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Thesis Title:

**Detecting temporal and spatial trends of aquatic communities of subtropical and temperate ecosystems
using multi-variate analyses**

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Abstract

Aquatic ecosystems are undergoing rapid change due to climate pressures, habitat degradation, and human activity. Understanding how biological communities respond across different environments is crucial for conservation and management. This thesis applies a consistent multivariate analytical framework to explore patterns of community structure and change in a wide range of aquatic systems, spanning marine, coral reef, and freshwater environments.

Using long-term and large-scale datasets, I analyzed spatial and temporal dynamics of fish and benthic communities across the Red Sea, the Mediterranean Sea, the Caribbean, and the freshwater river networks of Emilia-Romagna (Italy). Each chapter investigates different ecological gradients, such as protection status, land based human impact, and climate variability, while emphasizing the importance of community composition beyond traditional biodiversity metrics. Results reveal system-specific responses: temporal shifts, functional redundancy, resilience to disturbance, and species-level sensitivity to environmental change. Together, these studies demonstrate that community-level analyses can reveal ecological processes that may otherwise remain undetected through more traditional summary approaches.

By integrating citizen science data, and long-term ecological monitoring, this work highlights the potential of multivariate tools to detect subtle but meaningful changes in aquatic communities. It contributes to both methodological development and applied ecology by offering scalable strategies for assessing ecosystem health across diverse aquatic contexts.

Keywords

Citizen science monitoring; community structure; coral reef ecosystems; freshwater fish communities; functional traits; human impacts; long-term ecological monitoring; management strategy; marine protected areas (MPAs); multivariate analysis; temporal dynamics

Abbreviations and Acronyms

Abbreviation	Full Term	Brief Description
AD	Average Depth	Mean depth recorded per transect or survey.
BEST	BIOENV/BVSTEP Environmental Matching Procedure	A procedure that identifies variables (biotic or environmental) best explaining patterns in the multivariate community structure.
BIOENV	Biota-Environment Matching	Exhaustive routine used in the BEST procedure to identify all combinations of variables.
BVSTEP	Best Variable Subset Selection	Stepwise routine used in the BEST procedure to identify subsets efficiently.
CAP	Canonical Analysis of Principal Components	A constrained ordination method that finds axes best separating predefined groups based on resemblance matrices. CAP is useful for visualizing differences among groups and assessing classification success in multivariate datasets.
dbRDA	Distance-Based Redundancy Analysis	A constrained ordination method that visualizes the variation in a community matrix explained by predictor variables. It uses principal coordinate analysis followed by multiple linear regression and is often used to display results from a DistLM.
DistLM	Distance-Based Linear Model	A multivariate regression method that models the relationship between a community resemblance matrix and one or more predictor variables. It partitions variation and tests significance using permutations, allowing both marginal and sequential tests.

FD	Fraction of Day	Time proxy variable representing when, during the day, a survey occurred (00:00 = 0, 23:59 = 1)
MD	Maximum Depth	Maximum depth recorded per transect or survey
MDS	Non Metric Multidimensional Scaling	An ordination technique that visualizes the similarity or dissimilarity among samples in a reduced-dimensional space, preserving the rank order of their distances
PERMANOVA	Permutational Multivariate Analysis of Variance	A non-parametric test used to assess differences in community composition among groups, based on a resemblance matrix. It partitions variation according to experimental factors and uses permutations to test for significance
PY	Percentage of Year	Seasonality proxy variable representing when, during the year, a survey occurred (January 1 st = 0, December 31 st = 100)
RA	Relative Abundance	Proportion of individuals belonging to a given species, relative to the total count at a site or a time point.
RELATE	RELATE Test (Matrix Rank Correlation)	A test that compares two resemblance matrices (e.g. biological vs. environmental) to assess overall correlation using Spearman's rho and permutations. It evaluates whether two datasets share similar multivariate structure
RTD	Real Time Dive	Total dive time recorded for a survey, used as a proxy for observer effort.
SF%	Sighting Frequency	Percentage of surveys or transects in which a species was observed; used as a proxy for commonness or detectability.

SIMPER	Similarity Percentage Analysis	A procedure that identifies which taxa contribute most to observed dissimilarity between groups in multivariate data, based on pairwise comparisons of community structure. Often used to interpret PERMANOVA results.
STE	Scuba Tourism for the Environment	A Red Sea recreational citizen science project for biodiversity monitoring, launched by the University of Bologna's Marine Science group in 2007.
T	Temperature	In situ water temperature

Forward

Aquatic ecosystems are some of the most dynamic and sensitive environments on the planet, playing key roles in global climate regulation, nutrient cycling, and supporting food webs (Dudgeon et al. 2006; Woodhead et al. 2019). Yet they are facing increasing pressure from climate change, habitat degradation, and anthropogenic activity (IPCC 2021, 2023; Fine et al. 2019; Filipe et al. 2013). Traditionally, biodiversity metrics such as species richness or evenness have been used to track ecosystem changes, but these can fail to capture deeper shifts in ecological function or community organization (McGill et al. 2006). Two systems may host a similar number of species but differ entirely in which species are present, how those species interact, and what functional roles they perform. A coral reef dominated by hard corals and one overgrown by algae might have similar richness, but they reflect very different ecological states. For this reason, examining community composition and structure is central to understanding ecosystem condition and change.

Community structure describes not only which species are present in an ecosystem, but also their relative dominance and interactions, factors that strongly influence how ecosystems respond to disturbance and management (Pimm 1984; Jackson and Blois 2015). Shifts in community composition can signal degradation, recovery, or adaptation, and community-level analyses allow exploration of how environmental drivers such as temperature, nutrient input, or human pressure shape ecological outcomes (Tilman 1982). Functional trait-based approaches further extend this perspective by focusing on what organisms do, rather than solely who they are, providing complementary insight into ecosystem resilience and redundancy (McGill et al. 2006; Brandl et al. 2019).

Understanding community change requires attention to both temporal and spatial scales. Long-term datasets allow the detection of persistent trends, regime shifts, and recovery trajectories in both marine (Tsounis et al. 2017; Receveur et al. 2024) and freshwater (Danet et al. 2024) systems. Spatial comparisons, meanwhile, reveal how communities respond across gradients of protection, development, and exploitation from well-managed protected areas to more heavily used or degraded sites (Fraschetti et al. 2002; McLeod et al. 2009). Together, these dimensions provide a framework for examining how chronic human pressure, management intervention, and broader environmental forcing interact to structure ecological communities, a synthesis that remains rare across different types of aquatic ecosystems.

To address this gap, my work applies a consistent multivariate, community-level analytical framework across a series of case studies spanning marine and freshwater ecosystems. Multivariate tools are well suited to ecological data because they capture complex, non-linear, and high-dimensional patterns in community organization (Clarke and Gorley 2015). They allow us to ask questions about overall community similarity, the drivers of change, and the role of environmental gradients. Throughout most of the thesis, I applied these methods

to taxonomic data to examine shifts in species composition and dominance, while the final chapter extends this framework to a functional trait-based perspective. These approaches enable not only the analysis of individual systems, but also comparative inference about recurring patterns of community response across ecosystems and monitoring contexts.

The chapters are organized to build progressively in both analytical complexity and ecological scope. Chapter 1 focuses on the SS *Thistlegorm*, a World War II shipwreck in the Red Sea that has become a vibrant artificial reef. Using eight years of data from the Scuba Tourism for the Environment (STE) citizen science program, this chapter evaluates the stability and interpretability of community-level patterns derived from recreational monitoring, establishing a methodological foundation for the analyses that follow.

Subsequent chapters apply this framework to increasingly complex ecological and management contexts. Chapter 2 builds on the Red Sea dataset from the same citizen science program by incorporating spatial complexity, examining how reef communities vary across a gradient of coastal developmental impact and how these patterns evolve over time. Chapter 3 moves to the Mediterranean Sea, drawing on an older sister project of the Red Sea monitoring program, Divers for the Environment (Goffredo et al. 2010), to investigate benthic community structure across gradients of management strategy, cumulative human impact, and ecosystem health. Chapter 4 centers on coral reef fish communities in the Cayman Islands and examines how they responded over time to differing management strategies. Chapter 5 transitions to a freshwater system, analyzing more than thirty years of fish monitoring data from the Emilia-Romagna region of northern Italy. This chapter brings together spatial zones, long-term data, and environmental covariates to explore how inland fish communities are responding to climatic and anthropogenic pressures, representing the most complex integration of data types and scales in this dissertation.

Finally, Chapter 6 returns to the SS *Thistlegorm* but shifts the analytical perspective from taxonomic composition to functional trait structure. By applying the same multivariate framework through a different lens, this chapter explores whether changes in species identity correspond to shifts in ecological roles. In doing so, it builds directly on the earlier chapters while also extending the dissertation's scope, demonstrating how the same methodological approach can be adapted to address new ecological questions.

Together, these chapters form a comparative framework for understanding how aquatic communities reorganize under interacting gradients of human pressure, protection, and environmental change. While each study stands independently, collectively they reveal recurring tendencies toward increased dominance by generalist taxa, altered trophic structure, and context-dependent management effectiveness in systems exposed to chronic disturbance. By pairing long-term and participatory monitoring with multivariate and functional approaches, this dissertation demonstrates how generalizable ecological patterns can emerge from diverse data sources, contributing both to ecological theory and to the design of more integrated, conservation strategies.

