water

Article

Troubles Never Come Alone: Outcome of Multiple Pressures on a Temperate Rocky Reef

Annalisa Azzola [1,*], Virginia Picchio [1], Valentina Asnaghi [2], Carlo Nike Bianchi [1,3], Carla Morri [1,3], Alice Oprandi [1] and Monica Montefalcone [1,4]

1 Seascape Ecology Laboratory, DiSTAV (Department of Earth, Environment and Life Sciences), University of Genoa, Corso Europa, 26, 16132 Genova, Italy
2 Benthic Ecology Laboratory, DiSTAV (Department of Earth, Environment and Life Sciences), University of Genoa, Viale Benedetto XV, 16132 Genova, Italy
3 Department of Integrative Marine Ecology (EMI), Stazione Zoologica Anton Dohrn–National Institute of Marine Biology, Ecology and Biotechnology, Genoa Marine Centre (GMC), Villa del Principe, Piazza del Principe 4, 16126 Genoa, Italy
4 NBFC, National Biodiversity Future Center, 90133 Palermo, Italy
* Correspondence: annalisa.azzola@edu.unige.it

Abstract: Climate change is affecting rocky reef ecosystems in a multitude of ways at global scale. During summer 2018, the rocky reef communities of Portofino Marine Protected Area (MPA) (NW Mediterranean) were affected by thermal anomalies, a mucilaginous event, and the seasonal expansion of *Caulerpa cylindracea*. Moreover, a severe storm occurred on 29 October. The effects of these pressures on the rocky reef communities were analysed at different depths (10 m, 20 m, 30 m, and 40 m) and at three times (June, October, December) to evaluate change at short temporal scale. Portofino MPA's communities have significantly changed: thermal anomalies mostly affected the biota living above the summer thermocline (ca 20 m depth); mucilaginoius aggregates first impacted the communities in shallow waters and only later those in deep waters, where they typically fall in late summer; the greatest impact by *Caulerpa cylindracea* was detected at 20 m depth; the storm directly impacted communities in shallow and intermediate waters by uprooting algal species, while it had indirect effects at greater depths through sediment redistribution. Disentangling the effects of multiple pressures on coastal ecosystems is one of the most pressing goals in marine ecology and biodiversity conservation. This study represents an attempt in this direction as applied to the short-term dynamics of rocky reef communities under a climate change scenario.

Keywords: rocky reef communities; thermal anomalies; mucilage events; alien species; stress and disturbance; Mediterranean Sea

check for updates

Citation: Azzola, A.; Picchio, V.; Asnaghi, V.; Bianchi, C.N.; Morri, C.; Oprandi, A.; Montefalcone, M. Troubles Never Come Alone: Outcome of Multiple Pressures on a Temperate Rocky Reef. *Water* **2023**, *15*, 825. https://doi.org/10.3390/w15040825

Academic Editor: José Luis Sánchez-Lizaso

Received: 27 January 2023
Revised: 14 February 2023
Accepted: 16 February 2023
Published: 20 February 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Rocky reef ecosystems are exposed to several co-occurring impacts [1] due to many different global and local pressures (e.g., pollution, overfishing, coastal works, spread of alien species, ocean acidification, and climate change), which threaten their state of health [2]. Any abiotic or biotic agent exceeding its range of normal variation and capable of altering the ecosystem's state is frequently defined as a stressor [3]. However, a distinction between stress and disturbance is necessary, as they have different ecological meanings: the former refers to a condition that restricts production, while the lattercauses partial or total destruction of biomass [4]. In the context of ecological dynamics, they are also distinguished based on their frequency [5], intensity, duration, and effects ([6], and references therein). For this reason, the new term 'affectors' has been proposed [6,7] to embrace both stressors (i.e., environmental factors that reduce the productivity within the ecosystem because of the energetic cost of adaptation) and disturbances (i.e., unpredictable, episodic events that disrupt the state of the ecosystem, causing abrupt mortality and a decrease in biomass).

When subjected to a single affector, ecosystems are generally able to recover, but under the pressure of multiple affectors they may undergo irreversible change [8]. The sequential and interactive consequences of multiple affectors alter the mechanisms that regulate community composition, leading to phase shifts [9]. To date, most ecological studies have investigated the responses of ecosystems to one single affector; however, the dynamics and structure of an ecosystem are rarely influenced by a single environmental factor [10]. The need to better understand the cumulative effects of multiple affectors is one of the most pressing goals in marine ecology and conservation [11].

Climate change is perhaps the most serious threat to marine ecosystems, triggering unpredictable consequences on their structure and functioning [12]. Both on land and in the ocean, the first direct effects of climate change are due to positive thermal anomalies such as the so-called heat waves—abnormally high temperatures for a short period in a particular region [13,14]. In recent decades, abrupt increases in sea water temperature have caused mass mortality of marine species [15–17] and changes in community composition and structure [18]. In addition, high temperatures have promoted the development of mucilaginous aggregates, with negative effects on benthic ecosystems [19,20].

Another worrisome effect of the increasing temperature is the range expansion of invasive alien species [21], which represents a worldwide threat to the integrity of ecosystems, to economy, and even to human health ([22], and references therein]. The impact of these species can be context-dependent, varying at different spatial and temporal scales [23]. In the Mediterranean Sea, one of the worst invasive species is the green alga *Caulerpa cylindracea* [24–26]. Its success has been facilitated by habitat loss due to human-related activities. As a consequence of this further pressure, the composition of native benthic assemblages changes [27] toward homogenisation [28]. As another consequence of climate change, the intensity and frequency of extreme events are increasing [29]. Hurricanes and storms have caused important impacts on marine ecosystems [30,31] by damaging coastal communities with the mechanical removal of benthic species [32,33].

Like many other coastal habitats, rocky reefs are subjected to both local and global pressures. While many studies have considered intertidal communities [34–36], the effects of multiple affectors on subtidal communities have rarely been investigated [37,38]. Rocky reefs represent environments of great scientific and economic value [39], due to the high biodiversity they host [40] and the ecosystem services they offer [41]. Understanding the effects of multiple affectors on rocky reefs is of fundamental importance for conservation policies [42].

This paper aims at evaluating the short-term changes (June to December) undergone by the rocky reef communities of the Portofino MPA during 2018, when different stressors (i.e., thermal anomalies, a mucilaginous event, and the seasonal expansion of *C. cylindracea*) came into action contemporaneously (mostly in full summer), while a disturbance, i.e. a severe storm, occurred on 29 October. The study was carried out at four different depths (10 m, 20 m, 30 m, and 40 m), under the hypothesis that the expected consequences are different along a vertical gradient.

The starting assumptions on which this study was based were: (i) the main driver of change in a temperate rocky reef should be seasonality [43], which mainly affects shallow water communities (at 10 m and 20 m depths); (ii) mucilage should affect first the communities at the thermocline (where mucilagethe aggregates form) and later the deep water ones (at 30 m and 40 m depths), where the mucilaginous aggregates typically fall in late summer, covering erect organisms, such as gorgonian corals [44]; (iii) the greatest impacts due to the expansion of *C. cylindracea* were expected at the 20 m depth, as already reported by previous studies [20]; (iv) the severe storm was expected to have mainly caused impacts on shallow water communities, most subjected to wave action.

2. Materials and Methods

2.1. Study Area

The Portofino Marine Protected Area (MPA), established in 1999, covers an area of 346 ha in the Ligurian Sea (NW Mediterranean) and encircles a rocky promontory, which spans out towards the sea for about 5 km. The southern front of the promontory is characterised by high vertical or sub-vertical cliffs, which continue underwater to a depth of about 50 m, while the eastern and western sides are comparatively shallower [44].

Increasing urbanisation of the Ligurian coast has amplified large-scale and chronic impacts, affecting marine ecosystems. Before the protection regime came into effect, climate change and local human pressures have led to a phase shift in the rocky reef communities of Portofino MPA in the 1990s [20,45].

2.2. Sea Surface Temperatures Data Management

Trends of the yearly mean sea surface temperatures (SST) from 1948 till 2019 were examined to evaluate the potential role of temperature as a driver of change in the rocky reef communities. As 2018 had been one of the warmest years on record [46], we compared the monthly SST values for 2018 with the climatological mean (monthly mean values) of the previous 70 years (1948 to 2017). For each month of 2018, thermal anomalies (a_{SST}) were detected by applying the follow formula:

$$a_{SST} = \frac{SST_{2018} - SST_{mean}}{\sigma}$$

where SST_{2018} is the monthly mean (±SD) for 2018, SST_{mean} is the monthly climatological mean, and σ is the standard deviation of the climatological mean. The differences between 2018 and the climatological mean were evaluated through a Mann–Kendall test [47].

The SST data were derived from NOAA (US National Oceanic and Atmospheric Administration; https://psl.noaa.gov/cgi-bin/data/timeseries/timeseries1.pl, accessed on 1 September 2022) satellite records.

