



Article

# Effects of Habitat Fragmentation of a Mediterranean Marine Reef on the Associated Fish Community: Insights from Biological Traits Analysis

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**Abstract:** Habitat fragmentation (HF) is an ecological process, which is potentially also one of the main causes of diversity loss. Many studies have debated the best tools to adopt for assessing the effects of HF. The traditional application of biodiversity metrics might not fully describe the biotic community associated to a particular habitat or the ongoing ecological processes. The community-weighted mean (CWM) seems to be a valid investigation index, since biological traits (BTs) of the associated community are selected by local environmental factors. Furthermore, by combining species with common BTs into Functional Groups (FGs), it is possible to account for ecological functions that are supported by the inclusion of the response of key species within the same context. In our case study, we investigated the possible effect of HF of different Sicilian vermetid reefs on the associated infralittoral fish community based on the (i) vermetid fragmentation level, (ii) nature of the infralittoral substratum and (iii) conservational level of protection. We expected HF to be the main factor in shaping the local fish community; however, the nature of the infralittoral substratum proved to be the principal driver of the ichthyofaunal community. By analysing separately the two infralittoral substrata considered in the study, we observed how HF might affect the associated fish community differently. A pristine vermetid reef seems to sustain a higher number of FGs when established on a rocky substratum. On the other hand, in the presence of a sandy substratum, a fragmented vermetid reef seems to attract a more functionally rich fish community than those accounted for a pristine status. Our results provide some evidence in support of the need to include a broad spectrum of community function descriptors for a more comprehensive characterisation of a habitat and for the assessment of the functioning of its ecosystem.

**Keywords:** habitat fragmentation; vermetid reef; biological traits analysis; functional groups; marine fish community



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## 1. Introduction

Globally, habitat fragmentation (HF) is one of the major causes of biodiversity loss [1]. Its community-shaping action has been demonstrated across all ecosystems, from terrestrial to aquatic ones [2]. HF is at the core of a long debate that started with MacArthur and Wilson's [3] theory of island biogeography and that extended over the successive six decades up to the recent work by Riva and Fahrig [4]. Riva and Fahrig hypothesised that the ecological role played by patchiness, although raising a number of questions, is addressed within a considerable body of literature confirming that the assessment of HF is a complex exercise [5].

Across the most recent literature, biological traits (BTs), i.e., functional and life history traits that control the interaction of every organism with its environment, shaping intra- and interspecific relationships [6], emerged as an effective tool to disentangle the influence of HF from that of other stressors on biodiversity loss.

BTs allow a close examination of the relationship between functional traits and the environment, leading to the concept of habitat filtering [7,8]: “the species with traits less adapted to a given condition are filtered out” [6] effecting the local species in terms of abundance and diversity.

Since this filtering is a function of the multiple abiotic and biotic factors in an ecosystem, it is feasible to expect that anthropogenic factors acting at the local (e.g., contamination, hypoxia, etc.) or at the global (e.g., increasing temperature, sea level rise, etc.) level may filter out species depending on their functional traits [6], in addition to acting on their morphological, physiological and behavioural characteristics [9]. An environment shaped by multiple anthropogenic factors generates local conditions that increase the probability of losing a species or of decreasing the abundance of those species whose traits are more sensitive to the effect of a suite of stressors acting at the local scale [6,10]. For this reason, BTs are thought to be effective in guiding and prioritising conservation measures and management actions [6,11–19]. BTs have also been applied in marine environments, with remarkable examples such as the study of Bremner et al. [20], who investigated the influence of three decades of fisheries activity in filtering the traits of benthic species. More recent works used BTs to explain the effects of anthropogenic disturbance on the functional diversity of estuarine waters [10] and on the benthic functional diversity through the effects of sedimentation [11] and acidification [21] as well as to study the effect of habitat connectivity in surf zone ecosystems [22]. In this context, a marine reef habitat could be a good model to study the effect of HF, due to multiple anthropogenic drivers of biodiversity loss operating within such an ecosystem, and to increase our understanding of whether BTs may improve our ability to better decipher underlying ecological mechanisms and processes.

In the Mediterranean Sea, there are sensitive and unique bio-calcareous intertidal reefs deposited by bio-constructor organisms (the vermetid mollusc *Dendropoma cristatum* [Biondi, 1859] and the encrusting red alga *Neogoniolithon brassica-florida* [Harvey] [23], extending along the 38° parallel [17,18,24] and with a structural complexity and heterogeneity comparable to those of the infralittoral coral reefs of the Pacific and Indian oceans. This habitat is subjected to several direct anthropogenic environmental pressures, including shoreline reclamation, trampling, urbanisation and pollutant inputs [18,25,26]. Climate change represents a further threat, as the rise in water temperatures [17], ocean acidification [27] and the spread of invasive species [24,28] negatively affect vermetid reefs.

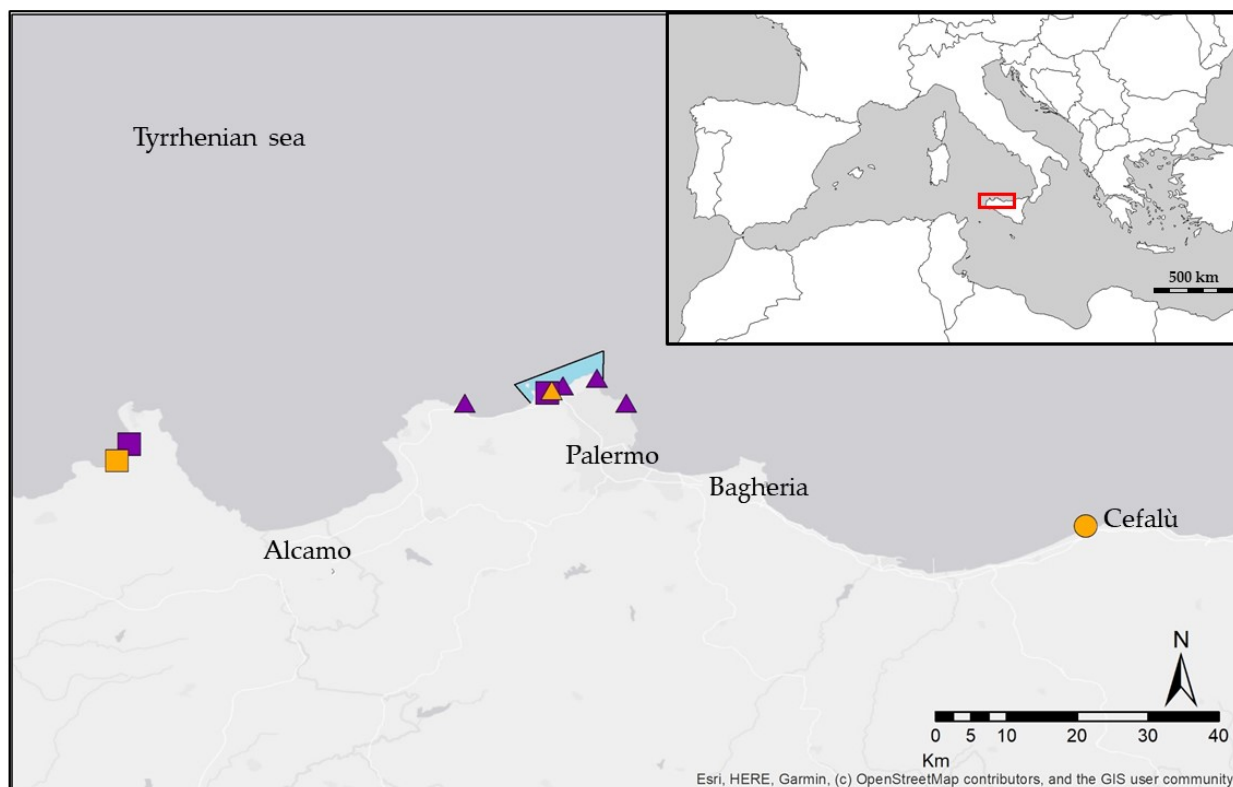
The combination of these threats significantly reduces the efficiency of the main bio-constructor species in depositing calcium bicarbonate whilst also affecting rates of larval recruitment and adult mortality [29]. The resultant regression in bioconstruction activity may cause an impairment of ecosystem functioning through an increment of the erosion processes. The latter, resulting from heightened wave action and carbonate dissolution, lead, in turn, to a gradual fragmentation of vermetid structures. Such fragmentation is a threat to the associated sessile and vagile biodiversity [30] as it promotes a reduction in habitat patch size [31], altering the habitat configuration that drives the filtering of species at the local level. The loss of these species impairs the amount of ecosystem functions expressed by local communities [32]. As a main consequence, this can lead to a decrement in the number of supporting ecosystem services such as shoreline protection, primary production, carbon and nutrient sequestration. The depletion of a number of culturally attractive services (e.g., SCUBA diving) based on the species richness of the reef-associated fish assemblages [33–36] also generates direct socio-ecological impacts, since these services represent the “core business” of those Marine Protected Areas where reefs are present. Thus, if the current biodiversity loss trend witnessed within vermetid reefs is not mitigated, we will register direct negative repercussions on the socio-ecological value of these habitats.

