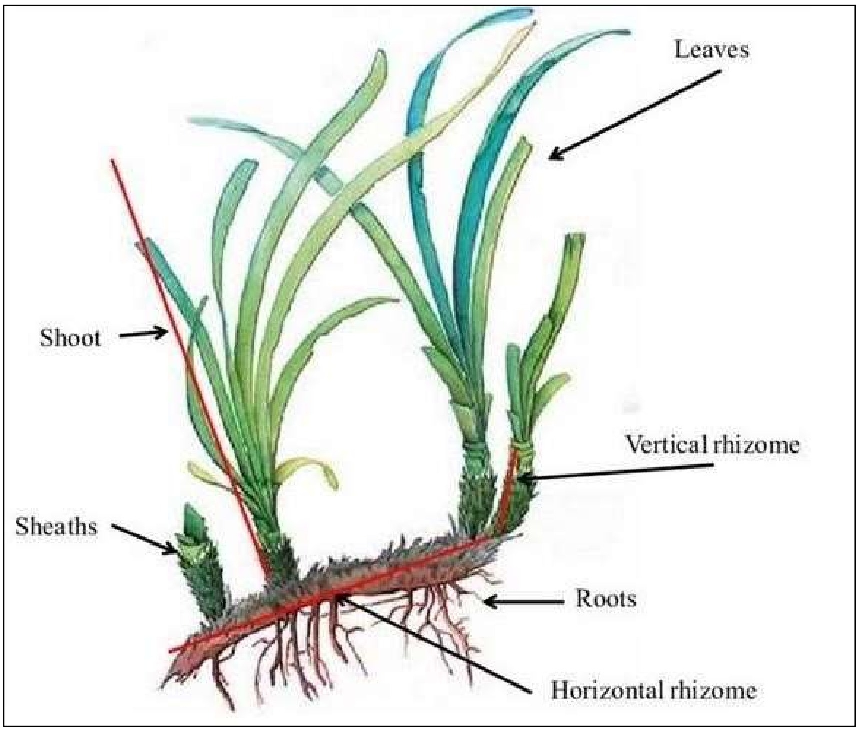


Figure S 3: Schematic overview of P. oceanica seagrass. For the present study, leaves were sampled as displayed. "Rhizomes", as defined in the 'Material' section, consisted of the sheaths, both vertical and horizontal rhizome, as well as the upper layers of the roots. Figure taken from Vasapollo (2010) [2]*.*

SM 7 – Identification Literature

Table S 4: Literature used for identification of species/phenotypes in P. crispa and P. oceanica samples.

Author(s)	Year	Title
Riedl R	2011	Fauna und Flora des Mittelmeeres [3]
Stresemann E	1992	Wirbellose [4]
Milker Y, Schmiedl G	2012	A taxonomic guide to modern benthic shelf foraminifera of the western Mediterranean [5]
Murray JW	1979	British Nearshore Foraminifera [6]
Cimerman F, Langer MR	1991	Mediterranean Foraminifera [7]
Holbourn A, et al.	2013	Atlas of Benthic Foraminifera [8]
Zabala M, Maluguer P	1988	Illustrated keys for the classification of Mediterranean Bryozoa[9]
Hayward JP, Ryland JS	1999	Marine Fauna of North-West-Europe [10]
Bianchi, CN	1981	Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane [11]
Ten Hove HA, Kupriyanova AK	2009	Taxonomy of Serpulidae (Annelida, Polychaeta): the state of affairs [12]
Zibrowius H	1968	Etude mophologique, systématique et écologique des Serpulidae (Annelida Polychaeta) de la région de Marseille [13]
Zibrowius H	1972	Mise au point sur les especes mediterraneennes de Serpulidae (Annelida Polychaeta) déecrites par Stefano delle Chiaje (1822-1829, 1841-1844) et Oronzio Gabriele Costa (1861) [14]

SM 8 - Subset dataset "Polychaeta"

Table S 5: Observed Polychaeta phenotypes that were identified to species (green), genus (orange), family (blue), order (grey) and class (yellow) level based on visually distinct morphological characteristics. Modified after Rossbach et al. (2021) [15].