2.3. Field Activities and Data Collection

Data on the rocky reef communities of the Portofino MPA were collected by scuba diving using photoquadrats in three sites (Figure 1): Altare (44°18′20″ N, 9°11′48″ E), Colombara (44°18′35″ N, 9°10′38″ E), and Torretta (44°18′45″ N, 9°10′03″ E). The three sites have similar environmental and morphological characteristics and are equally exposed to the considered affectors. To assess the differences in community composition and structure before and after the onset of seasonal and recurrent stressors (i.e., thermal anomalies, mucilaginous events, expansion of *Caulerpa cylindracea*), data were collected from 19 to 21 June and from 18 to 23 October 2018. Following the unpredictable storm that hit the Ligurian coast on 29 October, further data were collected between 12 and 13 December 2018 to quantify the impact of this disturbance. To analyse the effects of all affectors in relation to the depth, data were collected at 10 m, 20 m, 30 m, and 40 m depths. In each combination of sites and depths, 32 random photoquadrats (each covering an area of 50 × 50 cm^2) were taken (Figure 2).

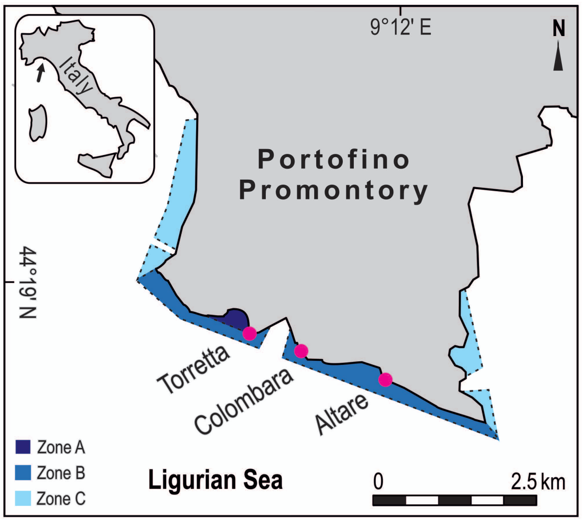

Figure 1. Map of the Portofino Marine Protected Area (Ligurian Sea, NW Mediterranean) with the three sites surveyed (Altare, Colombara, and Torretta) represented by hot pink circles. Geographic coordinates in WGS84.

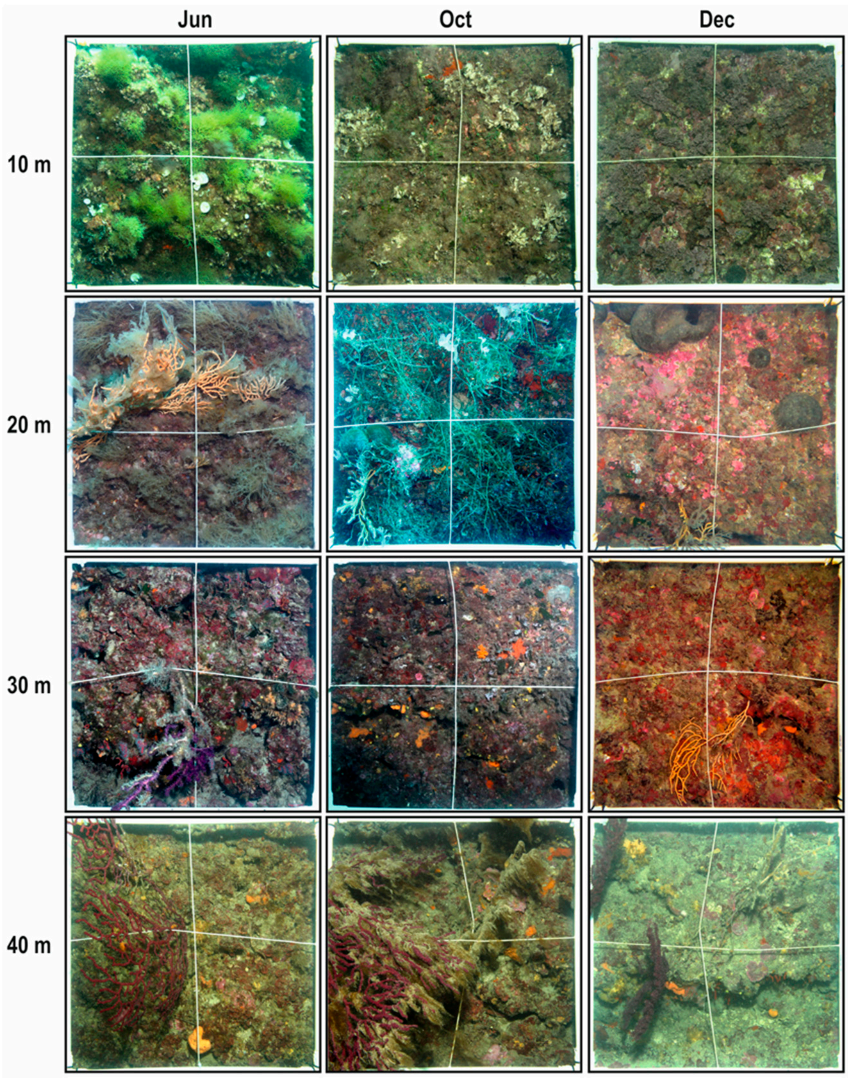

Figure 2. Examples of photoquadrats (each covering an area of 50 × 50 cm^2) carried out at three times (June, October, and December) and at four depths (10 m, 20 m, 30 m, and 40 m).

2.4. Photographic Analysis

Each photoquadrat was analysed using the software Image J [48] to calculate the percent cover of any organism present at the lowest possible taxonomic level (usually species). When species identification was not possible, lumped categories based on similar morphological and structural characteristics (i.e., OTUs, operational taxonomic units) were adopted [49,50]. The abiotic component (sediment and bare rock) was also recorded.

2.5. Community Data Management

Cover data of species and OTUs were organised in a matrix ((time × site × depth) × species/OTUs) and transformed by applying arcsine$\sqrt{(x/100)}$. A non-metric multidimensional scaling (nMDS) based on the Bray–Curtis index was applied to highlight potential changes over time and depth in rocky reef communities.

To test the differences in percent cover over time, a two-way permutational multivariate analysis of variance (PERMANOVA), followed by a PAIRWISE test for significant terms, was performed on orthogonal factors; 'time' (3 levels: June, October, and December) and 'depth' (4 levels: 10 m, 20 m, 30 m, 40 m) were fixed, while 'site' (3 levels: Altare, Colombara, and Torretta) was random.

Two time intervals were considered to highlight when the major changes occurred in the Portofino MPA rocky reef communities: June to October and October to December. The Pythagoras theorem was applied to the first two nMDS axis scores to measure the time trajectories between the two time intervals at the four depths [51]. For example, the time trajectory between June and October at 10 m depth was calculated as the geometric distance between the 10 m centroids of June and October as follows:

$$Tt_{10} = \sqrt{(x_O - x_J)^2 + (y_O - y_J)^2}$$

where Tt_{10} is the length of the time trajectory at 10 m, x_J and y_J are the axis scores of the 10 m centroid of June, and x_O and y_O are the axis scores of the 10 m centroid of October. Analogous formulas were applied to measure the time trajectories between June and October and between October and December at all the depths surveyed. Differences between time trajectories at each depth were evaluated by Student's *t*-test.

A SIMPER analysis based on the Bray–Curtis index was applied to identify the descriptors (species or OTUs) that mostly contributed to differences in community composition among the three times at each depth.

To assess the relative importance of the dominant species (cover > 5%) in determining overall changes in community composition and structure, a graphical display was produced, where each time (June, October, December) was represented by the species/OTUs mean value. In addition, the Shannon–Wiener diversity index (computed with the natural logarithm ln) was applied to evaluate whether changes in the community composition were reflected in the community structure (diversity).

Analyses were performed using the software PaSt [52], Primer 6 + PERMANOVA [53], and RStudio [54] packages.

2.6. Role of Seasonality in Community Change

Change in cover data of seasonal species (i.e., the sum of all the seasonal species, the alien *C. cylindracea* excluded) were considered as the community response to variation in the thermal regime; these data were analysed to highlight their trend in the investigated period. Differences among the three times at each depth were evaluated by Kruskall–Wallis test.

A new simulated data matrix ((time × depth) × species/OTUs), excluding seasonal species, was generated to distinguish the community changes due to seasonality from those referable to the other affectors. We used the term 'simulated' to make clear that we did not perform a real, physical removal experiment in the field. This procedure has already been applied for studies involving a so-called 'inclusion versus exclusion' approach, in which the descriptor of interest is an integral part of the response ([28], and reference therein). An nMDS analysis based on the Bray–Curtis index was applied to the simulated data matrix, and the time trajectories between June and October and between October and December were measured using the axis scores, as done for the original data matrix. Differences between the time trajectories of the original data matrix and the simulated one were evaluated by Student's *t*-test.

2.7. Data Management for Mucilage, Caulerpa cylindracea, and the Severe Storm

The cover data for mucilage and *C. cylindracea* were analysed to highlight their trends in the investigated period. Differences among the three times at each depth were evaluated by Kruskall–Wallis test.

As done for seasonal species, two new simulated data matrices ((time × depth) × species/OTUs), each excluding one stressor (mucilage or *C. cylindracea*), were generated to test the effect of such stressor on the rocky reef community composition. An nMDS analysis based on the Bray–Curtis index was applied to each simulated data matrix, and the time trajectories between June and October and between October and December were measured using the axis scores, as done for the original data matrix. Differences between the time trajectories of the original data matrix and the simulated ones were evaluated by Student's *t*-test.