We hypothesised vermetid HF to be the main factor in shaping the infralittoral fish community that is usually associated with the same reef by evaluating how such a community responds to a natural gradient of vermetid reef fragmentation [13,37,38]. In order to test such a hypothesis and that HF might not be the only factor playing a role in influencing the infralittoral fish community, we studied it against two other factors: the nature of the infralittoral substratum and the protection level of the sites under study. Thus, the objective of this study was to identify and distinguish from other factors the impact of fragmentation on a reef habitat due to the loss of a bioengineer species (vermetid). In order to obtain a more holistic understanding of the ongoing ecological processes, we employed a comprehensive approach by combining (i) traditional diversity indexes, (ii) biological traits (BTs) to perform a community-weighted analysis, (iii) the assessment of key species [39] and (iv) the quantity and composition of functional groups (FG), all of which are proxies for ecosystem functioning [40,41].

## 2. Materials and Methods

### 2.1. Rationale, Study Area and Sampling Design

The habitat fragmentation process leads a habitat from a pristine and continuous status to a habitat reduced to a number of smaller patches [5]. One of the constraints to the study of the process of HF is that it normally occurs over a broad time scale [1], making it difficult to follow. Therefore, we tend to compare geographically different sites characterised by the same habitat but with different fragmentation levels. Such an approach can lead to misinterpretation. In fact, Fahrig [1] suggested that the study of HF should always take into account the full landscape supporting the habitat in question trying to avoid wrong conclusions in terms of putative impacts on associated biodiversity. This study was carried out along the northern coast of Sicily, which was explored during pre-surveys in order to identify the most suitable experimental sites among those available (Figure 1).



**Figure 1.** Map of the sites investigated during this study. Squares represent Pristine Vermetid Reef sites (PVR); Triangles represent Fragmented Vermetid Reef sites (FVR); Circles represent No Vermetid Reef sites (NVR). Purple sites are on rocky substratum; Yellow sites are on sandy substratum.

In particular, nine sites along the coast between Cefalù and San Vito Lo Capo (collectively stretching for approximately 120 km) were selected and classified according to three main selection criteria: (i) the fragmentation level of the vermetid reef, (ii) the nature of the infralittoral substratum up to a maximum distance of 25 m from the reef and (iii) depending on the reef's inclusion within a Marine Protected Areas (MPA), the protection or not. At the selected sites, vermetid reefs have been qualitatively classified into three levels: "pristine" (PVR) when characterised by a continuous extension along the shoreline showing the complete "trottoir" structure [42] and not having undergone significant fragmentation; "fragmented" (FVR) when the reef was not continuous and was characterised by an interrupted margin such that it was not possible to distinguish the typical structure of a well-developed vermetid reef [43]; and "no reef" (NVR) when, along the rocky shoreline, a vermetid reef was completely absent. The nature of the infralittoral substratum extending up to 25 m from the shoreline was classified into two levels: "rocky substratum" when it was hard, consolidated and covered in macroalgae and "sandy substratum" when it was mobile, soft, incoherent and unvegetated. The levels for the factor protection were also two: Protected (PR) for all the sites lying within the Capo Gallo-Isola delle Femmine Marine Protected Area, and Not Protected (NP) for those not included. The proposed study design is asymmetrical [44] since, given the availability of accessible coastline with vermetid reefs, it was not possible to provide a location for each combination of factors and levels. Therefore, the list of the sites included in this study with respective factors, levels and coordinates is provided in Appendix A. After the identification of the sites, three subsites at a distance of between 100 and 200 m from each other were randomly selected for the conduction of the underwater visual census activities, with three different replicates being adopted at each subsite (nine transects per site).

## 2.2. Field Sampling

The sampling activities were carried out during August 2017, through the conduction of a standard underwater fish visual census along 25 m-long transects oriented in a shore-normal direction, up to a maximum water depth of 10 m. During the visual census, all the fish individuals spotted within three meters from the observer were identified and quantified. In the case of fish schools featuring high individual quantities, the quantity was estimated. When the exact species could not be identified, a lower taxonomical degree was assigned (e.g., genus or family). Two SCUBA divers were involved during these activities, one responsible for fish identification and the other responsible for underwater orientation. A total of 81 observation units were sampled following the same methodology.

Since at these Mediterranean latitudes, coastal fish assemblages are characterised by a high density of a nonmigratory key species, the damselfish *Chromis chromis* [45], we assessed its putative interaction with the reef. To evaluate such a relationship, two variables were analysed: (i) the densities of individuals and (ii) the distance from the shore in which the schools of *C. chromis* were found. Densities of the damselfish were estimated during the visual census activities, while distances were assessed in all of the nine sites and at all three different subsites with two replicates each. In order to increase replicability and discern variability at different times of the day, this exercise was repeated during the "morning" (07:00–09:00), "noon" (11:00–13:00) and "afternoon" (17:00–18:00).

## 2.3. Biodiversity Metrics, Biological Traits and Functional Groups

Traditional biodiversity metrics were extracted and calculated from the raw data matrix, in particular: (i) species richness (S), (ii) number of individuals (N) and (iii) the Shannon index (H). In order to study the distribution of the demersal fish communities associated with vermetid reef discontinuities in response to HF of the same reefs, a list of eleven biological, but not limited, traits was compiled for the fish species observed in our surveys (Table 1). In fact, although not considered BTs, we included in the list some fish generalisations (e.g., commercial interest, preferred habitat, etc.), which allow us to provide supplement insights in terms of ecological functionality and management. The information on the BTs and generalisations of

our species was extracted from <https://www.fishbase.se/search.php> and, being categorical variables, an arbitrary value was assigned to each (Table 1).

**Table 1.** List of the eleven identified Biological Traits (BT) with relative categories. Each trait was arbitrarily categorised with a value in brackets.

BTs	Categories				
Reproduction	Dioic (1)	Protandry (2)	Protogyny (3)		
Relationship with the substratum	Open Water Swim (OP) (1)	Open Water Sedentary (OPS) (2)	Necto-Benthonic with Horizontal Swim (NBO) (2)	Necto-Benthonic Sedentary (NBS) (3)	Necto-Benthonic Highly Sedentary (NBHS) (4)
Commercial interest	Commercial (1)	Not Commercial (0)			
Habitat	Cave/ <i>Posidonia</i> (1)	Pelagic/ <i>Posidonia</i> (2)	Rock/ <i>Posidonia</i> (3)	Sand (4)	Sand/Rock (5)
Trophic level	Consumer 1° (1)	Consumer 2° (2)	Consumer 3° (3)		
Feeding behaviour	Benthivorous (1)	Detritivorous (2)	Herbivorous (3)	Piscivorous (4)	Zooplanktivorous (5)
Eggs	Parental care (1)	Free in the water (2)			
Spawning frequency	1 peak per year (1)	2 peaks per year (2)	Multiple spawners (3)		
Gregariousness	Couples (1)	Gregarious (2)	Gregarious/Solitary (3)	Solitary (4)	
Spawning season	Autumn (1)	Summer (2)	Winter (3)	Spring (4)	
Max size	Small (1)	Medium (2)	Big (3)	Very big (4)	

Furthermore, we computed the community-weighted mean index (CWM) [6,46], which is a reliable index used to describe the functional structure of fish communities. Such an index includes both fish species' quantitative or qualitative traits and is an estimate of the average trait values of a community weighted by the species' relative quantities [6]. This means that the more abundant the species, the greater the average weight that it will contribute, according to the following formula:

$$CWM = \sum_{i=1}^N p_i x_i$$

with N representing the number of species found in a certain community,  $p_i$  representing the proportional abundance of species i in that community (ranging from 0 to 1), and  $x_i$  representing the trait value of species I [6]. Since *C. chromis* is considerably more abundant than other fish species and given the weight of such a key species in the community, we included it separately when computing the CWM.