Chapter 1

Eight years of community structure monitoring through recreational citizen science at the “SS Thistlegorm” wreck (Red Sea)

Abstract

Large artificial coral reef communities, such as those thriving on sunken shipwrecks, tend to mirror those of nearby natural coral reefs and their long-term dynamics may help future reef resilience to environmental change. We examined the community structure of the world renowned “SS Thistlegorm” wreck in the northern Red Sea from 2007 through 2014, analyzing data collected during the recreational citizen science Red Sea monitoring project “Scuba Tourism for the Environment”. Volunteer divers collected data on 6 different diving parameters which included the date of the dive, maximum depth, average depth, temperature, dive time, hour of dive, and gave an abundance estimation of sighted taxa from a list of 72 target taxa. Although yearly variations in community structure were significant, there was no clear temporal trend, and 71 of all 72 target taxa were sighted throughout the 8 years. The 5 main taxa driving variations among year clusters in taxa presence/absence (Soft Tree Coral—*Dendronephthya* spp., Giant Moray—*Gymnothorax javanicus*, Squirrel Fish—*Sargocentron* spp., Humpback Batfish—*Platax* spp., and Caranxes—*Carangidae*) and taxa abundance (Soft Tree Coral, Giant Moray, Red Sea Clownfish—*Amphiprion bicinctus*, Napoleon Wrasse—*Cheilinus undulatus*, and Caranxes) data were determined. The “SS Thistlegorm” provides a compelling example of how artificial coral reefs can sustain a well-established community structure similar to those of their natural counterparts.

Introduction

The biological communities of tropical and sub-tropical coral reefs support some of the highest biodiversity in the world (Bellwood and Hughes 2001) and provide a wide array of socio-economic services including coastal protection, water quality and chemical cycling, fisheries and materials markets (e.g. sponges, ornamental use, medicine, etc.), and experiential benefits (e.g. education, recreation) (Woodhead et al. 2019). Despite their assortment of values, coral species homogenization (Aronson et al. 2005), species extinctions (Carpenter et al. 2008), and overall changes of coral communities are expected in future (Kubicek et al. 2019). In fact, the Intergovernmental Panel on Climate Change projections (Bindoff et al. 2019) and current research indicate a multitude of threats to coral reefs including but not limited to ocean warming and acidification, overfishing, and rise in coastal human populations and impacts (Fine et al. 2019).

One of the largest coral reef systems of the world, with notably high endemism, lies within the Red Sea (DiBattista et al. 2015). Due to its peculiar geo-evolutionary past resulting in large latitudinal gradients of sea surface temperature and salinity (DiBattista et al. 2015) the Red Sea provides an attractive location for studying the response of coral reefs to threats of their projected immediate future. The northern Red Sea is considered a coral refuge because, although it has experienced some of the highest sea surface temperature anomalies, there have been very few coral bleaching events compared to its southern portion (Fine et al. 2019; Kleinhaus et al. 2020). Notwithstanding the relative resistance of reefs in the northern Red Sea, there is still evidence of general coral colony size decline and species homogenization throughout (Riegl et al. 2012), having the potential to induce changes in the biological communities they support. The importance of monitoring community variation lies not only in its fundamental value but also the economic income from fisheries (Tesfamichael et al 2006) and especially the tourist industry, which provide an array of socio-economic opportunities for many coastal countries (Gladstone et al. 2013). Unfortunately, there is general lack of information regarding Red Sea coral communities, which are relatively understudied when compared to the Great Barrier Reef or reef systems of the Caribbean Sea (Berumen et al. 2019). This can pose conservation challenges when attempting to monitor and analyze crucial changes in the biological communities of the Red Sea.

An innovative and upcoming approach to gathering large amounts of data in a time efficient way is the practice of citizen science. This approach is not so novel in terms of human history, but current technological advances of citizen science in the spreading and sharing of information and data (Sauermann et al. 2015; Silvertown et al. 2009) have benefits and uses that are now an integral part of modern science (Kobori et al. 2015). Citizen science can be a critical asset in helping researchers, policy makers, and stakeholders overcome resource limitations (e.g., economical, temporal, geographical). Community-based management, and community-based monitoring are two of the main methodologies of the citizen science approach. The former involves direct participation of citizens and stakeholders in management decisions (Keough et al. 2015), while the latter implies

the collaboration of citizens and stakeholders for data collection to monitor, track, and respond to areas of concern (e.g., environmental health) (Whitelaw et al. 2003). Scuba Tourism for the Environment, which was launched in 2007 (STE; www.steproject.org), provides a great example of involving large numbers of volunteers for community-based monitoring throughout much of the Red Sea (Branchini et al. 2015a). Divers participating in the STE program collected ecological community data with the assistance of active dive centers and trained guides throughout Egypt. Recreational citizen science was applied throughout the project. This approach to citizen science allows participants to carry out their normal activities (volunteer behavior is unchanged throughout the survey), and to collect casually observed data. To analyze the reliability of data collected by the participants, data were compared with those collected by control divers (marine biologists of the Marine Science group at the University of Bologna) (Meschini et al. 2021). Consistency, or the similarity of data collected by individual volunteers during the same dive, was the lowest ranking parameter. The percentage recorded of the total number of taxa present (acquired from control diver data), or the Percent Identified, was the highest-ranking parameter (Meschini et al. 2021). This indicates that while divers can accurately identify most taxa there is a tendency to focus on certain organisms due to personal interest. However, with correct development to the needs of specific projects, data collected through citizen science can reliably support monitoring efforts (Kobori et al. 2015; Keough et al. 2015; Meschini et al. 2021).

The SS Thistlegorm, a world class dive site (El Gohary 2013) and one of topmost visited wreck dives in the world (Motawea Hussein Shaikhon 2019), was one of the dive locations monitored in the STE project and is the focus of this study. The SS Thistlegorm was built in 1940 as a steal cargo steamship and was quickly taken over for the war efforts of the British military. On her final voyage from Glasgow (UK) to Alexandria (Egypt) she was met with two German Heinkel HE 111 bombers. The 128 m long and 17 m high cargo steam ship was split in two by munitions explosions and sunk within minutes (Brown et al. 2021). Her ultimate resting ground is in the Straights of Gubal 30 meters below the sea surface (Brown et al. 2021). In the 80 plus years since the “discovery” of the wreck by Jacques-Yves Cousteau in 1955, and the development of the Sharm el-Sheikh resort in the 1990s, the SS Thistlegorm has recruited up to 175,000 visiting divers per year from around the world (Brown et al. 2021). Due to its high popularity and frequent visitors, there are rising concerns regarding the structural longevity of this historic site. Irresponsibly moored boats, inexperienced divers, and even air bubbles can cause irreparable damage to the structural integrity of underwater relics (Edney and Boyd 2020) as well as the biological community residing there (Maynard 2008). Additionally, because the wreck is afforded no legal protection, it is prey to looting and souvenir plundering (www.thethistlegormproject.com). A dedicated team of divers, archeologists, and researchers developed the Thistlegorm Project (www.thethistlegormproject.com) to survey the site using 360 videography for the purposes of creating an accurate archeological 3D survey and to raise awareness for the protection of underwater cultural heritage sites.

Aside from the historical importance of the SS Thistlegorm wreck, the ship has recruited numerous marine life forms through the decades spent on the sandy bottom close to a natural coral reef and far from touristic resorts (70 km by boat from Sharm el Sheikh). We report here a first investigation on the temporal community trends of biological organisms at the SS Thistlegorm wreck. Scientific literature concerning long-term (more than 5 years) temporal trends and monitoring of community structure on wreck and/or artificial reefs is scarce (Asner et al. 2022). There is extensive interest, however, in the role of artificial reefs, (including wrecks and scuttled ships) as a conservation tool, potentially serving as a compensatory habitat for anthropogenically damaged natural reefs (Perkol-Finkel et al. 2004; Walker and Schlacher 2014; Higgins et al. 2019), and as possible refugia assisting corals in the colonization of cooler waters in response to ocean warming (Asner et al. 2022). Shipwrecks in particular have been shown to significantly increase biodiversity of both fish and benthic species on soft bottom environments (Renzi et al. 2017, Arreola-Robles and Elorduy-Garay 2002) along the coast and represent key microhabitats in the open ocean (Ogden and Ebersole 1981). The material of the wreck, the bottom type it lies upon, as well as its structural complexity play key roles in determining marine community and have been shown to be fundamental in epi benthic colonization patterns (Peirano 2013). The aim of this study was to characterize the community structure and its temporal trends of the SS Thistlegorm, over 8 years of monitoring through recreational citizen science.

Materials and Methods

The study site

The SS Thistlegorm lies 27° 48' 30.59" N and 33° 55' 7.19" E, at 30 m depth on sandy bottom substrate within the Straights of Gubal. The bow of the ship faces S40°W, with tidal currents flowing from Northeast to Southwest at varying strength, from stern to bow (Siliotti 2005). Her final voyage entailed a resupply mission to the British Armed Forces and was destined for Alexandria (Egypt). The cargo onboard the SS Thistlegorm included Bedford trucks, BSA motorcycles, Wellington rubber boots, aircraft parts, rifles, land mines, ammunition, and other weaponry, as well as two LMS Stanier Class 8F steam locomotives among other goods (Perkol-Finkel et al. 2004), much of which is potentially hazardous to the marine environment.

Data collection and isolation

The Scuba Tourism for the Environment—Red Sea Biodiversity Monitoring Program (www.steproject.org), included the collection of large scale spatial-temporal biodiversity data within the Red Sea from 2007 to 2015 using a recreational citizen science approach where the behavior of the underwater tourists is unaltered during surveys (Branchini et al. 2015a). Participant volunteers completed questionnaires that contained general demographic information (name, country of residence, address, diving certification, etc.), dive site and

environment type (sandy bottom, wreck, blue, or coral reef), six diving parameters (date (PY), maximum depth (MD), depth where they spent most of their time (AD), temperature (T), dive time (RTD), and hour (FD)), and the sightings of 72 target taxa which were recorded on the questionnaires immediately following the dive and without the use of underwater marking materials during the dive (Branchini et al. 2015a). For each of the taxa sighted, the divers also recorded an estimated Sighting Abundance according to 3 classes: 1, 2, and 3 (rare, frequent, and very frequent respectively) (Branchini et al. 2015a). The classes were weighted to each taxon's individual expected occurrence (Branchini et al. 2015a). The 72 faunal taxa were chosen because they are representative of the main ecosystem trophic levels within the Red Sea, they are common/abundant, and they are easily identifiable by recreational divers (Branchini et al. 2015a). Data coming from the Thistlegorm wreck site were aggregated per year.