Differences in the cover of abiotic components and perennial species, which are not expected to show seasonal variation, between October and December at each depth were analysed by Student's *t*-test to evaluate the effect of the storm. The species or OTUs that most contributed to differences were identified through a SIMPER analysis.

3. Results

3.1. Sea Surface Temperature and Thermal Anomalies

An analysis of the climatological mean SST showed a positive trend for the last 70 years (Figure 3A). In 2018, the SST yearly mean value was 17.4 ± 1.42 °C, 1.2 °C higher than the yearly mean for 1948–2017. The yearly mean SST for 2018 was slightly higher than that of the year 2003 (17.2 ± 1.4 °C), when a severe mass mortality event occurred in the northwestern Mediterranean rocky reef communities [15]. Comparing the monthly SST values for 2018 with the monthly SST mean values of the previous 70 years unveiled uninterrupted thermal anomalies from August to November (Figure 3B, Table 1).

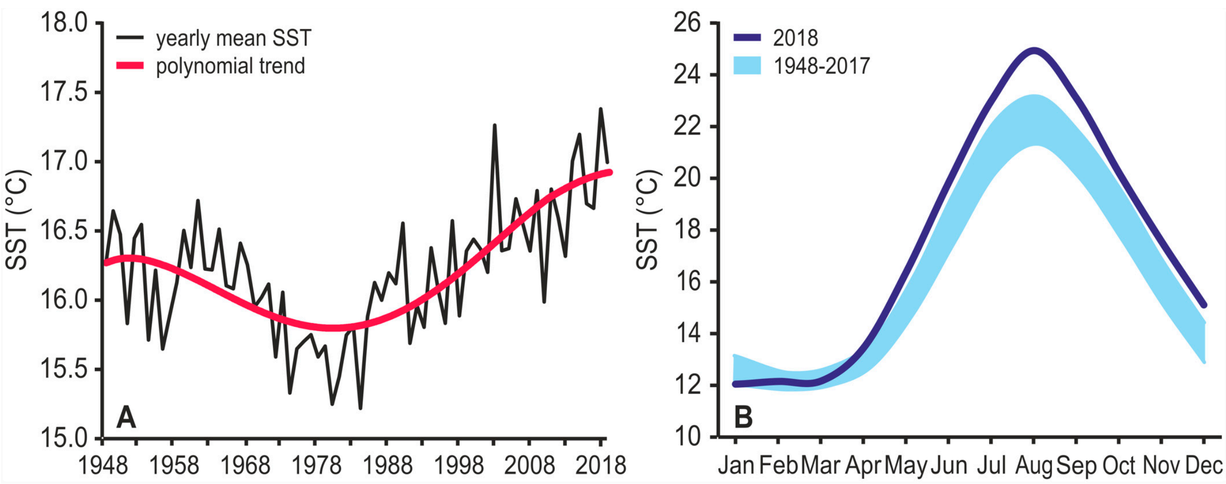

Figure 3. (**A**) Fourth-degree polynomial trend (r = 0.75) of the yearly mean sea surface temperature (SST) values in the Portofino MPA from 1948 to 2019. (**B**) The 2018 SST monthly values compared to the SST overall monthly mean values of the previous 70 years. The SST values were derived from NOAA satellite data (www.esrl.noaa.gov/psd/cgi-bin/data/timeseries/timeseries1.pl, accessed on 1 September 2022).

Table 1. The 2018 thermal anomalies (in bold) detected by applying the method proposed by Jiménez-Muñoz et al. [47].

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Thermal anomalies (°C)	−0.7	0.1	−0.4	0.6	1.4	1.4	1.8	**3.0**	**2.6**	**1.9**	**2.0**	2.0
Mann-Kendall test (*p*)	0.470	0.061	0.203	0.216	0.743	0.116	0.060	**0.001**	**0.009**	**0.001**	**0.003**	0.237

3.2. Changes in Community Composition and Structure

A total of 91 species and 7 OTUs were recorded (Supplementary Materials, Tables S1 and S2). The nMDS applied to the cover data ordered the photoquadrat points according to two gradients: a spatial one along the 1st (horizontal) axis, and a temporal one along the 2nd (vertical) axis (Figure 4A). The spatial gradient was an expression of increasing depths from the left (10 m) to the right (40 m) of the 1st axis.

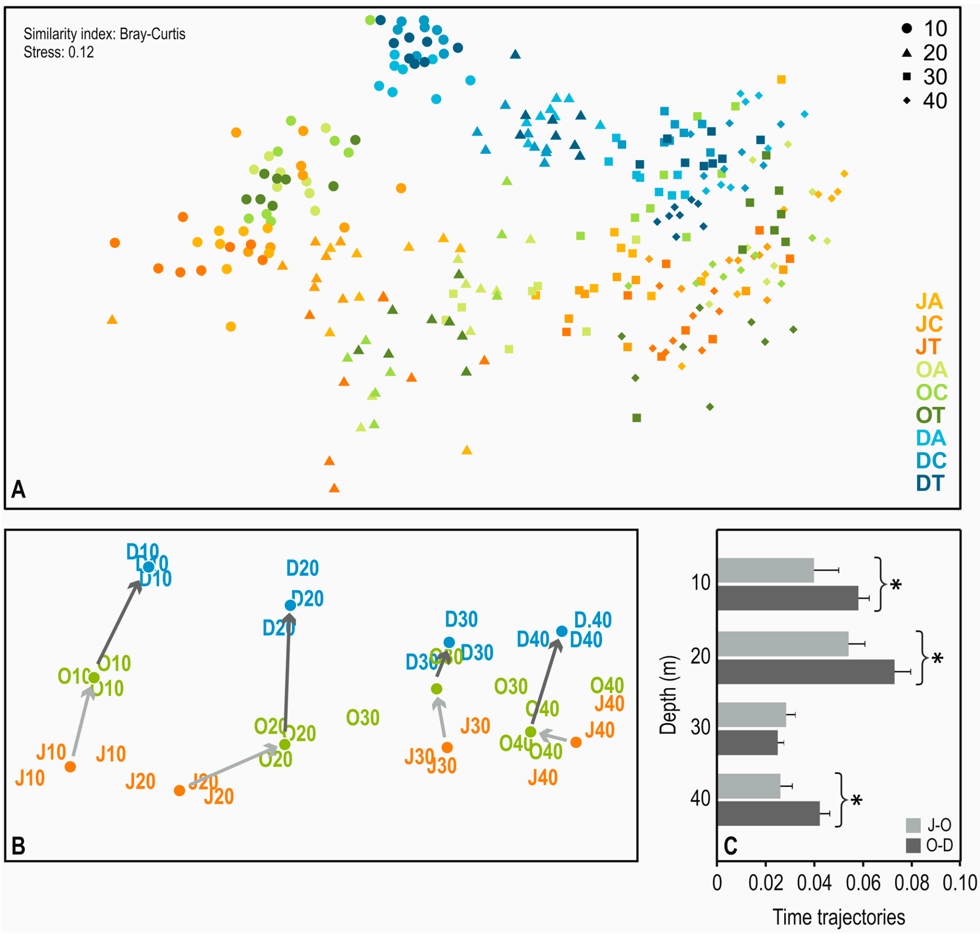

Figure 4. (**A**) Multivariate analysis (nMDS) plot of Portofino MPA species/OTU ratios. Individual observation points are represented by different colours and shapes according to time (J = June, shown in orange; O = October, shown in green; D = December, shown in blue) and depth. The colour gradations represent different sites at the three times (A = Altare; C = Colombara; T = Torretta). (**B**) The nMDS plot showing only the centroids and with time trajectories represented by arrows (light grey for trajectories between June and October; dark grey for trajectories between October and December). (**C**) Differences (* = significance $p < 0.001$) between time trajectory values (derived from axis scores and analysed with Student's *t*-test) for June to October and October to December at each depth (10 m, 20 m, 30 m, and 40 m).

The temporal gradient was expressed from bottom to top by seasonality, with the points of June, October, and December well separated from each other (Figure 4A,B). The nMDS did not show any ordination of photoquadrat points according to the factor site (Figure 4A).

The PERMANOVA results evidenced significant differences for the interactions between the time, site, and depth factors (Table 2). The PAIRWISE results showed significant differences for each combination of the three factors, indicating a pervasive change with time along the whole depth gradient in all the sites considered (Table 2).

Table 2. Results of the PERMANOVA and PAIRWISE tests applied on rocky reef communities of Portofino MPA. Times = June (J), October (O), and December (D); sites = Altare, Colombara, and Torretta; depth = 10, 20, 30, and 40 m.