Finally, out of the eleven BTs, five were individually studied, followed by the creation of a list of eleven functional groups (FGs) of fish species as combination of the selected BTs (Table 2) and by merging the quantities of the species belonging to the same FG.

**Table 2.** List of the eleven selected Functional Groups (FG) with the relative BTs in common. All the species belonging to each FG are listed in the last column.

	Reproduction	Relationship with the Substratum	Commercial Interest	Habitat	Feeding Behaviour	Species
FG 1	Dioic	NBHS	Commercial	Cave/ <i>Posidonia</i>	Benthivorous/ Zooplanktivorous	<i>Sciaena umba</i> <i>Apogon imberbis</i>
FG 2	Dioic	NBHS	Not Commercial	Sand/Rock	Benthivorous	<i>Gobius</i> sp. <i>Gobius niger</i> <i>Blennius</i> sp. <i>Tripterygion tripteronotus</i>
FG 3	Dioic	NBHS	Not Commercial	Sand	Piscivorous	<i>Synodus saurus</i> <i>Tracurus draco</i>
FG 4	Dioic	OP	Commercial	Pelagic/ <i>Posidonia</i>	Piscivorous	<i>Seriola dumerilii</i> <i>Caranx crysos</i>
FG 5	Dioic	NBO	Commercial	Rock/ <i>Posidonia</i>	Benthivorous	<i>Diplodus puntazzo</i> <i>Diplodus vulgaris</i>

Table 2. Cont.

	Reproduction	Relationship with the Substratum	Commercial Interest	Habitat	Feeding Behaviour	Species
FG 6	Dioic	NBO	Commercial	Sand/Rock	Detritivorous	<i>Mugil</i> spp. <i>Mullus surmuletus</i>
FG 7	Dioic	NBS	Not Commercial	Rock/Posidonia	Benthivorous/ Piscivorous	<i>Serranus cabrilla</i> <i>Serranus scriba</i> <i>Symphodus mediterraneus</i> <i>Centrolabrus melanocercus</i> <i>Symphodus roissali</i> <i>Symphodus rostratus</i>
FG 8	Dioic	NBS	Commercial	Rock/Posidonia	Benthivorous	<i>Labrus viridis</i> <i>Diplodus annularis</i>
FG 9	Protogyny	NBS	Not Commercial	Rock/Posidonia	Benthivorous	<i>Coris julis</i> <i>Thalassoma pavo</i> <i>Symphodus ocellatus</i>
FG 10	Protandry	NBO	Commercial	Rock/Posidonia	Benthivorous/ Herbivorous	<i>Diplodus sargus</i> <i>Sarpa salpa</i>
FG 11	Protogyny	NBS	Commercial	Cave/Posidonia	Piscivorous	<i>Epinephelus costae</i> <i>Epinephelus marginatus</i>

#### 2.4. Statistical Analysis

Prior to the statistical analysis, the species *Chromis chromis* was removed from the raw matrix and studied separately instead of downweighting its importance with the application of a dispersion weighting Primer 7 routine. The raw matrix of the fish assemblages was square root-transformed and ordered into a Bray–Curtis similarity matrix. Differences were tested within a multivariate framework using the Primer 7 and PERMANOVA+ software package within a three-factor analysis (Substratum, Vermetid Status, Protection) exploring any significant differences through a pair-wise test [47]. A SIMPER analysis [48] was computed so as to identify the species that characterised at least 80% of the assemblage for the factor “vermetid status”. Species richness, quantity and Shannon index were normalised and ordered into a Euclidean distance resemblance matrix testing for differences for the factors ‘Substratum’, ‘Vermetid Status’ and ‘Protection’ with any significant difference being explored through pair-wise test. The CWM was analysed after normalisation and generation of a Euclidean distance resemblance matrix against the already-mentioned three factors object of this exercise. The key species *C. chromis* was separately analysed for the response variables of quantities and distances of the schools from the shore with the use of PERMANOVA applied on the Euclidean resemblance matrix.

Finally, differences for the Functional Groups (FG) [49] were explored within a multivariate framework for the factors ‘Vermetid Status’ and ‘Protection’ after square-root transformation and ordered into a Bray–Curtis similarity matrix. For significant differences, further pair-wise tests assessed differences among the levels of the factors studied. FGs, were also individually studied within a univariate framework so to discern for significant differences among levels of the factor ‘Vermetid status’.

### 3. Results

A total of 12,902 individuals belonging to 43 fish species were recorded and identified during this study, including *Chromis chromis*, which accounted for 7436 individuals and represented more than 57% of the entire fish assemblage (the full list of species is reported in Appendix B). Differences in fish assemblages as a result of differences in ‘Substratum’, ‘Vermetid status’ and ‘Protection’ were explored, resulting in a significant difference for all the three factors (Table 3).

**Table 3.** Fish assemblage PERMANOVA for factors ‘Substratum’, ‘Vermetid reef’ and ‘Protection’ with pair-wise test between levels when a significant difference was detected. Level of significance is indicated ( $p < 0.001$  \*\*,  $<0.0001$  \*\*\*).

PERMANOVA Substratum X Vermetid Status X Protection			
Source	df	MS	P (perm)
Substratum Infralittoral	1	11351	0.0001
Vermetid Status	2	4552.7	0.0001
Protection Level	1	5424	0.0001
Substratum Infralittoral x Vermetid Status	1	3627.7	0.0001
Vermetid StatusxProtection Level	1	2160.3	0.0007
Pair-wise test within levels of ‘Rocky substratum’ and ‘Protection’ for ‘Vermetid Status’			
Source	t	P (perm)	
PR	2.2024	0.0001	PVR ≠ FVR ***
NP	2.3542	0.0001	PVR ≠ FVR ***
Pair-wise test within levels of ‘Sandy substratum’ and ‘Protection’ for ‘Vermetid Status’			
Source	t	P (perm)	
NP	2.3654	0.0008	PVR ≠ NVR ***
Pair-wise test within levels of ‘Vermetid Status’			
Source	t	P (perm)	
	2.3654	0.0011	PVR ≠ NVR **
	1.8834	0.0016	FVR ≠ NVR **
	2.3682	0.0002	PVR ≠ FVR ***

Subsequently, rocky and sandy assemblages were analysed separately. The entire rocky assemblage showed differences in terms of the status of vermetids (fragmented vs. pristine) within the two levels of protection (Table 3). From the sandy substratum, vermetid status proved also to be a key factor with significant differences recorded between pristine vermetid reefs, fragmented vermetid reefs and reefs with no vermetids, while the “level of protection” was not a significant factor (Table 3). SIMPER analysis results obtained for the factor “vermetid status”, both for rocky and sandy substrata, are reported in Tables 4 and 5.

**Table 4.** SIMPER for the rocky substratum between the two levels of vermetid fragmentation. Listed are the most abundant species of the assemblages with relative Average Abundance (Av. Ab.) and Percentage Contribution (%).