Preliminary Treatment and Analysis

Seven of the dives within the Thistlegorm dataset were viable for validation trials and were analyzed to measure the quality of volunteer collected data. The mean similarity index, accuracy, and correctness of abundance ratings (CAR) parameters were tested in accordance with the standard methodology for this citizen science project (Meschini et al. 2021).

Thistlegorm data were then split into three data sets: 1) taxa abundance; 2) taxa presence/ absence (p/a), obtained through an overall transformation of taxa abundance; and 3) diving parameters. The sighting frequency (SF%) and Relative Abundance (RA) for each taxon were calculated as such (Branchini et al. 2015a):

$$SF\% = \left(\frac{\text{Number of sightings per taxon}}{\text{Number of dives}} \right) \times 100$$

$$RA = (SF\%)(\text{Average Sighting Abundance})$$

The analyses below were completed using PRIMER-E version 6 with PERMANOVA+ version 1 software (PRIMER-E, Ltd., Ivybridge, UK).

For a general picture of the trophic community composition and to identify outliers within each year, 2-dimensional plots were created using non-metric multidimensional scaling (MDS) from the Bray-Curtis resemblance matrices of both taxa data sets (Shepard 1962, Kruskal 1964). A BVSTEP “Best” test was performed on both taxa data sets to find the subset of taxa that best represented the community of each year (Clarke and Ainsworth 1993). These subsets of taxa were later used for aggregation of the data in the temporal analysis.

A distance matrix of normalized diving parameters was created using Euclidean distances. Both taxa distance matrices were tested for a relationship with the distance matrices of the diving parameters using the Relate test to determine whether the variation between years was affected only by the change in time or also by

diving parameters (Clarke and Gorley 2006). Additional distance based linear models distLM; McArdle and Anderson 2001) were run for each year with a significant relationship between the taxa data and diving parameters, providing the individual parameters that significantly affected the variation of community structure in each year.

To test the differences in diving parameters over the years, a permutational multivariate analysis of variance (PERMANOVA, (McArdle and Anderson 2001)) was designed with the fixed factor of “year” and run on the Euclidean resemblance matrix of the normalized aggregate diving parameters. Both main, and pairwise tests were run. Additional PERMANOVAs were created for individual diving parameters to test whether each different diving parameter was significantly different among the years.

Temporal Analysis

Two reduced datasets containing only the BVSTEP best subsets of taxa representing the community structure from any individual year were obtained, and resemblance matrices were created from Bray-Curtis distances of both the abundance taxa data and the overall-transformed p/a taxa data (Shepard 1962). To test significant difference among years, PERMANOVAs (McArdle and Anderson 2001) were run with the fixed factor “year”. For a sharper visualization of temporal variation in community structure, distances between centroids were obtained from the resemblance matrices using “Year” as the grouping factor and visualized with 2D and 3D MDS plots. 3D plots were used to better interpret the direction of vectors in 2D plots and were not included as figures in the current manuscript. Any similar groupings of the centroidal “years” were found with a hierarchical cluster analysis (Kaufman et al. 1990). The average sample taxa abundance and presence/absence from each year were calculated, and a BVSTEP Best analysis was run from the Bray-Curtis resemblance matrix to find the subset of taxa that best explained the trophic community structure variance within centroid data sets (Clarke and Ainsworth 1993).

Results

Data collection and isolation

Nine questionnaires from 2015 were removed from the analysis, as per lack of efficacy for less than 10 questionnaires per year (following Branchini et al. 2015a). While Branchini and colleagues (2015a) examined the “coral reef” environment questionnaires, this study reports findings from an unpublished data set within the “wreck” environment type. Data from a total of 390 questionnaires between 2007 and 2014 were included in this analysis (Table 1.1). Questionnaire counts per year ranged from 12 to 84 and significantly decreased over time ($R^2 = 0.78$, $P < 0.05$). Over the 8 years, 71 (97%) of the 72 target taxa present on the STE questionnaire were sighted (all taxa excluding the Manta Ray).

Table 1.1. Yearly counts of completed questionnaires.

Year	2007	2008	2009	2010	2011	2012	2013	2014	Total
Number of Questionnaires	84	62	59	59	72	29	12	13	390

Preliminary treatment and analysis

According to the RELATE test, years 2007 ($\rho_s = 0.162$, $N = 6$, $P = 0.007$), 2008 ($\rho_s = 0.157$, $N = 2$, $P = 0.003$), 2009 ($\rho_s = 0.111$, $N = 45$, $P = 0.046$), and 2012 ($\rho_s = 0.217$, $N = 9$, $P = 0.01$) showed In all other years there was no significant relationship (Table 1.2; S1 Table). The DistLM of taxa abundance data in 2007 showed a significant relationship ($P < 0.05$) with all 6 diving parameters: date (PY), maximum depth (MD), depth where they spent most of their time (AD), temperature (T), dive duration (RTD), and hour (FD) (Table 1.2; S2 Table). In 2008 all the diving parameters except FD were significantly correlated ($P < 0.05$) to the taxa abundance (Table 1.2; SI Ch.1, Table S2). PY, AD, and FD were significantly related ($P < 0.05$) to taxa abundance in 2009 (Table 2; SI Ch.1, Table S2). In 2012 only PY and T diving parameters were significantly related to the community structure ($P < 0.05$; Table 1.2; SI Ch.1, Table S2).

Table 1.2. SS Thistlegorm dive parameters with significant ($P < 0.05$) sequential DistLM test to community structure by year for abundance and presence/absence data. PY as date (expressed as the percentage of year), MD as maximum depth, AD as depth at most times pent, T as temperature, RTD as dive duration, and FD as hour (expressed as percentage of day).

Year	Abundance	Presence/Absence
2007	PY, MD, AD, T, RTD, FD	PY, AD, T, RTD, FD
2008	PY, MD, AD, T, RTD	PY, MD, T, RTD
2009	PY, AD, FD	
2010		
2011		
2012	PY, T	PY, T, RTD
2013		
2014		

Regarding taxa presence/absence (p/a) data, the RELATE results indicated that in 2007 ($\rho_s = 0.18$, $N = 1$, $P = 0.002$), 2008 ($\rho_s = 0.136$, $N = 13$, $P = 0.014$), and 2012 ($\rho_s = 0.209$, $N = 4$, $P = 0.005$) there was a significant relationship between the community structure and dive parameters (Table 1.2, SI Ch.1, Table S1). The DistLM in 2007 revealed that all dive parameters except MD had significant relationships to the taxa p/a data ($P < 0.05$; Table 1.2, SI Ch.1, Table S3). In 2008 PY, MD, T, and RTD were significant ($P < 0.05$; Table 2, S3 Table), while only PY, T and RTD resulted significant in 2012 ($P < 0.05$).

Aggregated diving parameters showed significant differences among years (PERMANOVA, $F = 9.25$, $df = 7$, $P = 0.001$). PY, MD, AD, T and RTD all differed significantly among years ($P < 0.05$) FD was homogenous among years.

Of the seven validation trial dives, the average mean similarity index score was 98.8%, the average mean accuracy score was 57.1% and ranged from 40.4 to 70.0%, and the mean average CAR was 73.8%.

Temporal Analysis

According to the PERMANOVA from both the taxa abundance and p/a data, the community structure was significantly different between each pair of years ($F = 4.88$, $df = 7$, $P = 0.001$ and $F = 4.88$, $df = 7$, $P = 0.001$ respectively). The variation in community structure of taxon abundance and p/a through years 2007–2014 was represented as an MDS (Figs 1.1a and 1.2a). Given the high stress value (> 0.3) of these MDS plots, further centroidal MDS plots of both taxa data sets were generated to have a clearer, even if more general, visualization highlighting the similar groupings of years within their respective distances resulting from the hierarchical cluster analysis (Figs 1.1b and 1.2b).