PERMANOVA					
Source	**SS**	**Df**	**R^2**	**F**	***P***
Time	6.032	2	0.135	65.185	**0.001**
Site	1.018	2	0.023	11.002	**0.001**
Depth	17.854	3	0.399	128.626	**0.001**
Time × Site	0.433	4	0.009	2.342	**0.002**
Time × Depth	4.072	6	0.091	14.670	**0.001**
Site × Depth	2.090	6	0.047	7.527	**0.001**
Time × Site × Depth	1.532	12	0.034	2.7592	**0.001**
Residual	11.660	252	0.260		
Total	44.492	287			

PAIRWISE test									
	Altare			**Colombara**			**Torretta**		
	J ≠ O	**J ≠ D**	**O ≠ D**	**J ≠ O**	**J ≠ D**	**O ≠ D**	**J ≠ O**	**J ≠ D**	**O ≠ D**
10 m	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001
20 m	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001
30 m	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.001
40 m	0.022	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001

Comparing the time trajectories between June and October and between October and December at each depth by Student's *t*-test, significant differences were observed at 10 m (p = 0.004), 20 m (p = 0.009), and 40 m (p = 0.200). The magnitude of change between October and December was always greater than that between June and October, highlighting that the major changes in rocky reef communities occurred from October to December (Figure 4C).

The SIMPER analysis identified the descriptors that mostly contributed to community change at each depth in the two time intervals considered (Table 3): (i) *Dictyota dichotoma* at 10 m and 20 m depth between June and October; (ii) *Jania* spp. at 10 m depth in both time intervals and at 20 m depth between June and October; (iii) *Padina pavonica* at 20 m depth between October and December; (iv) mucilage at 40 m in both time intervals; (v) *Caulerpa cylindracea* especially at 20 m, and secondarily at 10 m and 30 m, in both time intervals; (vi) *Ellisolandia elongata* at 10 m in both time intervals; (vii) encrusting corallines at 10 m and secondarily at 20 m between October and December; (viii) *Halopteris scoparia* at 10 m and 20 m in both time intervals; (ix) sediment at all depths investigated in both time intervals.

Table 3. Descriptors that mostly contributed (>5%) to the similarity between June–October and between October–December identified by SIMPER analysis (based on the Bray–Curtis index). J-O: June to October; O-D: October to December.

SIMPER Analysis								
	10 m		20 m		30 m		40 m	
	J-O	O-D	J-O	O-D	J-O	O-D	J-O	O-D
Seasonality								
Dictyota dichotoma	30.6		18.0					
Jania spp.	10.7	16.5	5.1					
Padina pavonica				6.3				
Stressors								
Mucilage			7.9		7.7		19.4	15.6
Caulerpa cylindracea		5.0	19.4	24.6	7.0	8.4		
Others								
Ellisolandia elongata	6.0	12.2						
Encrusting corallines		11.8		9.0		6.2		
Halopteris scoparia	15.2	14.6	5.3					
Sediment	5.8	5.3	5.7	6.6	10.3	7.5	13.3	14.9

The species composition of the Portofino MPA community changed over time, mainly due to both the descriptors that represent the effect of change (i.e., seasonal species and perennial species after the storm) and the descriptors that are at the same time the cause and the effect of change (i.e., mucilage and *Caulerpa cylindracea*) (Figure 5).

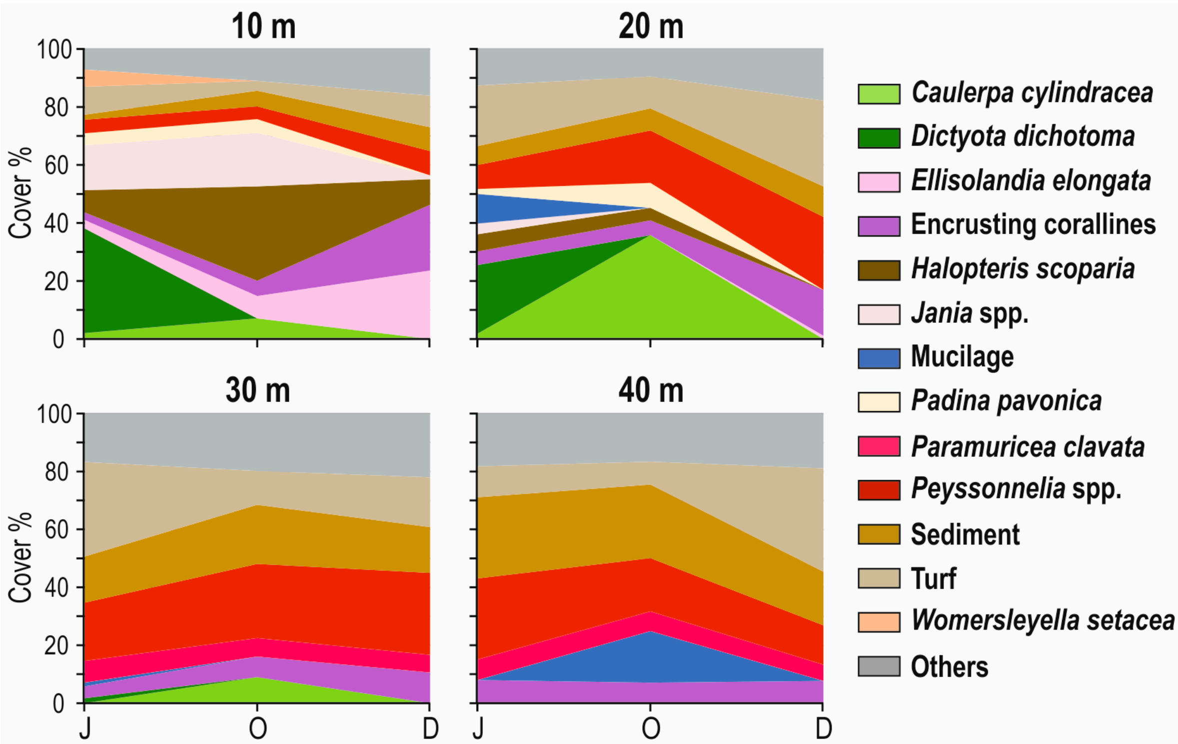

Figure 5. Compositional change in the rocky reef communities of Portofino MPA at the three times investigated (J = June; O = October; D = December). The descriptor 'others' includes species whose cover was always <5%.

The Shannon–Wiener diversity index did not show relevant differences between the communities at the three time points investigated, with the partial exception of 20 m depth, where a slight decrease was observed in October (Figure 6).

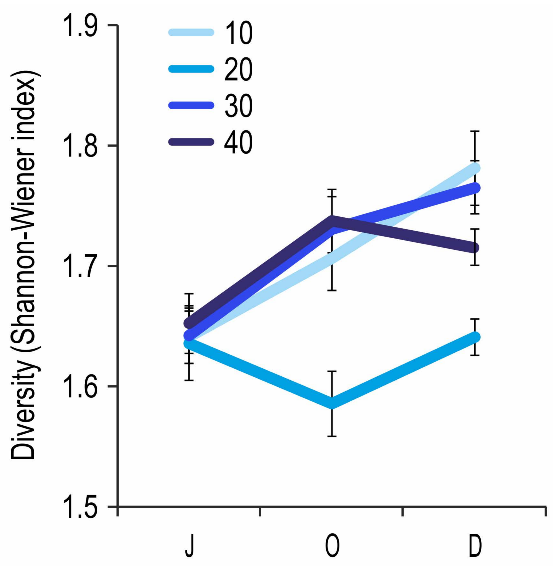

Figure 6. The community structure, expressed by mean (±se) diversity (Shannon–Wiener index), of the Portofino MPA at the three time periods investigated (J = June; O = October; D = December).

3.3. Seasonal Variations

The Kruskall–Wallis test applied to cover data for seasonal species at the three times evidenced significant differences at 10 m, 20 m, and 30 m depth (Figure 7A). The greatest changes were observed at 10 m, where the cover of seasonal species decreased significantly ($p = 3.36 \cdot 10^{-14}$) from June (67.7 ± 1.9) to October (37.1 ± 2.1) and again in December (5.3 ± 0.5). Significant changes also occurred at 20 m, where cover increased significantly ($p = 2.42 \cdot 10^{-11}$) from June (33.1 ± 4.2) to October (41.5 ± 3.5) and decreased from October to December (1.4 ± 0.2). The algal species that exhibited the greatest variations were *Dictyota dichotoma*, *Jania* spp., and *Padina pavonica*, which were abundant in June and October and then disappeared in December at both 10 m and 20 m depth (Figure 5).

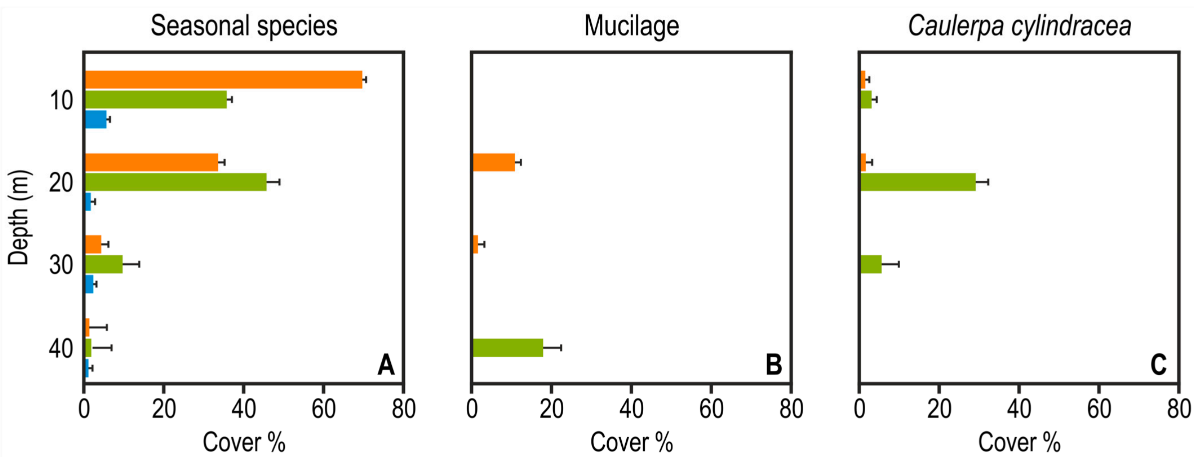

Figure 7. Percent cover (+ se) of (**A**) seasonal species, (**B**) mucilage, and (**C**) *Caulerpa cylindracea* at the three times (June in orange, October in green, December in blue) at the four depths surveyed (10 m, 20 m, 30 m, and 40 m).

Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix confirmed significant change in rocky reef communities between October and December at 10 m (p = 0.02) and 20 m (p = 0.02) depth (Table 4).

Table 4. The p values of Student's *t*-test, applied to analyse the differences between time trajectories (J-O: June to October; O-D: October to December) of the original data matrix and the simulated ones at the four depths investigated. Significant differences are in bold.

	***p* Values ANOVA**					
	Seasonal Species		**Mucilage**		***Caulerpa cylindracea***	
	J-O	**O-D**	**J-O**	**O-D**	**J-O**	**O-D**
10 m	0.265	**0.021**	0.102	0.956	0.375	0.181
20 m	0.547	**0.022**	0.665	0.710	0.391	**0.002**
30 m	0.531	0.547	**0.037**	0.183	0.109	0.342
40 m	0.256	0.094	**0.005**	**0.000**	0.115	**0.003**

3.4. Proliferation of Mucilaginous Aggregates

The Kruskal–Wallis test applied to mucilage cover data at the three times evidenced major differences at 20 m and 40 m (Figure 7B). The mucilage decreased significantly ($p = 1.10 \cdot 10^{-6}$) from June (10.2 $\pm$ 4.1) to October (0.2 $\pm$ 0.1) at 20 m; on the contrary, it increased significantly ($p = 9.82 \cdot 10^{-14}$) from June (0.1 $\pm$ 0.1) to October (17.7 $\pm$ 2.2) at 40 m. At both depths, the mucilage disappeared completely in December.

Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix showed significant differences at 40 m depth (Table 4), both between June and October (p = 0.005) and between October and December ($p = 7.89 \cdot 10^{-5}$).

3.5. Caulerpa cylindracea Expansion

The Kruskall–Wallis test applied to the cover of *C. cylindracea* at the three times evidenced major changes at 20 m depth, where *C. cylindracea* increased significantly ($p = 5.24 \cdot 10^{-13}$) from June (1.8 $\pm$ 0.3) to October (35.8 $\pm$ 3.4), disappearing in December (Figure 7C).

The Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix confirmed significant differences between October and December at the 20 m (p = 0.002) depth (Table 4).

3.6. Effects of the Storm

The SIMPER analysis highlighted the following perennial species and abiotic components as responsible for the main significant differences betweencommunities between October and December: *Ellisolandia elongata*, encrusting corallines, *Halopteris scoparia*, and sediment (Table 3). All exhibited significant variations in cover between the two times (Figure 8). *E. elongata* increased significantly from 7.7 $\pm$ 1.2 to 23.6 $\pm$ 1.9 ($p = 1.69 \cdot 10^{-6}$) at 10 m depth. The encrusting corallines increased significantly from 5.3 $\pm$ 0.6 to 22.7 $\pm$ 1.4 ($p = 2.71 \cdot 10^{-10}$) at 10 m depth and from 5.1 $\pm$ 0.4 to 15.8 $\pm$ 1.3 ($p = 3.18 \cdot 10^{-7}$) at 20 m. *H. scoparia* decreased significantly from 32.5 $\pm$ 2.5 to 8.8 $\pm$ 0.5 ($p = 7.19 \cdot 10^{-9}$) at 10 m and from 4.3 $\pm$ 1.8 to zero ($p = 2.11 \cdot 10^{-2}$) at 20 m. The sediment increased significantly from 5.4 $\pm$ 0.7 to 8.8 $\pm$ 0.6 ($p = 1.18 \cdot 10^{-3}$) at 10 m depth and from 7.6 $\pm$ 1.4 to 11.5 $\pm$ 1.4 ($p = 5.42 \cdot 10^{-2}$) at 20 m depth, while it decreased significantly from 20.8 $\pm$ 1.3 to 16.2 $\pm$ 0.7 ($p = 9.93 \cdot 10^{-3}$) at 30 m depth and from 25.9 $\pm$ 2.1 to 19.1 $\pm$ 3.3 ($p = 9.36 \cdot 10^{-2}$) at 40 m depth.

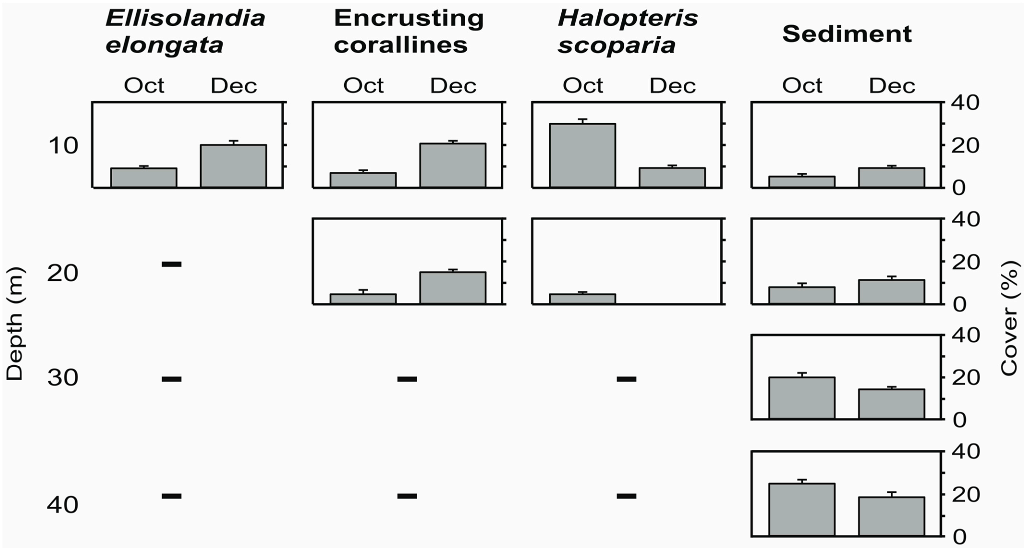

Figure 8. Percent cover (+se) of the species or abiotic components, indicating the effects of the storm and identified by the SIMPER analysis (see Table 3), before (Oct = October) and after (Dec = December) the storm occurrence at four different depths (10 m, 20 m, 30 m, and 40 m).

3.7. Magnitude of Pressures According to the Depth

Each single affector exhibited different impacts along the vertical gradient (Figure 9). The seasonality contributed mainly to the changes in shallow water communities (10 m and 20 m). The mucilage affected benthic communities at different times and depths: in June at 20 m and in October at 40 m. The highest cover of *C. cylindracea* was recorded at 20 m. The severe storm had impacts along all the water column, but especially at 10 m and 20 m depth.

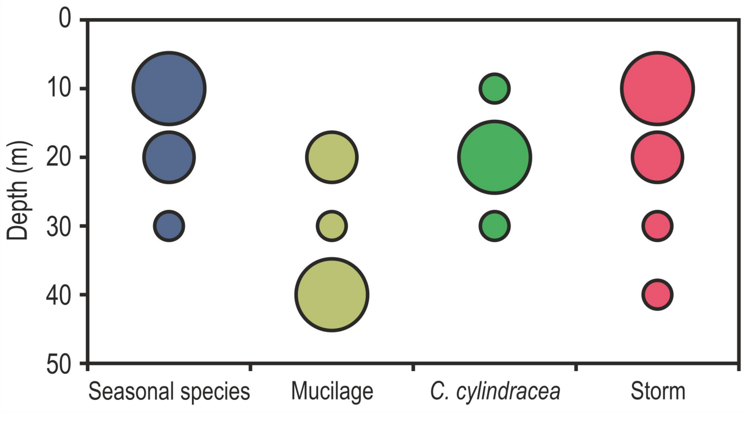

Figure 9. A schematic illustration of the level of pressure exerted by affectors considered (seasonality, mucilage, *Caulerpa cylindracea*, and the storm of 29 October) on the rocky reefs of Portofino MPA at the four depths investigated (circle sizes are roughly proportional to their intensity).

4. Discussion

In 2018, the rocky reef communities of Portofino MPA suffered three major stressors (high temperature, mucilage, and the invasive alien alga *Caulerpa cylindracea*) and were subjected to a severe disturbance (the storm of 29 October). Sea surface temperature (SST) was the highest over the last 70 years, slightly surpassing the hot season of 2003 [15]; similarly, the mucilaginous event equalled the one in 2003 [19,20], and a peak in the cover of *C. cylindracea* occurred [55]. The October storm was the most severe that ever hit the Ligurian coast [33]. The cumulative action of all these affectors triggered significant changes in the rocky reef communities in all sites considered from June to December. However, although changes in community composition were evident, this was not reflected in a change in community structure (diversity). Species diversity is an emergent property of ecosystems that is often maintained within narrow limits, notwithstanding change in species composition [56].