PVR			FVR		
Species	Av. Ab.	Contr. %	Species	Av. Ab.	Contr. %
<i>Coris julis</i>	3.29	19.72	<i>Thalassoma pavo</i>	2.93	18.39
<i>Sarpa salpa</i>	3.61	17.26	<i>Coris julis</i>	2.90	17.48
<i>Diplodus vulgaris</i>	2.63	15.26	<i>Sarpa salpa</i>	3.18	13.27
<i>Thalassoma pavo</i>	2.03	11.72	<i>Serranus scriba</i>	1.83	10.29
<i>Symphodus mediterraneus</i>	1.82	9.87	<i>Symphodus mediterraneus</i>	1.79	8.44
<i>Diplodus sargus</i>	1.33	5.31	<i>Diplodus vulgaris</i>	1.49	6.71
<i>Serranus scriba</i>	1.10	5.06	<i>Diplodus annularis</i>	1.33	5.26
<i>Symphodus roissali</i>	1.07	4.22	<i>Diplodus sargus</i>	1.26	4.51
<i>Oblada melanura</i>	1.17	2.71	<i>Oblada melanura</i>	1.64	4.42
			<i>Symphodus tinca</i>	1.16	4.12

**Table 5.** SIMPER for the sandy substratum between the three levels of vermetid fragmentation. Listed are the most abundant species of the assemblages with relative Average Abundance (Av. Ab.) and Percentage Contribution (%).

PVR			FVR			NVR		
Species	Av. Ab.	Contr. %	Species	Av. Ab.	Contr. %	Species	Av. Ab.	Contr. %
<i>Thalassoma pavo</i>	3.34	27.14	<i>Sarpa salpa</i>	4.80	19.00	<i>Coris julis</i>	2.51	18.02
<i>Diplodus vulgaris</i>	2.46	16.13	<i>Oblada melanura</i>	4.53	17.94	<i>Thalassoma pavo</i>	2.46	17.16
<i>Diplodus sargus</i>	1.58	10.61	<i>Diplodus sargus</i>	2.89	12.60	<i>Oblada melanura</i>	2.21	13.04
<i>Gobius</i> sp.	1.26	9.69	<i>Diplodus vulgaris</i>	2.64	11.21	<i>Diplodus vulgaris</i>	2.16	12.42
<i>Mullus surmuletus</i>	1.29	8.85	<i>Coris julis</i>	2.12	8.41	<i>Diplodus sargus</i>	2.06	11.06
<i>Oblada melanura</i>	1.74	8.44	<i>Symphodus mediterraneus</i>	1.79	7.77	<i>Sarpa salpa</i>	2.81	7.84
<i>Sarpa salpa</i>	1.55	6.25	<i>Serranus scriba</i>	1.38	6.05	<i>Serranus scriba</i>	1.16	5.89
<i>Symphodus mediterraneus</i>	0.97	4.98	<i>Thalassoma pavo</i>	1.61	5.92	<i>Symphodus tinca</i>	1.00	3.88
			<i>Mullus surmuletus</i>	1.39	5.41	<i>Diplodus annularis</i>	0.88	3.21

For the rocky substratum, the dominant species across all the assemblages were *Coris julis* and *Thalassoma pavo*, followed by common species belonging to the Sparidae family (e.g., *Sarpa salpa*, *Diplodus* sp.) (Table 4). In contrast, for the sandy substratum, we recorded a higher level of dominance by Sparidae species over wrasses in FVR, followed by PVR (Table 5).

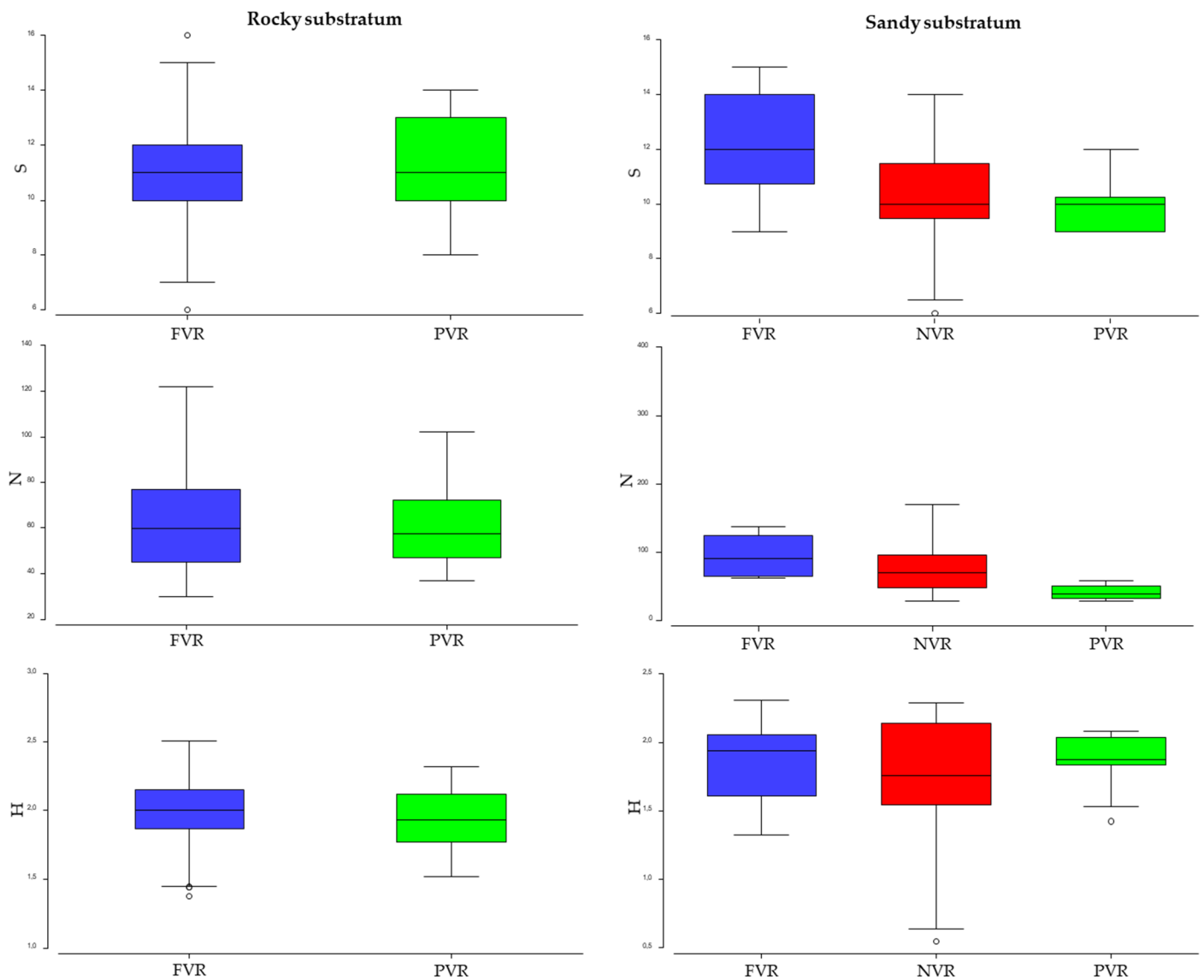
Traditional diversity community metrics accounted for differences among different substrata and for protection. On the other hand, no significant differences were found among rocky substratum, while, in contrast, sandy substratum fish assemblages were significantly different for all the indices tested (Table 6, Figure 2). For example, species richness (S) was significantly lower for pristine vermetid habitats when compared with fragmented or no vermetid sites, while Shannon evenness was higher for no vermetid reefs than fragmented or pristine ones (Figure 2).

**Table 6.** Diversity metrics PERMANOVA for factors ‘Substratum’, ‘Vermetid reef’ and ‘Protection’ with pair-wise test between levels when a significant difference was detected. Level of significance is indicated ( $p < 0.0001$  \*\*\*).