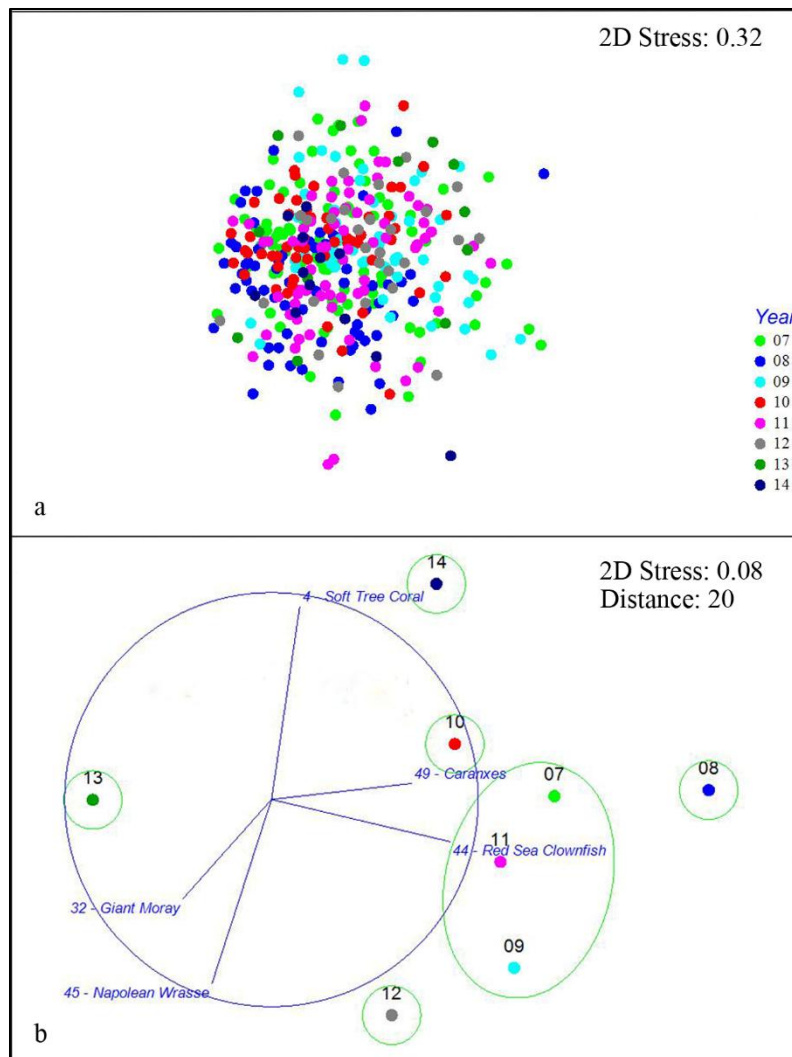


Figure 1.1 a) MDS plot of the SS Thistlegorm taxa abundance community structure over 8 years. Each point represents an individual questionnaire while the different shapes/colors indicate the different years. b) Centroidal MDS of SS Thistlegorm taxa abundance community structure variation over 8 years with overlaid vectors of aggregated best taxa and the indicated trajectories from 2007–2014. Each point represents the central location of the groups "Year". The green circles surround the groups resulting from the hierarchical cluster analysis.

The BVSTEP subset of taxa that explained the distances between yearly centroids of community structure for the taxa abundance data were the Soft Tree Coral (*Dendronephthya* spp.), Giant Moray (*Gymnothorax javanicus*), Red Sea Clownfish (*Amphiprion bicinctus*), Napoleon Wrasse (*Cheilinus undulates*), and Caranxes (*Carangidae*). The BVSTEP best subset of taxa explaining the distances between yearly centroids of community structure for the taxa p/a data were the Soft Tree Coral, Giant Moray, Squirrel Fish (*Sargocentron* spp.), Humpback Batfish (*Platax batavianus*), and Caranxes. Tables 1.3 and 1.4 show the relative abundance and SF% of the best taxa through the 8 years.

Table 1.3. Yearly relative abundance values of best species subset from aggregated taxa abundance data.

Year	Soft Tree Coral	Giant Moray	Red Sea Clownfish	Napoleon Wrasse	Caranxes
2007	77	48	64	26	117
2008	69	26	98	27	94
2009	41	78	80	59	125
2010	98	93	44	37	154
2011	61	63	49	47	114
2012	31	121	35	55	93
2013	50	67	17	50	8
2014	154	62	31	0	115

Table 1.4. Yearly sighting frequencies of best species subset from centroid aggregated data.

Year	Soft Tree Coral	Giant Moray	Squirrelfish	Humpback Batfish	Caranxes
2007	46%	38%	33%	70%	55%
2008	53%	21%	16%	61%	53%
2009	34%	61%	24%	56%	71%
2010	54%	70%	42%	86%	75%
2011	44%	47%	38%	61%	65%
2012	28%	66%	52%	62%	52%
2013	33%	67%	50%	33%	8%
2014	92%	54%	8%	70%	69%

Years 2007, 2009, and 2011 cluster around a general community structure in the taxa abundance data (Fig 1B). The Red Sea Clownfish (*Amphiprion bicinctus*) was overrepresented in 2008 (98 in 2008 vs 49–80 in 2007, 2009, and 2011; Table 1.3). The year 2010 was overrepresented in Soft Tree Coral (*Dendronephthya* spp.) (98 in 2010 vs 41–77 in 2007, 2009, and 2011; Table 1.3), and Giant Moray (*Gymnothorax javanicus*) (93 in 2010 vs 48–78 in 2007, 2009, and 2011; Table 1.3) and underrepresented in Red Sea Clownfish (44 in 2010 vs 49–80 in 2007, 2009, and 2011; Table 1.3). In 2012 there was an overrepresentation of Giant Moray (121 in 2012 vs 48–78 in 2007, 2009 and 2011; Table 1.3) and Napoleon Wrasse (*Cheilinus undulatus*) (55 in 2012 vs 26–59 in 2007, 2009, and 2011; Table 1.3) with an underrepresentation of Soft Tree Coral (31 in 2012 vs 41–77 in 2007, 2009 and 2011; Table 1.3) and Red Sea Clownfish (35 in 2012 vs 49–80 in 2007, 2009 and 2011; Table 1.3). In 2013 there was an underrepresentation of Caranxes (Carangidae) (8 in 2013 vs 114–125 in 2007, 2009, and 2011; Table 1.3) and Red Sea Clownfish (17 in 2013 vs 49–80 in 2007, 2009, and 2011; Table 1.3). 2014 was overrepresented by Soft Tree Coral (154 in 2014 vs 41–77 in 2007, 2009 and 2011; Table 1.3) and underrepresented by Red Sea Clownfish (31 in 2014 vs 49–80 in 2007, 2009 and 2011; Table 1.3) and Napoleon Wrasse (0 in 2014 vs 26–59 in 2007, 2009, and 2011; Table 1.3).

The taxa p/a centroidal data (Fig 1.2b) displayed a general cluster in community structure, like the abundance data, but this general cluster was larger and represented by the years 2007 through 2012. With respect to this general cluster the year 2013 exhibited an underrepresentation in Humpback batfish (*Platax bativianus*) (33% in 2013 vs 56–86% in 2007–2012; Table 1.4) and Caranxes (8% in 2013 vs 52–75% in 2007–2012; Table 1.4). The following year showed a community similar to the general cluster but with an overrepresentation of Soft Tree Coral (92% in 2014 vs 28–54% in 2007–2012; Table 1.4) and an underrepresentation of Squirrel Fish (*Sargocentron* spp.) (8% in 2014 vs 16–52% in 2007–2012; Table 1.4).

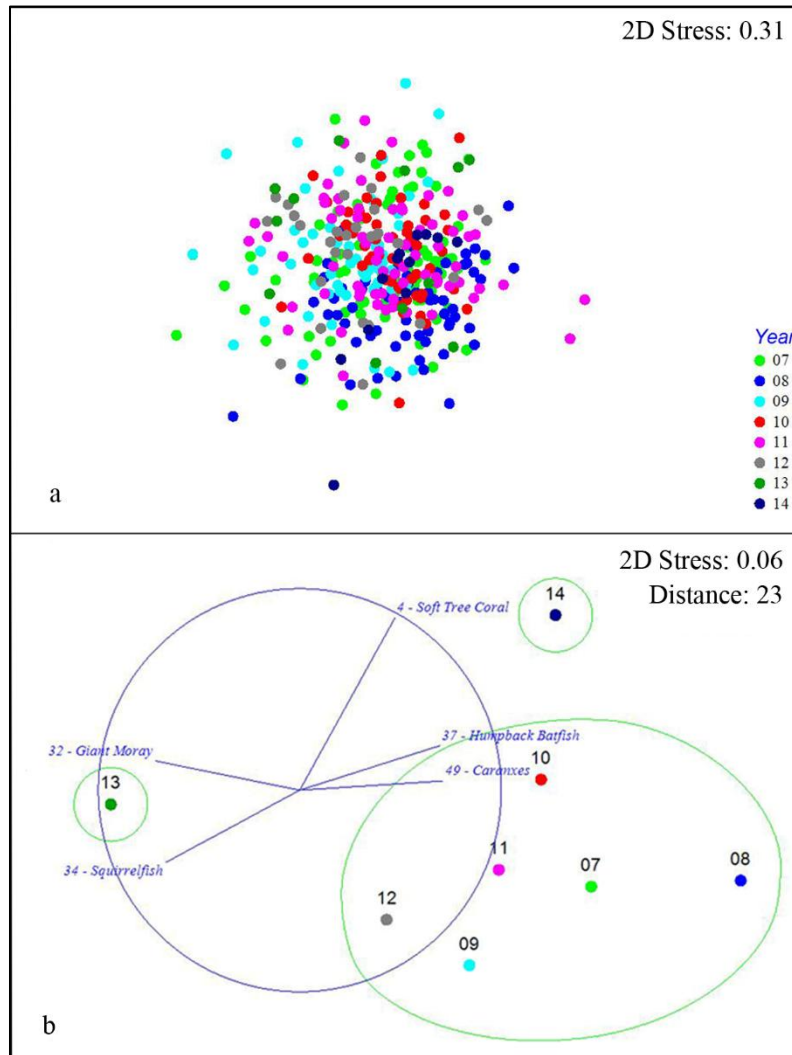


Figure 1.2. a) MDS plot of the SS Thistlegorm taxa presence/absence community structure over 8 years. Each point represents an individual questionnaire while the different shapes/colors indicate the different years. b) Centroidal MDS of SS Thistlegorm taxa presence/absence community structure variation over 8 years with overlaid vectors of aggregated best taxa and the indicated trajectories from 2007–2014. Each point represents the central location of the groups "Year". The green circles surround the groups resulting from the hierarchical cluster analysis.