When multiple affectors act simultaneously, as happened at Portofino MPA, each one may produce similar or distinct effects [57,58]. Distinguishing the outcome of every single affector, even excluding natural variability, is difficult [39,59]. Taking into account four depths, under the hypothesis that every single affector impacted differently along the vertical gradient, this study explored the possibility to face this difficulty.

Mediterranean coastal benthic ecosystems are strongly subjected to the seasonal variation of environmental factors [60], but the effects of seasonality are damped with depth [43]. As expected, at Portofino MPA, the major variation in the cover of seasonal species was recorded at the 10 m depth. However, the observed decrease in perennial species, such as *Halopteris scoparia*, and the increase in *Ellisolandia elongata* and encrusting corallines cannot be explained by the effects of seasonality alone. A possible explanation is that the October storm cleaned off the soft-bodied species *H. scoparia* from of the upper layer, making more visible the hard, calcified, and more resistant species (*E. elongata* and the encrusting corallines) of the basal layer.

Overall, the time trajectories illustrated that the greatest change in the rocky reef community between June and December occurred at 20 m depth, in response not only to seasonality but also to other affectors. The mucilaginous aggregates formed mostly at this depth, just below the summer thermocline [20]. The invasive alga *C. cylindracea* showed the highest cover there, conforming to previous years [28]. As observed at 10 m depth, the decrease in *H. scoparia* and the increase in calcified corallines in December point to the cleaning effect of the storm.

At 30 m depth, only small differences in community composition were observed from June to December; this result was consistent with the trend for all affectors that did not show noteworthy changes at this depth. A slight quantity of mucilage was observed only in October, and a negligible cover of *C. cylindracea* was recorded in June. Although significant, the variation in the cover of seasonal species was modest overall.

The significant change at 40 m depth was attributable to the fall of mucilage in late summer; while not being present in June, the mucilaginous aggregates covered a wide extent of the seafloor in October [20], only to completely disappear in December. The seasonal species and *C. cylindracea* were slight or not represented at this depth.

The only affector that impacted all four depths investigated was the severe storm that occurred at the end of October. Besides the cleaning effect on the species of the upper layer, the storm redistributed sediment along the depth gradient. In this case, the removal of the upper layer, which occurred mostly in the algal-dominated shallow-water communities, may also have made the sediment more visible on the rocky substrate.

Notwithstanding the significant interactions between the various sources of variability, the initial hypothesis that exploring different depths could have disentangled the effects of the four affectors was corroborated by the results of the present study, which adopted three different methodological approaches: (i) a multivariate analysis that allowed the time trajectories of change to be measured; (ii) the variation in cover of selected descriptors (species/OTUs) used as proxies for natural variability and the three affectors considered; (iii) the exploration of simulated matrices, where three of the proxies (i.e., seasonality, mucilage, and *C. cylindracea*) were excluded in turn.

No evident effect was observed due to the thermal anomalies recorded in the period investigated. The species more vulnerable to the increase in water temperature (e.g., *Paramuricea clavata* and sponges) did not show any decrease in cover following the thermal anomalies. The short period of the present study obviously did not allow an assessment of the long-term effects of such affector on vulnerable species. Unequivocal evidence of the effects of increasing temperature on rocky reefs has been reported elsewhere [15–17], and according to the predictions, thermal anomalies will lead to reductions in the number of taxa composing the benthic communities [61].

The effect of the storm was more evident at 10 m depth but involved all communities at the four depths investigated. Extreme storms are certainly disruptive events, and the increase in their frequency in recent decades raises concern for the future of biological communities [62]. In the marine realm, several studies about the effects of tropical hurricanes have been conducted on coral reefs (but less so in temperate reefs), highlighting species removal and sediment reshuffling [31,63], as observed at the Portofino MPA. This study analysed the effects of the severe storm shortly after (about one month later) its occurrence, and only through future monitoring will it be possible to assess the recovery capacity of benthic communities after this disturbance.

The mucilage, following a first bloom at 20 m, mostly impacted the community at 40 m depth covering erect species. Massive developments of mucilage are considered a worrisome ecological threat to marine ecosystems [64]. Covering wide areas of the substrate, mucilage limits light penetration and alters chemical and physical environments, causing anoxic conditions beneath [65]. Signs of necrosis due to mucilaginous events have been described for several benthic taxa in many different Mediterranean areas ([66], and references therein). However, no reduction in species cover was observed in December that was attributable to the mucilage, but once again sustained monitoring would be necessary to assess the long-term effects of this single affector.

C. cylindracea peaked at 20 m, where negative effects on diversity and spatial heterogeneity have already been demonstrated [28]. *C. cylindracea* was shown to easily colonise areas subject to loss of native species cover, and to prevent their recovery by facilitating the settlement and persistence of alternative assemblages dominated by stress-tolerant species [67,68]. Transient ecological effects, generated by a temporary deviation from the original disturbance regime, are, thus, transformed into permanent changes [25].

The cumulative effect of the considered pressures peaked at the 20 m depth, where the greatest changes in the rocky reef community were documented. Overall, these results suggest that the cumulative effect of multiple affectors is stronger than that of any individual affector, no matter how intense it is.

Notwithstanding the consistent results, our approach undoubtedly has several inherent limits: (i) despite the analyses conducted on the seasonal species, it is difficult to completely exclude natural variability when assessing changes over time due to the other affectors considered, two of which (mucilage and *C. cylindracea*) also exhibit seasonal trends; (ii) the lack of comparable information in the years before and after 2018 limits the possibility to infer cause–effect relationships between affectors and community change; (iii) considering only one sampling time before and after each affector may not be enough to thoroughly explore the sources of variation.

Interactions among multiple affectors, which can trigger abrupt changes in marine communities, are still poorly understood, and their consequences pose important challenges to researchers and conservationists alike [60,69]. Historical data series and the revisitation of sites already surveyed in the past are essential to illustrate ecological change [59,70], but short-term studies considering the multiple affectors acting almost simultaneously may help disentangle their effects. The importance of analysing ecological changes at different scales has been underlined on several occasions ([71], and reference therein). As a short-term scale example, this study may represent an attempt to describe the dynamics of temperate rocky reef communities following the impacts of co-occurring punctual events.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15040825/s1. Table S1: List of the 91 species and 7 OTUs recorded in the Portofino MPA. For each species the presence (1) and absence (0) in the three months surveyed at the four different depths are reported. Table S2: List of the conspicuous species and OTUs (cover > 1%) recorded in the Portofino MPA, ordered alphabetically. For each species the mean cover (±SE) values for the three months surveyed at the four different depths are reported.