Diversity PERMANOVA Substratum X Vermetid Status X Protection			
Source	df	MS	P (perm)
Substratum Infralittoral	1	7.5964	0.04
Protection Level	1	29.243	0.0001
Pair-wise test within levels of ‘Sandy substratum’ for ‘Vermetid Status’			
Source	t	P (perm)	
NP	2.6851	0.0007	PVR ≠ FVR ***

*Chromis chromis* exhibited different distribution patterns for rocky and sandy substratum sites, although in both cases, such a species appears to not be affected by the local protection. In fact, for the rocky substratum, we recorded significant differences for the factor “vermetid status”, with higher *C. chromis* individual quantities being recorded for FVR sites than for PVR ones (Table 7, Figure 3). Conversely, fish occurred further away from the shore at PVR sites when compared with FVR sites.





**Figure 2.** Boxplot of the significant differences between diversity indexes for rocky and sandy substratum for the levels of the factor ‘Vermetid status’. Comparison circles displays whether or not the mean values are significantly different from each other.

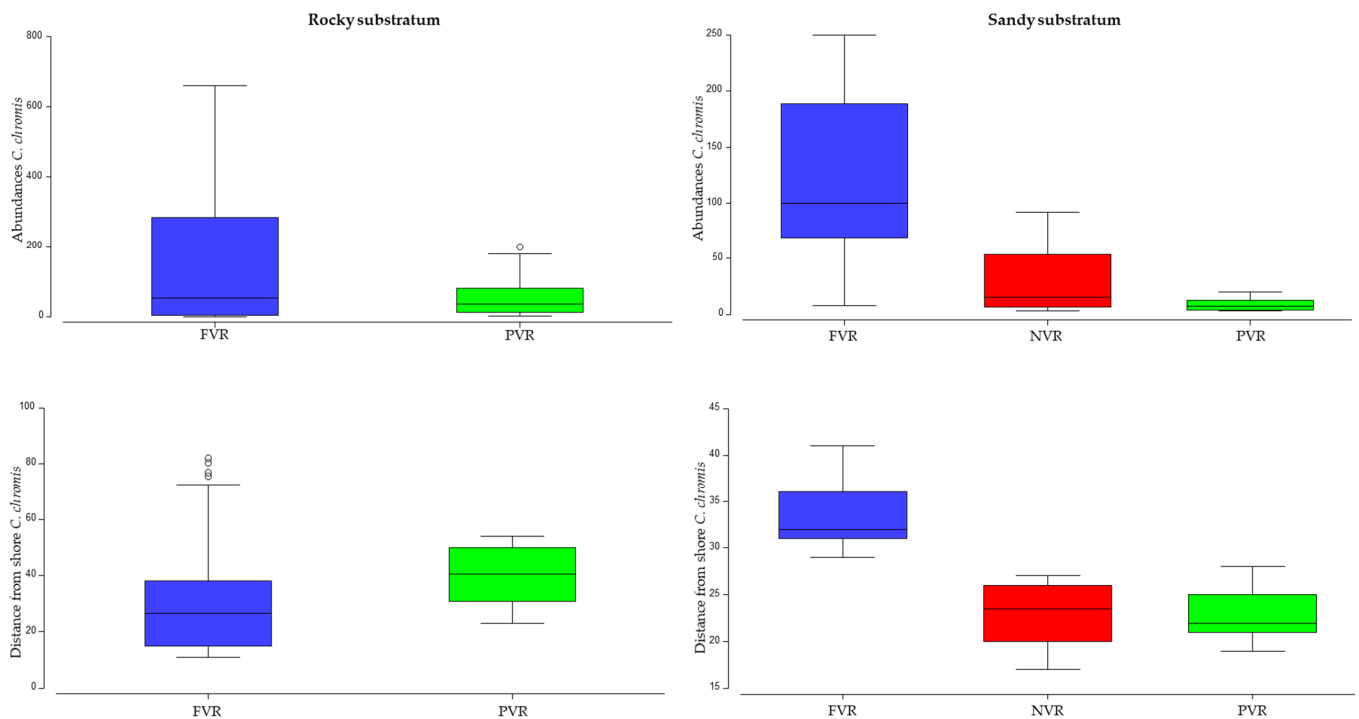
For sandy substratum, we recorded a different scenario (Figure 3). Indeed, the highest quantities of *C. chromis* were observed within FVR sites, with the fish schools occurring at significantly higher distances from the shore. For all the sites characterised by a PVR or a NVR reef, we recorded low quantities of damselfish schools occurring in near proximity to the shore (Table 7, Figure 3).

No significant differences were found among the three different sampling times in terms of the distances from the shoreline at which the *C. chromis* schools occurred.

The analysis of the CWM computed by using the 11 BTs showed differences in terms of substratum only when combined with the vermetid status, while protection accounted for small differences. As we look into the CWMs for the different substrata, the only one with differences in terms of vermetid status was within the sandy substratum, with PVR being significantly higher than FVR and NVR (Table 8).

**Table 7.** *C. chromis* school quantities and distances from shore PERMANOVA for factors ‘Substratum’, ‘Vermetid reef’ and ‘Protection’ with pair-wise test between levels when a significant difference was detected. Level of significance is indicated ( $p < 0.05$  \*;  $<0.0001$  \*\*\*).

Abundances <i>C. chromis</i> . PERMANOVA Substratum X Vermetid Status X Protection			
Source	df	MS	P (perm)
Vermetid Status	2	80,528	0.0106
Pair-wise test within levels of ‘Vermetid Status’ for ‘Substratum’			
Source	t	P (perm)	
Rocky substratum	2.0474	0.0422	PVR ≠ FVR *
Sand substratum	4.1208	0.0004	PVR ≠ FVR ***
Sand substratum	3.1266	0.0057	FVR ≠ NVR *
Sand substratum	1.9677	0.071	PVR = NVR
Distances <i>C. chromis</i> . PERMANOVA Substratum X Vermetid Status X Protection			
Source	df	MS	P (perm)
Substratum	1	3816.5	0.0001
Vermetid Status	2	837.65	0.0041
Substratum X Vermetid Status	1	1151.2	0.004
Pair-wise test within levels of ‘Vermetid Status’ for ‘Substratum’			
Source	t	P (perm)	
Rocky substratum	3.4646	0.0009	PVR ≠ FVR ***
Sand substratum	9.23	0.0001	PVR ≠ FVR ***
Sand substratum	9.804	0.0001	FVR ≠ NVR ***
Sand substratum	0.0535	0.975	PVR = NVR



**Figure 3.** Boxplot of the significant differences between *C. chromis* densities and distance from shore of the schools, respectively, for rocky and sandy substratum for the levels of the factor ‘Vermetid status’. Comparison circles displays whether or not the mean values are significantly different from each other.

**Table 8.** Community Weighted Mean (CWM) PERMANOVA for factors ‘Substratum’, ‘Vermetid reef’ and ‘Protection’ with pair-wise test between levels when a significant difference was detected. Level of significance is indicated ( $p < 0.05$  \*).