Discussion

We have described here the first investigation of temporal community trends of an historical relic, and world class dive site within the Red Sea, The SS Thistlegorm. Notwithstanding these merits, The SS Thistlegorm can also be regarded as an artificial reef community, though “accidental” in nature. Since the 1960’s, artificial reefs have been used for a variety of purposes including biodiversity conservation (Bombace 1989), fisheries management (Seixas et al. 2013), and tourist locations (Tessier et al. 2015). One study in Eilat (Red Sea) designed and submerged multiple concrete structures to test whether artificial reefs could decrease diving pressures on the surrounding natural reef areas, concluding that small scale structures, knowledge of such structures within the local diving community, and education of divers could be sufficient (Polak and Shashar 2012). The provision of migratory networks for coral movement and resettlement within changing oceans (Pinheiro et al. 2017) is another application for artificial reefs, though highly debated and rather unexplored. Organismal community recruitment and interchange is an evident and well observed occurrence when any new colonizable object, be it an island or a plastic bottle, appears near a source population. In accordance with island biogeography theory (Macarthur and Wilson 1967), the evolution of this community is much dependent on the size of the object in question, the time it is in existence, and its distance from the source. Sunken warships have been shown to serve as exceptionally good artificial substrates for coral reefs as their size and complexity offer a multitude of opportunities for micro habitats, and in cases where they extend down to 30–40 m, the cooler waters can provide respite to corals from warming oceans but still sufficient light for their symbiotic zooxanthellae (Brown et al. 2021). Many shipwrecks in the North Sea have supported hard substrate communities throughout the Belgian Continental Shelf which has otherwise been transformed into a soft-bottom environment due to anthropogenic activity (Lengkeek et al 2013; Mallefet et al. 2008). Thanks to the steel structure of the SS Thistlegorm, recruitment of various tolerant sessile species typical of hard substrates was enabled in the sandy bottom environment within the Straights of Gubal and a well-established coral community can now be witnessed, but no information is available in the scientific literature.

Much of the work regarding artificial coral reefs examine differences between colonization patterns on various substratum material or recently scuttled ships (less than 3 years) and natural reefs, which are largely short-term studies (Rilov and Benayahu 2000; Maynard 2008; Edney and Boyd 2020; Monchanin et al. 2021; Asner et al. 2022). The few studies that have investigated community structure change over the long term (more than 5 years) have primarily been snapshot comparisons between the virgin and mature communities (Abelson and Shlesinger 2002; Kotb 2013) or follow succession patterns of virgin artificial reefs (Nicoletti et al. 2007).

The present study begins to fill this gap through an 8-year monitoring of a well-established wreck community (i.e. > 80 years since the sinking of the SS Thistlegorm in 1941). Despite the internal fluctuation among years (50% in 2013, 93% in 2007), all target taxa, apart from the Manta Ray, were sighted throughout the

duration of this study. These findings indicate a well-developed, if variable in time, community structure at the Thistlegorm and are in line with the monitoring of natural reefs in the region where reef sites far from touristic facilities show higher biodiversity (Branchini et al 2015b). The Kingston, a 119-year-old shipwreck 10 km east of the Thistle gorm, mimics the surrounding natural reef communities due to its similarities in structural complexity (Perkol-Finkel et al. 2006). Using a visual census technique and the recordings of all species seen, oil jet ties in Eilat (northern Red Sea) were studied as artificial reef proxies and were shown to have even higher species richness in fish assemblages than neighboring natural reefs (Rilov and Benayahu 2000) due to increased structural complexity. Studies of artificial reefs and wrecks in other regions throughout the world have shown similarly promising results with respect to community development. In the Florida Keys, artificial reef fish communities mirror those of natural reefs in terms of fish numbers and species composition (Stone et al. 1979) and two thirds of genera from neighboring natural reefs can thrive on sunken shipwrecks within the Caribbean (Nicoletti et al. 2007). With the use of SCUBA diving video sampling, microbenthic species abundances on eight shipwrecks and one airplane of varying construction and bottom type were compared to those of sampling on nearby natural substrates in the Ligurian seabed (Mediterranean Sea) (Higgins et al. 2019). The findings of this study exhibited not only a strong correlation of wreck material and bottom type to the epibenthic community present but also revealed that some species abundances were greater on the wrecks than those of nearby natural rocky bottom substrates (Higgins et al. 2019). Even in temperate seas, such as the North Sea along the Belgian Continental Shelf, shipwrecks are described as biodiversity hot spots of hard substrate communities in an otherwise sandy bottom environment while protecting nearby soft-bottom communities from fishing activities (Lengkeek et al. 2013; Mallefet et al. 2008). The steel structured SS Thistlegorm lies upon a sandy bottom marine environment and while this study does not compare its taxa sightings to natural reefs nearby, it does provide key insights into the community dynamics of a well-established artificial reef.

The number of observations collected in this study through recreational citizen science methods decreased in time (Table 1.1). This is ascribable to the reduction in overall tourism due to political upheaval consuming Egypt during the revolution and the following transitional period of the “Arab Spring” (Esmail 2016; Wendt 2019). As tourists seek to vacation in more stable and safe countries, participation in citizen science is inherently affected. This temporal non-homogeneity is an evident and acknowledged limitation of citizen science, and thus it should not outweigh the benefits (e.g., increasing participant education and awareness, reduction of economic and time costs associated with environmental monitoring, and acceptable level of accuracy in data collection) of such a monitoring approach (Goffredo et al. 2010; Branchini et al. 2015a; Branchini et al 2015b; Meschin et al. 2021).

Diving parameters were significantly correlated with the taxa data in some of the years. PY was the most frequently related dive parameter, appearing in all years with a significant relationship to diving parameters for

both the taxa abundance and taxa p/a data sets (Table 1.2). The yearly average PY spans about half a year, ranging from early April in 2014 to late September in 2009, with little internal variation (S4 Table). Some of the variation in community structure over time in this study could thus be due to the differing seasonal environmental conditions in which questionnaires were collected. Total macroalgal biomass and community structure on coral reefs are strongly linked to seasonality and temperature variation in the Red Sea (Ateweberhan et al. 2006), where variation in benthic communities also affects the distribution of fish species (Eyal et al. 2019). Species richness, diversity, and evenness of reef fish assemblages vary significantly between summer and winter in the Red Sea (Megdadi et al. 2017), and these seasonal variations may be mirrored on wrecks and artificial reefs close to natural coral reefs.

Temperature, the next most linked dive parameter (Table 1.2) is generally influenced by both seasonality and depth. In this study, T is more likely a function of the seasonal variation because the range in average depth (AD) and Maximum Depth (MD) throughout the years is quite small (19–22 m and 25–28 m respectively) (SI Ch. 1, Table S4). In fact, coral species that have narrower depth ranges tend to occupy shallower depths, while species that can colonize deeper in the water column have wider depth ranges (Chow et al. 2019). This may suggest that the small range of average depth in surveys at the Thistlegorm, even if statistically significant, is trivial in terms of its effect on the community structure variation given the greater depth at which the surveys were conducted. This is especially true when considering non-sessile benthic and/or free-swimming taxa. The yearly average dive duration ranged from 41 to 55 minutes (SI Ch.1, Table S4) and presented a significant correlation in 2007 and 2008 for both data sets and in 2012 for the taxa p/a data (Table 1.2). Dive duration certainly plays a role in the number and amount of target taxa sighted. In validation trials of the STE project (Meschini et al. 2021), volunteer scores of mean accuracies, mean consistency, and mean percent identified are all positively correlated with dive time.

Following the temporal sequence of centroids of taxa abundance (Fig 1.1b), no clear trend emerged. Years 2007, 2009, and 2011 cluster around what could be interpreted as a general (even if variable) community composition. In the p/a centroidal data (Fig 1.2b) the years 2007 through 2012 displayed a relatively larger general cluster in community structure when compared with the abundance data.

The taxa abundance data revealed more variance among years than taxa p/a data, and even if the general trend is quite similar, two of the five best species best explaining community structure variations among years are different in the analysis of the two data sets (the Red Sea Clownfish and Napoleon Wrasse for p/a data vs. the Squirrel Fish and Humpback Batfish for abundance data). This partial inconsistency may depend on the different reliabilities of volunteer collected data between presence/absence and abundance data sets. In fact, the correctness of abundance parameter ratings ranges from 41 to 82 percent in validation trials of the entire STE project (and from 64 to 82 percent in the present analysis) while groups of volunteers show better performances in correctly

identifying the presence of taxa (Branchini et al. 2015a, Meschini et al. 2021). In 7 validation trials from the SS Thistlegorm, the parameters of data quality measurements were in line with previous analyses on coral reef environment types within the STE project (Meschini et al. 2021). The SS Thistle gorm average mean accuracy scored 57.1 percent within a range of 46 to 70 percent, coinciding with the results from (Meschini et al. 2021) where 94 percent of trials scored a mean accuracy between 40 and 70 percent. From the similarity index (SI) parameter all 7 of the dives analyzed here resulted in high levels of precision (SI, 95% CI lower bound $> 75\% \leq 100\%$), whereas only 0.4 percent of trials in (Meschini et al. 2021) obtained these similarity index scores. Notwithstanding the small number of validation trials ran on the SS Thistlegorm data set with respect to the entire STE project 20, the results highly resemble those of the more rigorous trials analyzed in Meschini et al. 2021 and can therefore provide enhanced reliability to the data that has been used for evaluating the community structure variance over time at this dive site.

In conclusion, there was no clear sequential shift of the SS Thistlegorm community structure over the eight years of monitoring, but some fluctuation around a general cluster characterized by a well-developed community, mainly driven by yearly relative changes in the frequency of a few species. The temporal analysis may have been slightly biased by the different average PY (i.e., seasonality) of collected data, but this bias is likely to be minimal, as the years in which PY was significantly related to taxa data are mainly included in the general cluster and do not display unusual patterns. The community structure at the SS Thistlegorm showed relative stability over time, making this artificial reef a possible and promising refugia for Red Sea communities. Further investigation on the influence of artificial reefs as an auxiliary tool for human-influenced decline could entail the comparison of multiple wreck sites within the northern Red Sea to nearby natural coral reef dive sites through species abundance analyses between and among groupings of sites, possibly by latitudinal and/or temperature gradients.