Author Contributions: A.A.: Investigation, conceptualisation, data curation, formal analysis, writing—original draft. V.P.: Investigation, data curation, formal analysis. V.A.: Data curation, methodology, formal analysis. C.N.B.: Conceptualisation, methodology, writing—review and editing. C.M.: Conceptualisation, methodology, writing—review and editing. A.O.: Investigation, writing—review and editing. M.M.: Conceptualisation, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Acknowledgments: Thanks are due to Lorenzo Meroni (University of Genoa) for his scientific advice. Alix Varenne, Chiara Redoano, and Silvia Mangraviti contributed to the photographic analyses. The logistical support during field activities provided by Andrea Galliadi (Diving Evolution, Santa Margherita Ligure) is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Korpinen, S.; Laamanen, L.; Bergström, L.; Nurmi, M.; Andersen, J.H.; Haapaniemi, J.; Harvey, E.T.; Murray, C.J.; Peterlin, M.; Kallenbach, E.; et al. Combined effects of human pressures on Europe's marine ecosystems. *Ambio* **2021**, *50*, 1325–1336. [CrossRef] [PubMed]
2. Halpern, B.S.; Selkoe, K.A.; Micheli, F.; Kappel, C.V. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. *Conserv. Biol.* **2007**, *21*, 1301–1315. [CrossRef] [PubMed]
3. Barrett, G.W.; Van Dyne, G.M.; Odum, E.P. Stress ecology. *BioScience* **1976**, *26*, 192–194. [CrossRef]
4. Grime, J.P. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* **1977**, *111*, 1169–1194. [CrossRef]
5. Borics, G.; Várbíró, G.; Padisák, J. Disturbance and stress: Different meanings in ecological dynamics? *Hydrobiologia* **2013**, *711*, 1–7. [CrossRef]
6. Montefalcone, M.; Parravicini, V.; Bianchi, C.N. Quantification of coastal ecosystem resilience. In *Treatise on Estuarine and Coastal Science*; Wolanski, E., McLusky, D.S., Eds.; Academic Press: Waltham, UK, 2011; Volume 10, pp. 49–70.
7. Montefalcone, M.; Morri, C.; Bianchi, C.N.; Bavestrello, G.; Piazzi, L. The two facets of species sensitivity: Stress and disturbance on coralligenous assemblages in space and time. *Mar. Pollut. Bull.* **2017**, *117*, 229–238. [CrossRef]
8. Jaiswal, D.; Pandey, U.; Mishra, V.; Pandey, J. Integrating resilience with functional ecosystem measures: A novel paradigm for management decisions under multiple-stressor interplay in freshwater ecosystems. *Glob. Change Biol.* **2021**, *27*, 3699–3717. [CrossRef]
9. Davis, J.; Sim, L.; Chambers, J. Multiple stressors and regime shifts in shallow aquatic ecosystems in antipodean landscapes. *Freshw. Biol.* **2010**, *55*, 5–18. [CrossRef]
10. Polazzo, F.; Rico, A. Effects of multiple stressors on the dimensionality of ecological stability. *Ecol. Lett.* **2021**, *24*, 1594–1606. [CrossRef]
11. Ellis, J.; Hewitt, J.; Clark, D.; Taiapa, C.; Patterson, M.; Sinner, J.; Hardy, D.; Thrush, S. Assessing ecological community health in coastal estuarine systems impacted by multiple stressors. *J. Exp. Mar. Biol. Ecol.* **2015**, *473*, 176–187. [CrossRef]
12. Henson, S.A.; Beaulieu, C.; Ilyina, T.; John, J.G.; Long, M.; Séférian, R.; Tjiputra, J.; Sarmiento, J.L. Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nat. Commun.* **2017**, *8*, 14682. [CrossRef]
13. Hobday, A.J.; Alexander, L.V.; Perkins, S.E.; Smale, D.A.; Straub, S.C.; Oliver, E.C.; Benthuysen, J.A.; Burrows, M.T.; Donat, M.G.; Feng, M.; et al. A hierarchical approach to defining marine heatwaves. *Prog. Oceanogr.* **2016**, *141*, 227–238. [CrossRef]
14. Frölicher, T.L.; Fischer, E.M.; Gruber, N. Marine heatwaves under global warming. *Nature* **2018**, *560*, 360–364. [CrossRef] [PubMed]
15. Garrabou, J.; Coma, R.; Bensoussan, N.; Bally, M.; Chevaldonné, P.; Cigliano, M.; Diaz, D.; Harmelin, J.G.; Gambi, M.C.; Kersting, D.K.; et al. Mass mortality in Northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Glob. Change Biol.* **2009**, *15*, 1090–1103. [CrossRef]
16. Garrabou, J.; Gómez-Gras, D.; Ledoux, J.-B.; Linares, C.; Bensoussan, N.; López-Sendino, P.; Bazairi, H.; Espinosa, F.; Ramdani, M.; Grimes, S.; et al. Collaborative database to track mass mortality events in the Mediterranean Sea. *Front. Mar. Sci.* **2019**, *6*, 707. [CrossRef]
17. Garrabou, J.; Gómez-Gras, D.; Medrano, A.; Cerrano, C.; Ponti, M.; Schlegel, R.; Bensoussan, N.; Turicchia, E.; Sini, M.; Gerovasileiou, V.; et al. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Glob. Change Biol.* **2022**, *28*, 5708–5725. [CrossRef]

18. Bianchi, C.N.; Azzola, A.; Parravicini, V.; Peirano, A.; Morri, C.; Montefalcone, M. Abrupt change in a subtidal rocky reef community coincided with a rapid acceleration of sea water warming. *Diversity* **2019**, *11*, 215. [CrossRef]
19. Schiaparelli, S.; Castellano, M.; Povero, P.; Sartoni, G.; Cattaneo-Vietti, R. A benthic mucilage event in North-Western Mediterranean Sea and its possible relationships with the summer 2003 European heatwave: Short term effects on littoral rocky assemblages. *Mar. Ecol.* **2007**, *28*, 341–353. [CrossRef]
20. Bianchi, C.N.; Azzola, A.; Bertolino, M.; Betti, F.; Bo, M.; Cattaneo-Vietti, R.; Cocito, S.; Montefalcone, M.; Morri, C.; Oprandi, A.; et al. Consequences of the marine climate and ecosystem shift of the 1980-90s on the Ligurian Sea biodiversity (NW Mediterranean). *Eur. Zool. J.* **2019**, *86*, 458–487. [CrossRef]
21. Galil, B.S. Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. *Mar. Pollut. Bull.* **2007**, *55*, 314–322. [CrossRef]
22. Streftaris, N.; Zenetos, A. Alien Marine Species in the Mediterranean—The 100 'Worst Invasives' and their Impact. *Mediterr. Mar. Sci.* **2006**, *7*, 87–118. [CrossRef]
23. Thomsen, M.S.; Wernberg, T.; Olden, J.D.; Griffin, J.N.; Silliman, B.R. A framework to study the context-dependent impacts of marine invasions. *J. Exp. Mar. Biol. Ecol.* **2011**, *400*, 322–327. [CrossRef]
24. Montefalcone, M.; Morri, C.; Parravicini, V.; Bianchi, C.N. A tale of two invaders: Divergent spreading kinetics of the alien green algae *Caulerpa taxifolia* and *Caulerpa cylindracea*. *Biol. Invasions* **2015**, *17*, 2717–2728. [CrossRef]
25. Piazzi, L.; Balata, D.; Bulleri, F.; Gennaro, P.; Ceccherelli, G. The invasion of *Caulerpa cylindracea* in the Mediterranean: The known, the unknown and the knowable. *Mar. Biol.* **2016**, *163*, 1–14. [CrossRef]
26. Tsirintanis, K.; Azzurro, E.; Crocetta, F.; Dimiza, M.; Froglia, C.; Gerovasileiou, V.; Langeneck, J.; Mancinelli, G.; Rosso, A.; Stern, N.; et al. Bioinvasion impacts on biodiversity, ecosystem services, and human health in the Mediterranean Sea. *Aquat. Invasions* **2022**, *17*, 308–352. [CrossRef]
27. Casoli, E.; Mancini, G.; Ventura, D.; Belluscio, A.; Ardizzone, G. Double trouble: Synergy between habitat loss and the spread of the alien species *Caulerpa cylindracea* (Sonder) in three Mediterranean habitats. *Water* **2021**, *13*, 1342. [CrossRef]
28. Morri, C.; Montefalcone, M.; Gatti, G.; Vassallo, P.; Paoli, C.; Bianchi, C.N. An alien invader is the cause of homogenization in the recipient ecosystem: A simulation-like approach. *Diversity* **2019**, *11*, 146. [CrossRef]
29. Stott, P. How climate change affects extreme weather events. *Science* **2016**, *352*, 1517–1518. [CrossRef]
30. Renaud, P.E.; Ambrose Jr, W.G.; Riggs, S.R.; Syster, D.A. Multi-level effects of severe storms on an offshore temperate reef system: Benthic sediments, macroalgae, and implications for fisheries. *Mar. Ecol.* **1996**, *17*, 383–398. [CrossRef]
31. Baird, A.H.; Álvarez-Noriega, M.; Cumbo, V.R.; Connolly, S.R.; Dornelas, M.; Madin, J.S. Effects of tropical storms on the demography of reef corals. *Mar. Ecol. Prog. Ser.* **2018**, *606*, 29–38. [CrossRef]
32. Betti, F.; Bavestrello, G.; Bo, M.; Enrichetti, F.; Cattaneo-Vietti, R. Effects of the 2018 exceptional storm on the *Paramuricea clavata* (Anthozoa, Octocorallia) population of the Portofino Promontory (Mediterranean Sea). *Reg. Stud. Mar. Sci.* **2020**, *34*, 101037. [CrossRef]
33. Oprandi, A.; Mucerino, L.; De Leo, F.; Bianchi, C.N.; Morri, C.; Azzola, A.; Benelli, F.; Besio, G.; Ferrari, M.; Montefalcone, M. Effects of a severe storm on seagrass meadows. *Sci. Total Environ.* **2020**, *748*, 141373. [CrossRef] [PubMed]
34. Thompson, R.C.; Crowe, T.P.; Hawkins, S.J. Rocky intertidal communities: Past environmental changes, present status and predictions for the next 25 years. *Environ. Conserv.* **2002**, *29*, 168–191. [CrossRef]
35. Micheli, F.; Heiman, K.W.; Kappel, C.V.; Martone, R.G.; Sethi, S.A.; Osio, G.C.; Fraschetti, S.; Shelton, A.O.; Tanner, J.M. Combined impacts of natural and human disturbances on rocky shore communities. *Ocean Coast. Manag.* **2016**, *126*, 42–50. [CrossRef]
36. Kunze, C.; Wölfelschneider, M.; Rölfer, L. Multiple driver impacts on rocky intertidal systems: The need for an integrated approach. *Front. Mar. Sci.* **2021**, *8*, 578. [CrossRef]
37. Rogers-Bennett, L.; Catton, C.A. Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Sci. Rep.* **2019**, *9*, 1–9. [CrossRef]
38. Moore, P.J.; Smale, D.A. Impacts of climate change on shallow and shelf subtidal habitats, relevant to the coastal and marine environment around the UK. *MCCIP Sci. Rev.* **2020**, *2020*, 272–292. [CrossRef]
39. Bianchi, C.N.; Morri, C. Climate change and biological response in Mediterranean Sea ecosystems—A need for broad-scale and long-term research. *Ocean Chall.* **2004**, *13*, 32–36.
40. Duffy, J.E.; Amaral-Zettler, L.A.; Fautin, D.G.; Paulay, G.; Rynearson, T.A.; Sosik, H.; Stachowicz, J.J. Envisioning a marine biodiversity observation network. *Bioscience* **2013**, *63*, 350–361. [CrossRef]
41. Paoli, C.; Montefalcone, M.; Morri, C.; Vassallo, P.; Bianchi, C.N. Ecosystem functions and services of the marine animal forests. In *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*; Rossi, S., Bramanti, L., Gori, C., Orejas, C., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 1271–1312.
42. Schiel, D.R. Multiple Stressors and Disturbances. In *Marine Hard Bottom Communities*; Wahl, M., Ed.; Springer: Berlin/Heidelberg, Germany, 2009; pp. 281–294.
43. Garrabou, J.; Ballesteros, E.; Zabala, M. Structure and dynamics of north-western Mediterranean rocky benthic communities along a depth gradient. *Estuar. Coast. Shelf Sci.* **2002**, *55*, 493–508. [CrossRef]