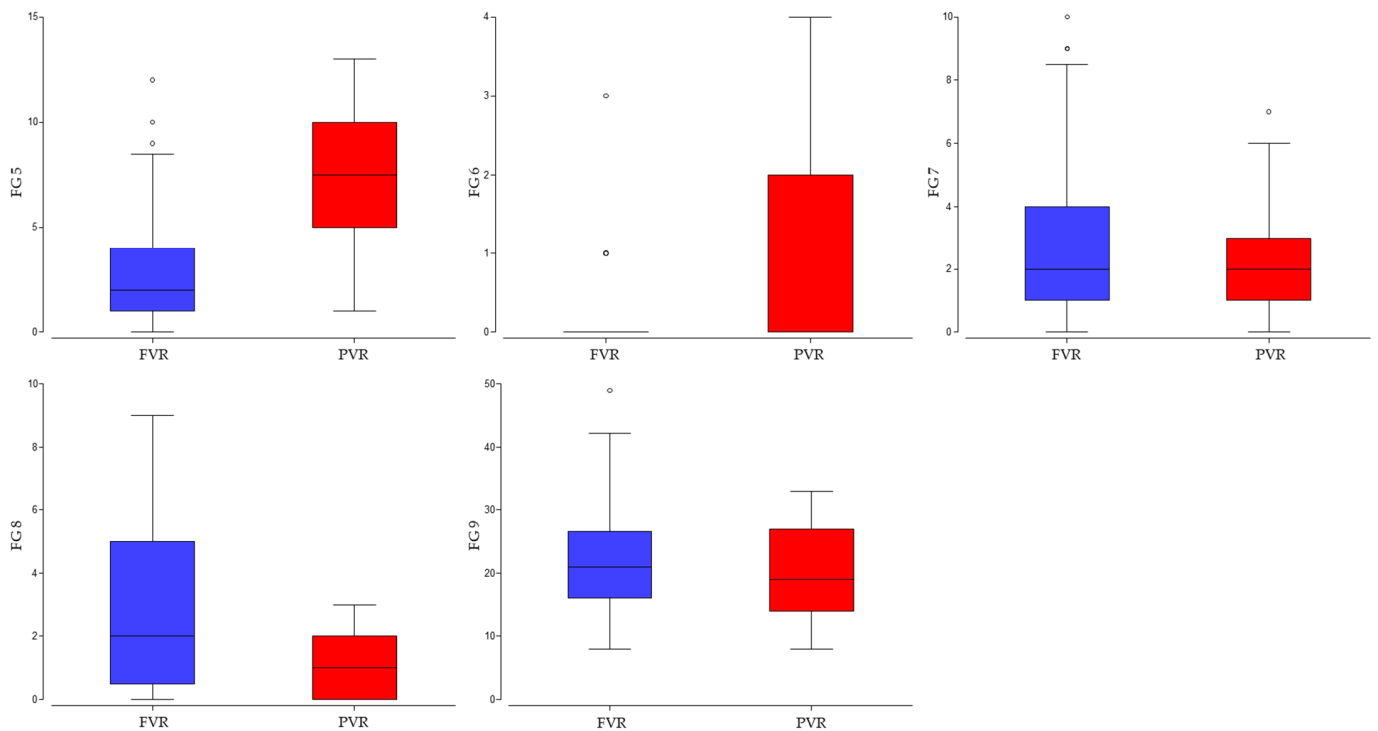
CWM, PERMANOVA Substratum X Vermetid Status X Protection			
Source	df	MS	P (perm)
Vermetid Status	2	4.197	0.0551
Protection Level	1	5.5821	0.0453
Substratum Infralittoral x Vermetid Status	1	6.5334	0.0329
Pair-wise test within levels of ‘Vermetid Status’			
Source	t	P (perm)	
Rocky substratum	0.6393	0.565	PVR = FVR
Sandy substratum	2.2094	0.0074	PVR ≠ NVR *
Sandy substratum	1.3463	0.1418	FVR = NVR
Sandy substratum	3.0882	0.0014	PVR ≠ FVR *

From the PERMANOVA carried out for the functional groups for the factors ‘Vermetid reef’ and ‘Protection’ for the two different substrata, significant differences were assessed (Table 9). In particular, within rocky substratum, significant differences were found for both ‘vermetid status’ and ‘protection’ supported from the results of the pair-wise tests from which differences were detected for the factors studied within their levels (Table 9). By looking at the FGs PERMANOVA for sandy substratum, significant differences were found for the ‘vermetid status’ factor, particularly, as indicated by the pair-wise test, with PVR being different from both FVR and NVR (Table 9).

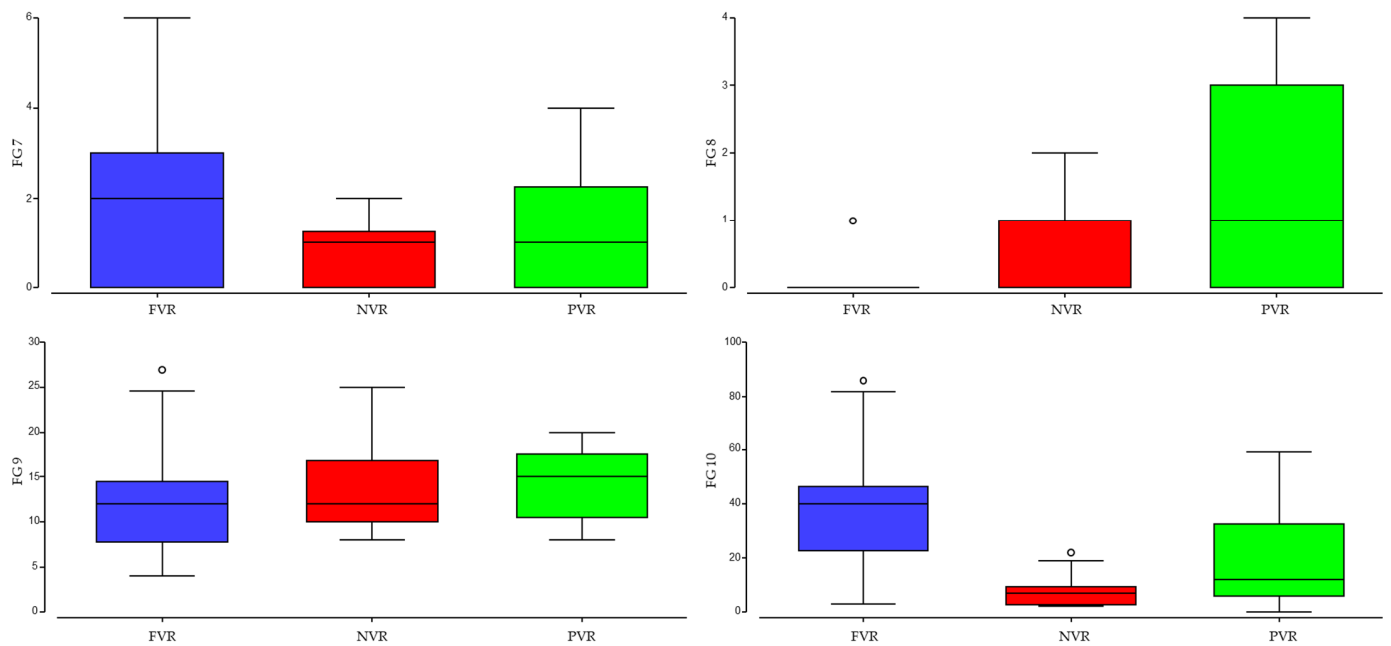
**Table 9.** Functional Groups PERMANOVA for the rocky and sandy substrata for factors ‘Vermetid reef’ and ‘Protection’ with pair-wise test between levels when a significant difference was detected. Level of significance is indicated ( $p < 0.05$  \*;  $< 0.001$  \*\*;  $< 0.0001$  \*\*\*).

Rocky Substratum; PERMANOVA ‘Vermetid Status’ X ‘Protection’			
Source	df	MS	P (perm)
Vermetid Status	1	2475.8	0.0001
Protection	1	2214	0.0001
Vermetid status X Protection	1	1653.4	0.0006
Pair-wise test within levels of ‘Protection Level’ for ‘Vermetid Status’			
Source	t	P (perm)	
NP	2.0872	0.001	PVR ≠ FVR **
PR	2.6182	0.0001	PVR ≠ FVR ***
Pair-wise test within levels of ‘Vermetid Status’ for ‘Protection’			
Source	t	P (perm)	
PVR	2.0779	0.004	PR ≠ NP **
FVR	2.3771	0.001	PR ≠ NP ***
Sandy substratum; PERMANOVA ‘Vermetid Status’			
Source	df	MS	P (perm)
Vermetid Status	2	1591.6	0.0024
Pair-wise test within levels of ‘Vermetid Status’			
Source	t	P (perm)	
Vermetid Status	1.5919	0.037	NVR ≠ PVR *
Vermetid Status	1.4579	0.0868	NVR = FVR
Vermetid Status	2.1838	0.0017	PVR ≠ FVR **

Analysis of the variance values for the single functional groups showed significant differences for the factor “vermetid status”, both for assemblages characterised by a rocky and by a sandy substratum (Figures 4 and 5).