Supplementary Information for Chapter 1

Table S1. Yearly sample statistics of the relate test and significance of the relation between taxa data sets (abundance and presence/absence) and diving parameters. * Indicates significant differences ($P < 0.05$).

Year	Rho	Significance Level (%)
Abundance data		
2007	0.162	0.7*
2008	0.157	0.3*
2009	0.111	4.6*
2010	0.009	42.6
2011	0.104	6.9
2012	0.217	1*
2013	0.242	6.4
2014	-0.128	70.8
Presence/Absence data		
2007	0.18	0.2*
2008	0.136	1.4*
2009	0.106	6.3
2010	0.015	40.7
2011	0.079	15
2012	0.209	0.5*
2013	0.194	10.4
2014	-0.097	64.4

Table S2. DistLM sequential test table of results for each year with a significant relate test between taxa abundance data and diving parameters data. * Indicates significant relationship ($P < 0.05$).

Variable	R ²	SS(trace)	Pseudo-F	P
2007				
Percentage of Year	3.24E-02	7139.6	2.7418	0.001*
Maximum Depth	5.24E-02	4424.3	1.7139	0.043*
Depth with Most Time Spent	8.08E-02	6259.6	2.4688	0.005*
Water Temperature	0.11721	8040.8	3.2609	0.001*
Dive Duration	0.1455	6242.9	2.5825	0.002*
Fraction of Day	0.17072	5565.3	2.3418	0.003*
2008				
Percentage of Year	6.59E-02	10516	4.2355	0.001*
Maximum Depth	0.10501	6231.5	2.5756	0.004*
Depth with Most Time Spent	0.13201	4307.2	1.8046	0.033*
Water Temperature	0.16697	5575.7	2.3921	0.008*
Dive Duration	0.19716	4814	2.1053	0.017*
Fraction of Day	0.21578	2970.5	1.3062	0.195
2009				
Percentage of Year	6.48E-02	9698.4	3.9507	0.001*
Maximum Depth	9.06E-02	3853.3	1.5858	0.102
Depth with Most Time Spent	0.13677	6912.2	2.9434	0.001*
Water Temperature	0.16299	3923.3	1.6917	0.053
Dive Duration	0.18001	2545.9	1.0998	0.329
Fraction of Day	0.22789	7165.5	3.2253	0.001*
2012				
Percentage of Year	0.10713	7681	3.2394	0.001*
Maximum Depth	0.15342	3319.4	1.4218	0.15
Depth with Most Time Spent	0.18313	2130.4	0.90932	0.549
Water Temperature	0.2546	5124.6	2.3012	0.004*
Dive Duration	0.30535	3638.6	1.6802	0.077
Fraction of Day	0.34348	2733.9	1.2777	0.239

Table S3. DistLM sequential test table of results for each year with a significant relate test between taxa presence/absence data and diving parameters data. * Indicates significant relationship ($P < 0.05$).

Variable	R ²	SS(trace)	Pseudo-F	P
2007				
Percentage of Year	3.19E-02	6025.3	2.6996	0.004*
Maximum Depth	5.01E-02	3448.6	1.5556	0.117
Depth with Most Time Spent	7.84E-02	5346.3	2.4549	0.007*
Water Temperature	0.11628	7161.3	3.3865	0.001*
Dive Duration	0.14611	5640.3	2.7254	0.003*
Fraction of Day	0.16696	3940.6	1.9268	0.032*
2008				
Percentage of Year	5.11E-02	6812.9	3.2286	0.001*
Maximum Depth	8.97E-02	5156	2.5047	0.004*
Depth with Most Time Spent	0.11369	3199.3	1.5691	0.114
Water Temperature	0.15081	4953.1	2.4918	0.007*
Dive Duration	0.1813	4068.6	2.0858	0.031*
Fraction of Day	0.20006	2502.8	1.2897	0.238
2012				
Percentage of Year	9.76E-02	6197.1	2.9202	0.003*
Maximum Depth	0.15262	3493.6	1.6882	0.087
Depth with Most Time Spent	0.17632	1505	0.71941	0.703
Water Temperature	0.25321	4882.3	2.4711	0.015*
Dive Duration	0.3159	3980	2.1074	0.049*
Fraction of Day	0.34834	2060.1	1.0953	0.375

Table S4. Yearly averages of all diving parameters with confidence intervals. PY as date of dive (expressed as percentage of year), MD as maximum depth, AD as average depth, T as temperature, RTD as dive time, and FD as hour (expressed as percentage of day).

Year	Average	Confidence Interval
PY (% of Year)		
2007	56.3	50.9-61.7
2008	73.4	71.2-75.6
2009	81.6	80.1-83.1
2010	62.4	58.9-67.7
2011	74.5	69.9-79.1
2012	52.7	46.5-58.9
2013	61.6	60.4-62.8
2014	35.3	35.4-35.3
MD (m)		
2007	27.3	26.8-27.8
2008	27.1	26.4-27.7
2009	28.4	27.7-29.0
2010	28.4	27.9-28.9
2011	27.1	26.2-28.0
2012	26.4	25.4-27.3
2013	28.9	26.8-31.1
2014	27.5	26.2-28.7
AD (m)		
2007	18.9	18.3-19.5
2008	19.3	18.5-20.0
2009	20.3	19.5-21.2
2010	22.3	21.5-23.2
2011	19.5	18.7-20.3
2012	20.0	18.6-21.3
2013	19.6	18.0-21.1
2014	20.1	18.3-21.8
T (°C)		
2007	25.3	24.8-25.8
2008	26.0	25.7-26.3
2009	26.3	26.2-26.5
2010	26.6	25.2-27.1
2011	24.8	24.5-25.1
2012	25.7	24.8-26.6
2013	27.5	27.1-27.9
2014	23.9	23.3-24.5
RTD (mins)		
2007	44.2	43.2-45.2
2008	44.8	43.4-46.2
2009	41.2	39.7-42.9
2010	49.9	47.5-52.4
2011	45.7	44.6-46.7
2012	43.5	41.8-45.3
2013	47.3	45.0-49.7
2014	54.8	51.8-57.7
FD (% of Day)		
2007	0.50	0.48-0.52
2008	0.46	0.45-0.48
2009	0.48	0.46-0.49
2010	0.46	0.43-0.48
2011	0.46	0.45-0.48
2012	0.46	0.44-0.48
2013	0.49	0.45-0.52
2014	0.49	0.46-0.51

Chapter 2

Land-sea protection affects Red Sea biological communities of coral reefs over eight years of monitoring

Abstract

Coral reefs, vital for biodiversity and ecosystem services, face numerous threats, making management practices like marine protected areas (MPAs) crucial for conservation. However, MPAs alone have limitations in achieving biodiversity goals. A holistic approach to environmental protection, especially for coastal marine ecosystems, is necessary. From 2007 to 2015, spatial-temporal data was collected through recreational citizen science by Scuba Tourism for the Environment (STE; www.steproject.org) in the Red Sea. A subset of this data analyzed coral reef community structure variations across three land-based management levels (protected, intermediate, impacted) along the Sinai Peninsula's Eastern coast from 2007 to 2014. The “Protected” sites were along a non-developed and tourist-restricted coastline, while the “Impacted” sites were along a heavily developed coastline. The “Intermediate” sites were roughly 5 kilometers offshore from a developed coastline. All sites were within the marine-protected park of Ras Mohammed. Distinct community structures were identified between all management levels. Protected sites had a higher representation of Plating Acropora, Soft Tree Coral, Map Angelfish, and Sohal Surgeonfish, demonstrating elevated site aggregation in the canonical analysis of principal components and increased stability. In contrast, impacted sites displayed lower site aggregation and were marked by the prevalence of lionfish and sea urchins. This research underscores the importance of integrating terrestrial elements into marine conservation planning within MPAs to enhance biodiversity and community stability.

Introduction

Coral reefs are highly productive marine ecosystems, hosting some of the world's richest biodiversity (Bellwood and Hughes 2001) and providing many socioeconomic benefits through various ecosystem services including fisheries support, tourism revenue, and important barriers to coastal erosion (Woodhead et al. 2019). Notwithstanding their array of benefits, many developing and existing threats related to climate change and anthropic actions including ocean warming, acidification, overfishing, coastal population growth, agricultural activity, urbanization, and tourism are linked to impacts on coral reefs (Fine et al. 2019).

In response to these threats, marine protected areas (MPAs) emerged as a cornerstone for essential conservation strategies to safeguard coastal biodiversity from human related pressures (Gladstone et al. 2003). These areas, when effectively managed, promote ecosystem health and resilience, assisting marine conservation efforts through time (Halpern and Warner 2002). However, their effectiveness, weighed with their ability to equally distribute benefits to various stakeholders, has been long debated (Pendleton et al. 2017). Regarding fisheries, a primary economic beneficiary of marine conservation, there is concern that once an MPA is established, the fishery is simply relocated, resulting in local economic loss and a mere displacement of exploitation (Favoretto et al. 2023). Perceived limitations on recreational opportunities (Lopes et al. 2015) are another concern regarding tourism. Despite the many controversies surrounding MPAs' impact on fishing and tourism, numerous studies have demonstrated their effectiveness in preserving ecosystems while continuing to yield long-term benefits to many economic sectors and coastal human populations (Favoretto et al. 2023).