44. Bavestrello, G.; Betti, F.; Bianchi, C.; Bo, M.; Cappanera, V.; Corradi, N.; Montefalcone, M.; Morri, C.; Relini, G. Il promontorio di Portofino: 150 anni di storia di biologia marina. *Not. Soc. Ital. Biol. Mar.* **2022**, *81*, 53–114.
45. Gatti, G.; Bianchi, C.N.; Montefalcone, M.; Venturini, S.; Diviacco, G.; Morri, C. Observational information on a temperate reef community helps understanding the marine climate and ecosystem shift of the 1980–90s. *Mar. Pollut. Bull.* **2017**, *114*, 528–538. [PubMed]
46. Cheng, L.; Zhu, J.; Abraham, J.; Trenberth, K.E.; Fasullo, J.T.; Zhang, B.; Yu, F.; Wan, L.; Chen, X.; Song, X. 2018 continues record global ocean warming. *Adv. Atmos. Sci.* **2019**, *36*, 249–252. [CrossRef]
47. Jiménez-Muñoz, J.C.; Mattar, C.; Sobrino, J.A.; Malhi, Y. A database for the monitoring of thermal anomalies over the Amazon forest and adjacent intertropical oceans. *Sci. Data* **2015**, *2*, 150024. [CrossRef]
48. Ferreira, T.; Rasband, W. ImageJ user guide. *ImageJ/Fiji* **2012**, *1*, 155–161.
49. Gimenez, G.; Corriero, G.; Beqiraj, S.; Lazaj, L.; Lazic, T.; Longo, C.; Mercurio, M.; Marzano, C.N.; Zuccaro, M.; Zuna, V.; et al. Characterization of the coralligenous formations from the Marine Protected Area of Karaburun-Sazan, Albania. *J. Mar. Sci. Eng.* **2022**, *10*, 1458. [CrossRef]
50. Longobardi, L.; Bavestrello, G.; Betti, F.; Cattaneo-Vietti, R. Long-term changes in a Ligurian infralittoral community (Mediterranean Sea): A warning signal? *Reg. Stud. Mar. Sci.* **2017**, *14*, 15–26. [CrossRef]
51. De Cáceres, M.; Coll, L.; Legendre, P.; Allen, R.B.; Wiser, S.K.; Fortin, M.J.; Condit, R.; Hubbell, S. Trajectory analysis in community ecology. *Ecol. Monogr.* **2019**, *89*, e01350. [CrossRef]
52. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PaSt: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 4.
53. Clarke, K.R.; Gorley, R.N. *PRIMER: Getting Started with v6*; PRIMER-E Ltd.: Plymouth, UK, 2005; Volume 931, p. 932.
54. R Core Team. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021.
55. Mancini, I.; Azzola, A.; Bianchi, C.N.; Morri, C.; Oprandi, A.; Montefalcone, M. The story of a successful invasion: *Caulerpa cylindracea* in the Marine Protected Area of Portofino (Ligurian Sea). In Proceedings of the 2nd Mediterranean Symposium on the Non-Indigenous Species, Genova, Italy, 21–23 September 2022.
56. Brown, J.H.; Ernest, S.M.; Parody, J.M.; Haskell, J.P. Regulation of diversity: Maintenance of species richness in changing environments. *Oecologia* **2001**, *126*, 321–332. [CrossRef] [PubMed]
57. Bianchi, C.N.; Morri, C.; Chiantore, M.; Montefalcone, M.; Parravicini, V.; Rovere, A. Mediterranean Sea biodiversity between the legacy from the past and a future of change. In *Life in the Mediterranean Sea: A Look at Habitat Changes*; Stambler, N., Ed.; Nova Science: New York, NY, USA, 2012; pp. 1–55.
58. Azzola, A.; Atzori, F.; Bianchi, C.N.; Cadoni, N.; Frau, F.; Mora, F.; Morri, C.; Oprandi, A.; Orrù, P.E.; Montefalcone, M. Variability between observers does not hamper detecting change over time in a temperate reef. *Mar. Environ. Res.* **2022**, *177*, 105617. [CrossRef] [PubMed]
59. Gissi, E.; Manea, E.; Mazaris, A.D.; Fraschetti, S.; Almpanidou, V.; Bevilacqua, S.; Coll, M.; Guarnieri, G.; Lloret-Lloret, E.; Pascual, M.; et al. A review of the combined effects of climate change and other local human stressors on the marine environment. *Sci. Total Environ.* **2021**, *755*, 142564. [CrossRef] [PubMed]
60. Coma, R.; Ribes, M.; Gili, J.M.; Zabala, M. Seasonality in coastal benthic ecosystems. *Trends Ecol. Evol.* **2000**, *15*, 448–453. [CrossRef] [PubMed]
61. Ceccherelli, G.; Pinna, F.; Pansini, A.; Piazzi, L.; La Manna, G. The constraint of ignoring the subtidal water climatology in evaluating the changes of coralligenous reefs due to heating events. *Sci. Rep.* **2020**, *10*, 17332. [CrossRef]
62. Garnier, E.; Ciavola, P.; Spencer, T.; Ferreira, O.; Armaroli, C.; McIvor, A. Historical analysis of storm events: Case studies in France, England, Portugal and Italy. *Coast. Eng.* **2018**, *134*, 10–23. [CrossRef]
63. Harmelin-Vivien, M.L. The effects of storms and cyclones on coral reefs: A review. *J. Coast. Res.* **1994**, 211–231, Special Issue No. 12 (Coastal Hazards: Perception, Susceptibility and Mitigation).
64. Innamorati, M.; Nuccio, C.; Massi, L.; Mori, G.; Melley, A. Mucilages and climatic changes in the Tyrrhenian Sea. *Aquatic Conservation: Mar. Freshw. Ecosyst.* **2001**, *11*, 289–298. [CrossRef]
65. Misic, C.; Schiaparelli, S.; Harriague, A.C. Organic matter recycling during a mucilage event and its influence on the surrounding environment (Ligurian Sea, NW Mediterranean). *Cont. Shelf Res.* **2011**, *31*, 631–643. [CrossRef]
66. Piazzi, L.; Atzori, F.; Cadoni, N.; Cinti, M.F.; Frau, F.; Ceccherelli, G. Benthic mucilage blooms threaten coralligenous reefs. *Mar. Environ. Res.* **2018**, *140*, 145–151. [CrossRef]
67. Piazzi, L.; Ceccherelli, G. Persistence of biological invasion effects: Recovery of macroalgal assemblages after removal of *Caulerpa racemosa* var. *cylindracea*. *Estuar. Coast. Shelf Sci.* **2006**, *68*, 455–461. [CrossRef]
68. Montefalcone, M.; Morri, C.; Peirano, A.; Albertelli, G.; Bianchi, C.N. Substitution and phase shift within the *Posidonia oceanica* seagrass meadows of NW Mediterranean Sea. *Estuar. Coast. Shelf Sci.* **2007**, *75*, 63–71. [CrossRef]
69. Turner, M.G.; Calder, W.J.; Cumming, G.; Hughes, T.P.; Jentsch, A.; LaDeau, S.; Lenton, T.M.; Shuman, B.; Turetsky, M.R.; Ratajczak, Z.; et al. Climate change, ecosystems and abrupt change: Science priorities. *Philos. Trans. R. Soc. B* **2020**, *375*, 20190105. [CrossRef] [PubMed]

70. Gatti, G.; Bianchi, C.N.; Parravicini, V.; Rovere, A.; Peirano, A.; Montefalcone, M.; Massa, F.; Morri, C. Ecological change, sliding baselines and the importance of historical data: Lessons from combining observational and quantitative data on a temperate reef over 70 years. *PLoS ONE* **2015**, *10*, e0118581. [CrossRef] [PubMed]
71. Bianchi, C.N.; Azzola, A.; Cocito, S.; Morri, C.; Oprandi, A.; Peirano, A.; Sgorbini, S.; Montefalcone, M. Biodiversity monitoring in Mediterranean marine protected areas: Scientific and methodological challenges. *Diversity* **2022**, *14*, 43. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.