**Figure 4.** Boxplot of the significant differences between Functional Groups for rocky substratum for the levels of the factor ‘Vermetid status’. Comparison circles displays whether or not the mean values are significantly different from each other.



**Figure 5.** Boxplot of the significant differences between Functional Groups for sandy substratum for the levels of the factor ‘Vermetid status’. Comparison circles displays whether or not the mean values are significantly different from each other.

Commercially exploited benthivorous and detritivorous species were significantly more abundant close to the pristine reefs and under rocky substrata than at sites where vermetids were fragmented. At these latter sites, FGs accounting species, including *Diplodus annularis* and *Coris julis*, exhibited significantly greater quantities than other FGs. Sandy substratum assemblages showed smaller significant differences in terms of FGs: for pristine vermetids, only the FG9, represented mostly by the wrasse species, exhibited significantly higher individual quantities than other FGs and under different levels/conditions. Conversely, FGs characterised by species like *Diplodus sargus* and *Sarpa salpa*, were more abundant under FVR conditions.

#### 4. Discussion

Historically, empirical studies have always suggested that HF has negative direct and indirect effects on biodiversity [50,51]; however, more recently, these theories have been the subject of renewed discussion [4] since a multitude of studies have yielded contrasting results, e.g., [52–54]. From our preliminary results, a major difference in terms of fish community, was detected between the two rocky and sandy substrata, irrespective of the HF state of the same sites. These two infralittoral substrata are considerably different in terms of complexity, functioning and resource availability, suggesting that this factor is the major driver in shaping the associated fish community and diversity [55,56]. The separation of fish assemblages in terms of substratum was crucial to disentangling the real role that HF can have in different landscapes. Following the Fagan [57] study, we categorised the landscape characterised by a vermetid reef facing a rocky substratum as dendric (highly complex and interconnected multitude of habitats) and the one with a vermetid reef contiguous to a sandy substratum as linear (very few habitats with little interconnection). Nevertheless, the outputs from empirical studies of HF are often difficult to interpret [1], with even the classic biodiversity metrics being unable to describe the real picture [15,58,59]. In fact, within our results, the biodiversity index values obtained for the rocky substratum did not show any significant differences under different fragmentation levels. Instead, the sandy substratum appeared to host a higher fish individuals abundance and plain species diversity than a pristine one, although the Shannon index was higher for no vermetid reef sites (NVR). Significant differences reported for different fragmentation levels in terms of traditional biodiversity indices, fail to contribute much to understanding the functioning of these two landscapes.

Habitat fragmentation seems to influence fish assemblages differently when operating on vermetid reefs occurring close to rocky or sandy substrata, even for populations of *C. chromis*. For rocky substrates, we observed how this species gathered in very small schools and relatively far from pristine sites (PVR), as opposed to fragmented reefs, where the schools were more abundant and occurred in close proximity to the shore [45]. Based on this observation, we suggest an indirect competition for resources between the vermetid reef and *C. chromis*. In fact, the vermetid reef's trophic web is largely based on marine organic matter in the form of phytoplankton [60]. A PVR might have a higher impact in terms of resource sequestration, affecting the infralittoral food web. We can infer that *C. chromis*, feeding mainly on zooplankton [45], moves further offshore from the vermetid reef since zooplankton, depending on the availability of phytoplankton, is less available in nearshore areas. At landscapes dominated by sandy substrates, we recorded a different situation, whereby the greatest quantities of *C. chromis* were still recorded for FVR sites but occurred at higher distances from the shore, when compared with PVR or NVR sites. We speculated that this behaviour might be explained in terms of a refuge effect and less in terms of a feeding ground [61]. According to this hypothesis, at FVR sites, *C. chromis* might not be able to find enough refuges for nesting, contrary to a well-developed vermetid reef structure or a complex rocky shoreline, a theory deserving further and ad hoc experimentation.

The analysis of biological traits has proven to be a strong tool for identifying functional differences [62]. In fact, from our results, we have observed important (and once again different) patterns for the two landscapes under study. Indeed, pristine vermetid reefs over a

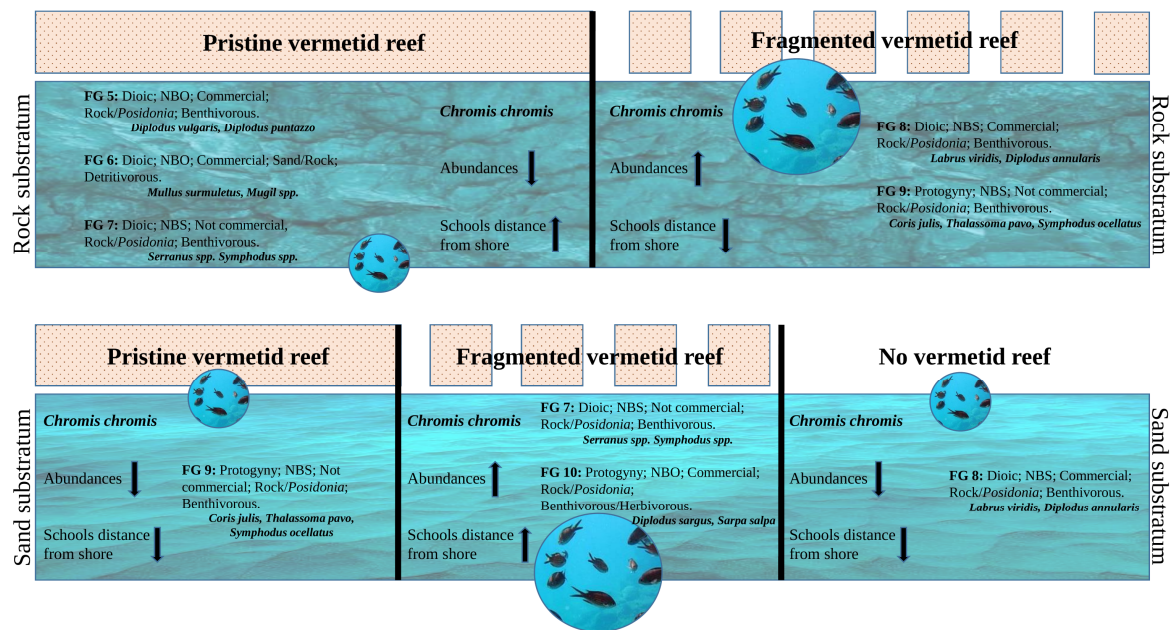
rocky substratum appeared to attract trophic resources [63], triggering an abundant presence of several commercially exploited species and, in particular, hermaphroditic, spring-spawning fish species, which prefer a heterogeneous habitat of rocks and *Posidonia oceanica* meadows (Supplementary Material, Table S1) [64]. Such availability of resources should attract even small-size fish species [33], which might explain the presence of a higher number of young pelagic fish-feeding species of high commercial value, including *Seriola dumerili* [65]. An opposite pattern emerged from the analysis of the biological traits for the fish assemblages recorded over a sandy substratum (Supplementary Material, Table S2). The FVR sites appear to gather greater quantities of commercially exploited fish species, in particular benthic ones with regular horizontal movements. The absence of significant differences between the fish assemblages patrolling in near proximity of PVR and NVR suggests that well-developed reefs may not be particularly attractive towards infralittoral fish species when compared with fragmented ones.

The analysis of the CWM based on an analysis of the functional traits returned significant results only in terms of the site protection factor. The reef fragmentation level was only significant when it was in combination with a high protection level for sites with a rocky substratum. Instead, over a sandy substratum, the fragmentation level showed significant differences. It appeared that the habitat fragmentation did not have a strong influence on dominant fish species at complex landscapes like that characterised by a rocky substratum, probably because of a putative buffer effect resulting from the occurrence of a highly diverse net of interconnected habitats (e.g., *Posidonia oceanica* meadow, different algal communities, caves, etc. [22,52]). A stronger effect occurred on the landscape characterised by a sandy substratum, where dominant fish species might rely to a higher degree on the vermetid habitat, given the scarce interconnectivity with other habitats rich in resources and complexity [22]. In our fish assemblages, the species *Chromis chromis* was by far the most abundant and, for this reason, the CWM index detected the dominant effect of this species and poorly considered all other fish species [66]. Hence, although the CWM index has been widely applied [46], several authors, e.g., [67] suggest that it might have descriptive limits. Wen et al. [67] compared CWM with functional diversity, concluding that the second was more effective in describing the effect of impacts (e.g., habitat fragmentation) on ecological variables.