Unfortunately, the increasing realization is that over half of the cumulative impact on coral reefs can be attributed to land-based activities (Loiseau et al. 2021). This underscores the need to incorporate effective land-based management strategies for safeguarding these vulnerable ecosystems. Traditional conservation approaches, such as MPAs or national parks, focus on marine and terrestrial ecosystems separately. Yet, numerous anthropic and environmental factors originating from terrestrial sources carry over to marine ecosystems and can directly influence coral reef health. These factors include but are not limited to urban runoff, wastewater pollution, nutrient loading, sediment input, peak and annual rainfall, and wave exposure (Gove et al. 2023). Given that ecological connections between land and sea are heavily down streamed toward the marine environment, ecosystem integration should be especially considered if a management project aims at prioritizing marine health (Stoms et al. 2005; Carlson et al. 2019). This perspective has given rise to innovative management techniques such as the "ridge-to-reef" approach or land-sea conservation planning (Alvarez-Romero et al. 2011). Although this approach is not new in terms of human societies, as many small and traditionally governed communities often view/ed environmental stewardship holistically (Fitzpatrick and Giovas 2021; Sandin et al. 2022), most modern governances participating in environmental protection are siloed, subsequently isolating the costs, efforts, and benefits of terrestrial and marine conservation (Ruttenburg and Granek 2011; Sandin et al. 2022).

Recognizing the linkages between land and sea in the conservation of marine ecosystems is essential, yet the integration of terrestrial components remains limited in contemporary governance. While there is evidence that some modern strategies acknowledge and account for terrestrial influences on marine health, the majority tend to concentrate on singular objectives like endangered species preservation or water quality in watersheds (Darimont et al. 2010; Alvarez-Romero et al. 2011; Covino T 2017). Unfortunately, many of these approaches fall short of incorporating terrestrial systems holistically within the broader planning framework for marine protection. Notably, the highest integration of terrestrial components with marine conservation planning is most often observed on small island regions like Hawaii, Fiji, Pacific Islands, and Samoa (where planning and governance tends to follow a bottom-up approach) (Comeros-Raynal et al. 2019; Sandin et al. 2022; Fache and Pauwels 2022). While this marks a positive stride in coral reef protection, existing literature predominantly focuses on coral-centric or fish-centric aspects, such as population dynamics and responses to nutrient or sediment runoff, often examining individual species responses in isolation (Roberts et al. 2017; Gove et al. 2023). Remarkably scarce is research that addresses the entirety of biological communities supported by coral reefs. A study in Fiji, however, explored how habitat mediates fish population responses to turbidity (run-off). It found that fish populations were most sensitive to overfishing and pollution via habitat effects. Notably, both increases and decreases in biomass were observed among various functional groups, suggesting that run-off doesn't simply reduce biomass uniformly across the community, but instead leads to shifts in the overall community structure (Brown et al. 2017). This underscores the importance of examining whole community responses, rather than solely individual species responses, to understand the impacts on coral reef ecosystems holistically.

The Red Sea provides a unique case study for examining the consequences of fully incorporating a terrestrial ecosystem into marine conservation planning (Gladstone et al. 2013). Rapid coastal expansion and tourism pose a substantial risk to the long-term survival of its coral reefs (Gladstone et al. 2013). Additionally, the northern Red Sea is recognized as a coral refugia due to lower bleaching incidences than its southern portion, emphasizing the need for monitoring to understand factors supporting its resilience (Gladstone et al. 2013). Furthermore, the amount of information regarding coral reef systems in the Red Sea is minimal when compared to the coral reef communities of the Great Barrier Reef or of the Caribbean Sea (Berumen et al. 2019). Given its unique status and potential threats, intensified research and monitoring are crucial for effective conservation and sustainable management.

Seeing the large-scale and rapid development in the area, the Egyptian government established Ras Mohammed National Park in 1983 with the foresight and objectives of safeguarding the biodiversity that would significantly contribute to its economic development (www.iucngreenlist.org). Its primary purpose being marine protection, the terrestrial areas of the park encompass the southernmost portion of the Sinai Peninsula in addition to both Tiran and Sanafir islands, while the surrounding waters of these islands were incorporated in 1989

(www.iucngreenliist.org). Just twelve kilometres above the northern terrestrial border of the park lies Sharm-El-Sheikh, the resort hot-spot of the region. While regulations are in place to inhibit permanent development within 500 meters of the highest water line and the surrounding waters are protected (no-take zones), the diverse management of the coastline in conjunction with the consistent management of the waters allows for a meaningful examination of the effects of integrating terrestrial territory into marine conservation planning through the comparison of its coral reef communities. Protected sites in this study are located along the southernmost portion of the eastern Sinai Peninsula. Only 12% of this area is accessible to visitors, with no development along the coastline or within the entire southernmost portion. North of the park's terrestrial boundary lie the “impacted” sites, situated in the most developed area of the peninsula, where all the dive resorts and the airport are located. Finally, an intermediate set of dive sites was selected further off the developed coastline, within the Straights of Tiran.

Since traditional scientific monitoring requires intensive training and high costs, citizen science has emerged as an innovative and cost-effective biodiversity monitoring approach that can provide valuable data to researchers, policymakers, and stakeholders. The project Scuba Tourism for the Environment (STE; www.steproject.org), which has involved divers as volunteers to collect large-scale biodiversity data (Goffredo et al. 2010; Branchini et al. 2015b), exemplifies this in the Red Sea. Volunteers collected ecological community data with trained guides and dive centres without altering their recreational behaviour (Branchini et al. 2015a; Lee et al. 2023). Using these data, this study aims to: 1) evaluate the impact of land-based management strategies on Red Sea coral reef communities in the eastern Sinai Peninsula over 8 years and 2) address data gaps and controversies related to various MPA management approaches with an emphasis on coral reef community responses to terrestrial integration.

Materials and Methods

Data collection and isolation

Data collection was performed during the Scuba Tourism for The Environment – Red Sea Biodiversity Monitoring Program (STE; www.steproject.org). STE collected large-scale spatial-temporal biodiversity data using recreational citizen science monitoring from 2007 to 2015 throughout the Red Sea (Branchini et al. 2015a; Meschini et al. 2021). The recreational approach to citizen science implies that the volunteers did not alter their behaviour for the purposes of data collection (Branchini et al. 2015a). Divers were asked by participating dive centres to complete questionnaires that included personal information (name, country of residence, address, diving certification etc.), environment type of the dive site (sandy bottom, wreck, blue, or coral reef), six diving parameters (date, maximum depth, depth where they spent most of their time, temperature, dive time, and hour of the dive), and a list of 72 easily recognizable target animal taxa with taxa specific scaled abundance ratings (1:

rare, 2: frequent, 3: very frequent), encompassing all levels of the trophic community (for detailed information see Branchini et al. 2015a). An example of the questionnaire and a list of all the taxa considered can be found on the STE website ([STE: Scuba Tourism For The Environment – download \(steproject.org\)](https://steproject.org)).

The reliability of data collected through the STE project has been established through validation trials by Meschini et al. (2021). Random groups of volunteer divers were accompanied by trained marine scientists from the University of Bologna, who served as control divers. Volunteer questionnaires were compared to those completed by control divers across several key parameters: Consistency, Percent Identified, Accuracy, Similarity, and Reliability. Findings demonstrated that volunteer data met acceptable reliability standards, supporting the utility of citizen science in marine research. Detailed results and analyses are available in Meschini et al. (2021).

Data from three zones along the southeastern coast of the Sinai Peninsula (northern Red Sea) were then isolated from the larger STE dataset and categorized by their land-based management strategies for this study. Although the marine segments of these zones are all included within the no-take marine reserve, coastal development regulations vary. The "Protected" strategy, represented by Ras Mohammed National Park, included three selected dive sites: Jackfish Alley, Ras Za'Atar, and Shark and Yolanda Reef (Fig. 2.1). The bulk of the terrestrially protected area lies here, where only 12% of the area is accessible to visitors and no development is present along its portion of the coastline (www.iucngreenlist.org). The "Impacted" strategy zone featured two dive sites: Near and Middle Garden (considered as one site due to proximity) and Temple, situated just off the coast of Sharm El-Sheikh which exhibits prominent levels of touristic exploitation and coastal development (Fig. 2.1). The "Intermediate" strategy zone, Tiran area, featured four dive sites: Jackson Reef, Woodhouse Reef, Thomas Reef, and Gordon Reef (Fig. 2.1). Although these sites are located near the developed coastline of Nabq, the substantially greater distance from the coastline (~5 km offshore) may provide geophysical protection from the influence of terrestrial anthropogenic stressors. All dive sites included in this study are exposed, meaning they are not sheltered within any bay or lagoonal area. Each questionnaire represents the entirety of the selected dive site, as they were completed using a recreational citizen science approach. Unlike transect-based methods, this approach encompassed all sections and habitat types of each dive site. Consequently, the data include observations from both the reef crest and slope of all sites, with no dive sites containing reef flats. Sites were chosen based on a minimum of 10 valid questionnaires per year to ensure data reliability (Meschini et al. 2021) and continuity throughout the STE monitoring program.