ID	Taxa
Polychaeta 1	*Amphiglena mediterranea*
Polychaeta 2	*Bathyvermilia* sp.
Polychaeta 3	*Bispira viola*
Polychaeta 4	*Brachiomma* sp.
Polychaeta 5	*Hydroides pseduouncinatus*
Polychaeta 6	*Hydroides* sp.
Polychaeta 7	*Janua* sp.
Polychaeta 8	*Josephella marenzelleri*
Polychaeta 9	*Pileolaria militaris*
Polychaeta 10	*Pileolaria* sp.
Polychaeta 11	Polychaeta sp. I
Polychaeta 12	Polychaeta sp. II
Polychaeta 13	Sabellida sp.
Polychaeta 14	Serpulidae sp.
Polychaeta 15	*Serpulum concharum*
Polychaeta 16	*Spirobranchus lamarckii*
Polychaeta 17	*Spirobranchus polytrema*
Polychaeta 18	*Spirobranchus triqueter*
Polychaeta 19	*Spirobranchus* sp.
Polychaeta 20	*Spororbis* sp.
Polychaeta 21	*Vermiliopsis infundibulum*
Polychaeta 22	*Vermiliopsis labiate*
Polychaeta 23	*Vermiliopsis striaticeps*
Polychaeta 24	*Vermiliopsis* sp.

SM 9 – Sample clustering based on Bray-Curtis similarities

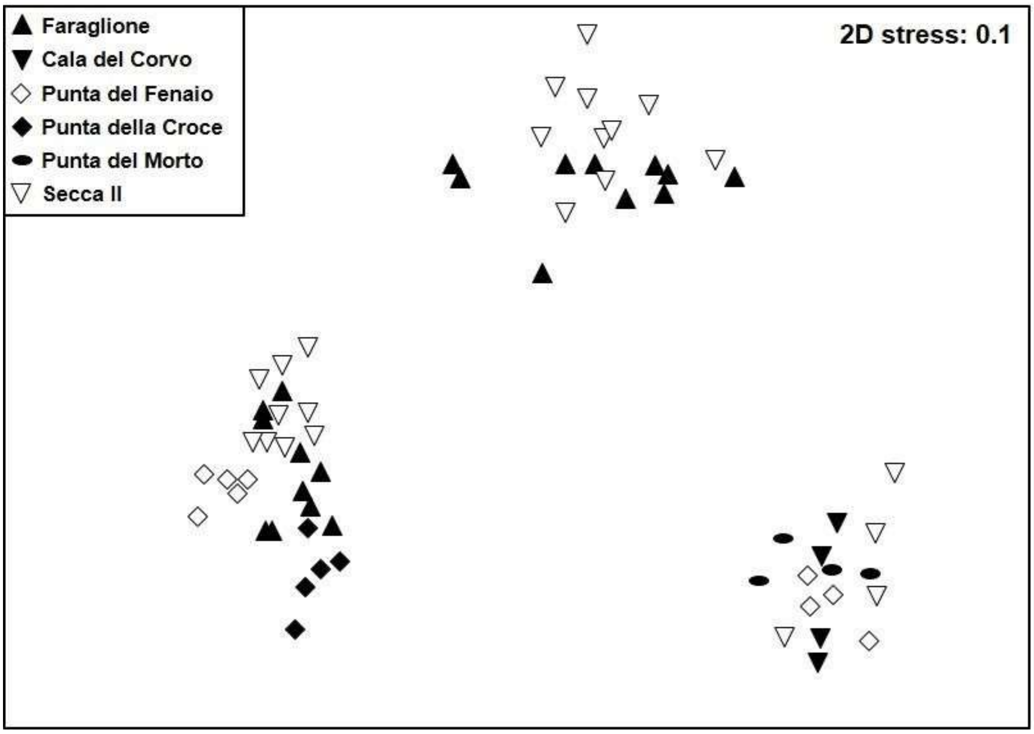
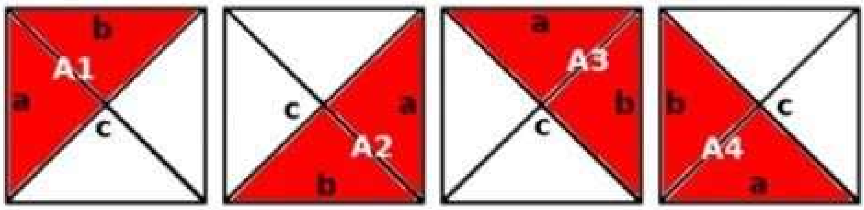


Figure S 4: Non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis similarities showing that sampling sites do not influence observed biodiversity clusters.