The functional group (FGs) analysis based on the shared biological traits allowed us to integrate the information collected by combining them with the observations made for the key species *C. chromis* (Figure 6), highlighting important patterns.

Within the landscape characterised by a rocky substratum and PVR, we identified a greater diversity of FGs [17], in particular those featuring benthivorous and detritivorous species having a high commercial and ecological value (e.g., *Mullus surmuletus*, *Diplodus vulgaris*, *Mugil* spp.) known for their bioturbation activities. On the other hand, the downscaling action of a FVR seems to be compensated by the higher presence of the key species *C. chromis*, resulting in a “takeover” in terms of dominating the associated fish community [68]. On the contrary, a higher number of FGs could be sustained within landscapes characterised by a sandy substratum in the presence of an FVR. Hence, herbivorous species here are more abundant and likely to play an important role in controlling algal coverage on the fragmented reef [69] in co-occurrence with the presence of a multitude of small-size predators. Given the limited availability of resources, in such a scenario, the occurrence of *C. chromis* is highly restricted, either in terms of abundance or in terms of proximity to the reef [68]. These results are supported by those from Fagan [57]. Accordingly, habitat fragmentation has different effects on dendric and linear landscapes. In a dendric context, arising, for example, from the combination between vermetid reefs and adjacent rocky substrata, the biotic community is expected to have a high resistance level as a result of the buffer effect of the multiple, well-connected habitats but with a very low resilience level. A linear context as the one represented by a vermetid reef with an adjacent sandy substratum has less habitats available other than the vermetid reef itself. In this latter case,

small but complex (based on the overall surface available) patches can host more diverse and multifunctional assemblages [4,12,70].



**Figure 6.** Schematical representation of the two landscapes studied under the condition of rock and sandy substratum with the different levels of fragmentation of the vermetid reef. For each reality are listed the Functional Groups (FGs) with significantly greater quantities with the specific traits and the fish species included. The quantities of *C. chromis* are also schematically represented through bubbles.

### 5. Conclusions

Through our study, we support the widely accepted statement that each landscape requires a full characterisation and ad hoc conservation strategies, rather than a single management measure [71]. It is crucial to include the habitat studied within the broader framework of the landscape it belongs to, taking into account all its local and geographically different parameters [70]. Through our empirical observations, we highlighted the importance of the use of multiple investigation tools in order to disentangle the role HF plays in shaping the local community. In fact, discerning between the effects of natural substratum and the effect of protection, we observed how the vermetid reef might shape the local community differently based on its fragmentation level. It becomes increasingly evident that intricate and interconnected habitats, typical of dendritic landscapes, bestow a remarkable level of resistance against resilience for those pristine and unbroken vermetid reefs [57].

Conversely, the more simplified and naturally less diverse linear landscapes appear to benefit when they embrace a habitat characterised by heightened patchiness, sustaining more complex communities [57]. The combination of traditional diversity metrics, BTs, FGs and the density of key species (Figure 6), we can acquire a good degree of understanding prior to formulating future MPA management and conservation measures [67,72] as well as fisheries management recommendations [73,74]. Thus, functional groups can be considered a proxy for ecosystem functioning and, according to the Habitats Directive (EC, 1992) and the European Union (2010), the conservation of coastal habitats should be prioritised in order to support the provision of a higher number of ecosystem services by the same habitats [75–78].

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse11101957/s1>, Table S1: ANOVA test for the rocky substratum for the diversity indexes, the selected biological traits and for the *C. chromis* quantities and distances from shore between the habitat fragmentation levels studied. ANOVA test and *p* value are indicated with relative SNK test and level of significance; Table S2: ANOVA test for the sandy substratum for the diversity indexes and the selected biological traits between the habitat fragmentation levels

studied. ANOVA test and  $p$  value are indicated with relative SNK test and level of significance. Table S3: ANOVA test for the selected functional groups for the two conditions of rocky and sandy substratum between the habitat fragmentation levels studied. ANOVA test and  $p$  value are indicated with relative SNK test and level of significance.

**Author Contributions:** A.M. participated in sampling and data collection. A.M., M.C.M., M.B. and G.S. performed data analyses, prepared the figures, and drafted the manuscript. G.S. and A.D. revised critically the intellectual content and the English language giving the final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original contributions presented in the study are available and it can be requested directly to the corresponding author.

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**Conflicts of Interest:** The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A

Site	Substratum Infralittoral	Vermetid Status	Protection	Sub-Site	Latitude	Longitude
Addaura	Rock	Fragmented	None	1	38,190,983	13,359,409
				2	38,186,131	13,362,946
				3	38,179,507	13,366,856
Capo Playa	Sand	No vermetid reef	None	1	38,029,974	13,945,312
				2	38,030,822	13,956,559
				3	38,035,039	13,972,531
Punta Raisi	Rock	Fragmented	None	1	38,185,770	13,146,196
				2	38,187,153	13,154,650
				3	38,185,669	13,161,603
Capo Gallo-Motomar	Rock	Fragmented	Protected	1	38,218,782	13,322,964
				2	38,221,092	13,321,641
				3	38,223,048	13,319,901
Macari	Rock	Pristine	None	1	38,132,237	12,726,255
				2	38,134,599	12,729,528
				3	38,136,992	12,733,803
Castelluzzo	Sand	Pristine	None	1	38,124,062	12,722,640
				2	38,118,760	12,719,328
				3	38,113,428	12,713,051
Capo Gallo-Bellevue	Sand	Fragmented	Protected	1	38,203,740	13,266,365
				2	38,202,445	13,266,185
				3	38,201,292	13,265,970
Capo Gallo-Isola	Rock	Pristine	Protected	1	38,201,304	13,259,515
				2	38,200,617	13,261,604
				3	38,200,464	13,263,855
Capo Gallo-Barcarello	Rock	Pristine	Protected	1	38,210,696	13,280,152
				2	38,209,193	13,280,283
				3	38,207,290	13,280,076



## Appendix B

Fish Species	
<i>Apogon imberbis</i>	<i>Oblada melanura</i>
<i>Atherina</i> sp.	<i>Sarpa salpa</i>
<i>Blennius</i> sp.	<i>Sciaena umbra</i>
<i>Boops boops</i>	<i>Scorpaena porcus</i>
<i>Bothus podas</i>	<i>Seriola dumerilii</i>
<i>Caranx crisos</i>	<i>Serranus cabrilla</i>
<i>Chromis chromis</i>	<i>Serranus scriba</i>
<i>Coris julis</i>	<i>Sparisoma cretense</i>
<i>Diplodus annularis</i>	<i>Spicara maena</i>
<i>Diplodus puntazzo</i>	<i>Spondiliosoma cantharus</i>
<i>Diplodus sargus</i>	<i>Symphodus mediterraneus</i>
<i>Diplodus vulgaris</i>	<i>Centrolabrus melanocercus</i>
<i>Epinephelus costae</i>	<i>Symphodus ocellatus</i>
<i>Epinephelus marginatus</i>	<i>Symphodus roissali</i>
<i>Gobius</i> sp.	<i>Symphodus rostratus</i>
<i>Gobius niger</i>	<i>Symphodus tinca</i>
<i>Labrus viridis</i>	<i>Synodus saurus</i>
<i>Lithognathus mormyrus</i>	<i>Tracurus draco</i>
<i>Mugil</i> spp.	<i>Tripterygion tripteronotus</i>
<i>Mullus surmuletus</i>	<i>Thalassoma pavo</i>
<i>Muraena helena</i>	<i>Xyrichtys novacula</i>

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