SM 10 - Herons Formula to determine surface area enlargement factor

Concept of the triangular partial surfaces (red) inside the quantification frame and the use of Herons' formula to calculate the total mean surface area of the square. a-c: sides of the respective triangle. A1-A4: respective triangle partial surfaces. Adapted from Klain (2004) [16].



Heron´s formula:

$$A_{(triangle)} = \sqrt{s * (s-a) * (s-b) * (s-c)} \qquad s = \frac{(a+b+c)}{2}$$

$$A_{(total)} = \frac{A1 + A2 + A3 + A4}{2}$$

SM 11 – Schematic figure of the utilised Gypsum Clod Card setup

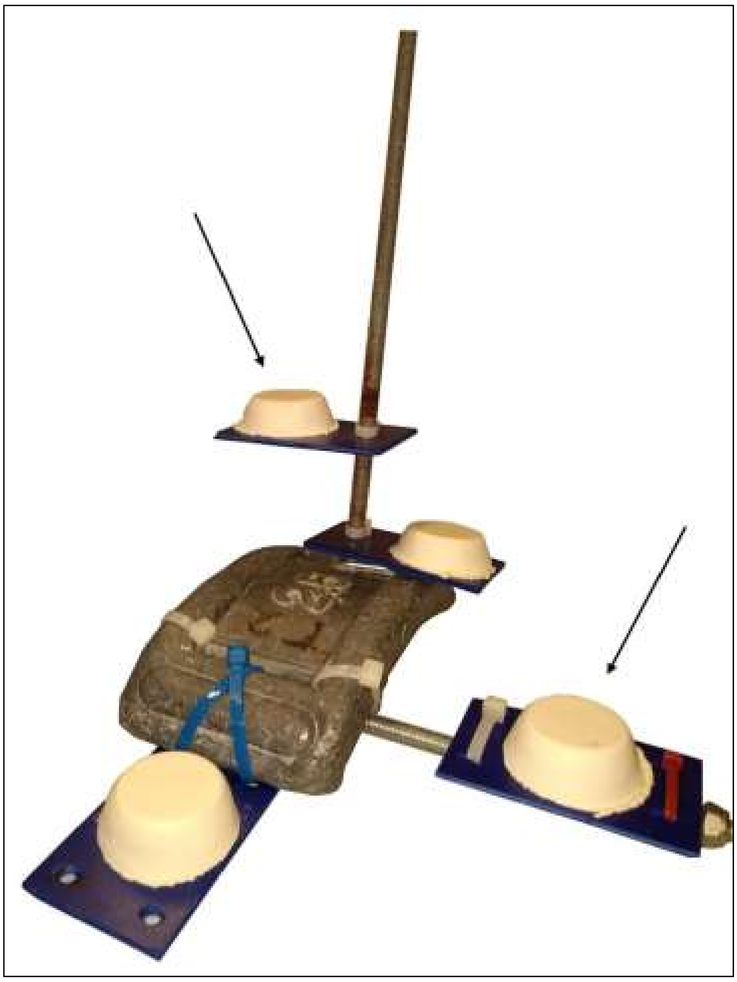


Figure S 5: Schematic figure of utilised gypsum clod card setup; data acquired for the present study based on indicated gypsum clod cards.

SM 12 – Data availability

All data are freely available from the corresponding author and available at DOI: http://doi.org/10.5281/zenodo.4896778.

SM 13 - References

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15. Rossbach, F. I., Casoli, E., Beck, M. & Wild, C. Mediterranean red macro algae mats as habitat for high abun- dances of serpulid polychaetes. **40**, 1–13 (2021).
16. Klain, D. A. An Intuitive Derivation of Heron’s Formula. *Am. Math. Mon.* **111**, 709– 712 (2004).

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Ich, Felix Ivo Roßbach, [redacted], versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbstständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegeben Literatur oder sonstiger Hilfsmittel bedient habe.

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____________________ ____________________

Ort, Datum Unterschrift

I, the undersigned Felix Ivo Roßbach, wohnhaft in [redacted] hereby affirm in lieu of oath that the present dissertation is my own work and that I have duly referenced all passages cited from publications, either literally or in content, and that I have not used any literature or aides other than those referenced.

I hereby affirm that to the best of my knowledge and belief, all the information I have supplied is true and that I have not failed to disclose any relevant facts.

I am aware that making false statements in lieu of oath is an offense that, in the event of intention, pursuant to § 156 StGB may be punished by fine or up to three years' imprisonment and, if committed by negligence, pursuant to § 161 Abs. 1 StGB by fine or up to one year's imprisonment.

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Place, Date Signature