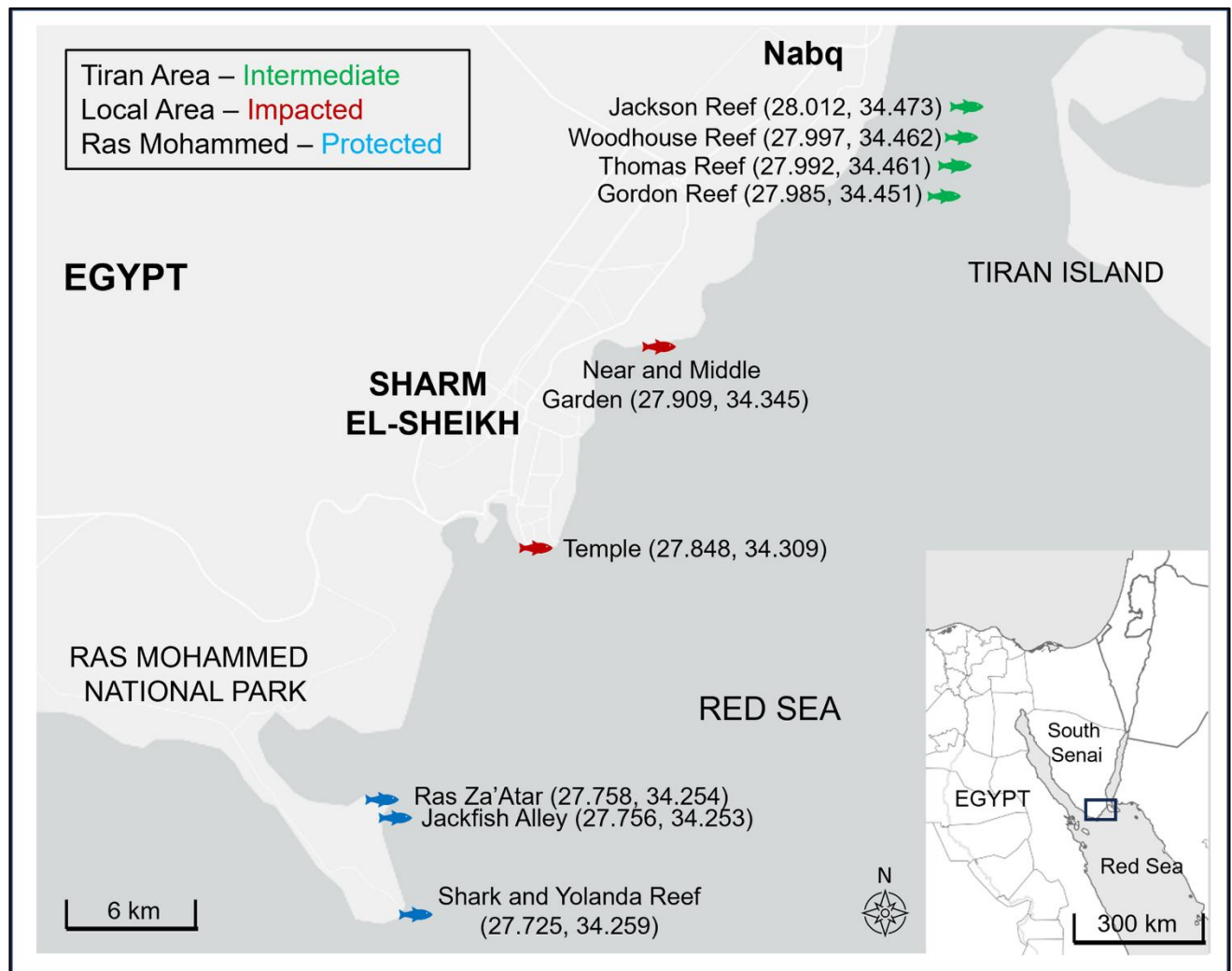


Figure. 2.1. Study area of the eastern coast of the Sinai Peninsula with relative locations of each dive site selected for the survey (exact coordinates shown in parentheses): Protected (blue; lowermost 3), Impacted (red; middle 2), and Intermediate (green; topmost 4) according to different management strategies.

Preliminary treatment and analyses

Data from each dive site were divided by year and used for preliminary analyses. Three datasets for each year of each dive site (216 total) were considered: two taxa datasets (abundance and presence-absence datasets) and an environmental dataset. The abundance datasets contained scaled abundance ratings for each taxon sighted during the dives while the environmental datasets were composed of the 6 diving parameters collected by the volunteer diver during the dive: date, expressed as Percentage of Year (PY), Maximum Depth (MD), Depth with Most Time Spent (AD), Water Temperature (T), Real-Time Dive (RTD) and Fraction of the Day (FD) (see Branchini et al. 2015a for details). The year 2015 was not included in this study due to insufficient questionnaires.

For a general picture of the yearly coral reef community structure and to recognize potential outliers of each dive site, figures were created using non-metric multidimensional scaling of Bray-Curtis resemblance matrices from both taxa datasets (Clarke and Gorley 2015). RELATE tests (Clarke and Gorley 2015) were then

conducted between the taxa and diving parameter resemblance matrices (Bray-Curtis and Euclidean, respectively) to investigate possible relationships between the variation of sighted taxa and dive parameters. The RELATE test provides the rho test statistic, a non-parametric correlation coefficient that indicates the strength and direction of the association between distance matrices. For any RELATE test resulting in a significant relationship, distance based linear models (DistLM) (Clarke and Gorley 2015) were then performed to individuate the specific diving parameters influencing reef community structure variation. A permutational multivariate analysis of variance (PERMANOVA) main test, with the factor "year" (2007-2014) and "management strategy" was then performed on the merged normalized diving parameters to test for significant differences in variation throughout the years and/or levels of management strategy.

Spatial-temporal analyses

Yearly Relative Abundances (RA) and Sighting Frequencies (SF%) of each taxon were calculated for each dive site as follows (Branchini et al. 2015a; Lee et al., 2023):

$$SF\% = \left(\frac{\text{Number of Sighting per taxon}}{\text{Number of Dives}} \right) \times 100$$

$$RA = (SF\%)(\text{Average Sighting Abundance})$$

Two datasets were elaborated using SF% values (relating to presence/absence) and RA values (relating to abundance) for each taxon. These datasets were derived from diver questionnaires completed annually at nine dive sites in the Red Sea. Dive sites were categorized into three management strategies: Protected (n = 3), Intermediate (n = 4), and Impacted (n = 2). At least ten questionnaires per dive site per year were used to calculate sighting frequency and relative abundance values, which were analysed using a single PERMANOVA design, treating dive sites as replicates in temporal-spatial analyses. These new taxa data from all dive sites were analysed collectively to explore the effect of land-based management strategies and their interaction on variations in reef community structure over time.

Ideally, an environmental matching method would have been employed to account for potential habitat-based variability; however, the 'recreational citizen science' (Goffredo et al. 2010) approach used in this study did not include protocols for collecting structural and biophysical habitat variables (e.g., distance to deep water, proximity to the nearest market, exposure type, slope gradient, etc.). Nonetheless, the questionnaires provided comprehensive coverage of each dive site, encompassing both reef slopes and crests and did not include any reef flats, minimizing potential confounding effects related to habitat type. However, the absence of detailed structural data (e.g. reef slope, angle, or rugosity) may limit the resolution of analysis and the ability to detect fine-scale habitat-specific variations.

For both datasets, two factors were considered: Years (with 8 levels: 2007-2014) and Management Strategy (with three levels: Protected, Intermediate, and Impacted). Main and post-hoc pairwise PERMANOVA tests (Clarke and Gorley 2015) were performed on Bray-Curtis resemblance matrices with the added Euclidean resemblance matrix of covariates to investigate differences between management strategies throughout the years. The main test returns a pseudo-F statistic, which indicates the ratio of variance between groups overall, while the pairwise test statistic, known as the T-statistic, measures the difference between two specific groups (Clarke and Gorley 2015). Latitude and PY were included as the covariates in the main test to account for possible spatial and/or seasonal confounding. Latitude values were sourced from the Professional Association of Diving Instructors (PADI) webpage (www.padi.com), and with estimated point locations from Google Maps when the dive site coordinates were not listed. For a visual representation of community variance among the 3 different levels of management strategy, a canonical analysis of principal coordinates (CAP; Clarke and Gorley 2015) was conducted on both datasets. Species vectors resulting from BioEnv "BEST" analyses (Clarke and Gorley 2015) were overlaid to show the 5 taxa most responsible for variation among the management strategies.

To explore temporal variation in community structure for both taxa RA and SF% datasets, in all dive sites, mean values of relative abundance and sighting frequency for each year were calculated for respective taxa subsets. Non-metric multidimensional scaling analysis (MDS) (Clarke and Gorley 2015) using Bray-Curtis resemblance matrices of the mean values was conducted to visualize the coral reef community composition variance through time. BioEnv "BEST" tests were performed on resemblance matrices of Average for the factor "Year," and the resulting taxa trajectory overlays were included in the plots to show their influence on yearly variation. The mean values of relative abundance and sighting frequency of every year were calculated for the respective subsets of each "best" taxa to visualize the individual temporal evolutions of each taxa.

As Plating Acropora emerged as a significant indicator species in the BEST analysis, a post-hoc exploratory correlation analysis was performed to assess its potential associations with other hard coral taxa abundances. These taxa included *Seriatopora hystrix* (Porcupine Coral), *Plerogyra spp.* (Bubble Corals), Mushroom Corals, *Turbinaria spp.* (Lettuce Corals), Pineapple Corals, and Other Coral (a combined category for corals not specified in the questionnaires). Draftsman plots confirmed positive associations between all other hard coral taxa and Plating Acropora (Draftsman Plot; Clarke and Gorley, 2015), consequently, scatter plots with best-fit lines and Pearson's correlation coefficients were then created to explore the strength and significance of these relationships (including both RA and SF% data sets). This analysis aimed to explore the role of Plating Acropora as a potential indicator of overall coral cover across sites.

All analyses were conducted using PRIMER-E v7 with PERMANOVA+ version 1 software (PRIMER-E, Ltd., Plymouth, UK).

Results

Data collection and isolation

From 2007 to 2014, 10,969 questionnaires were collected and distributed across areas as follows: 1097 in the Impacted area, 4351 in the Intermediate area, and 5521 in the Protected area (SI Ch.2, Table S1). Annual questionnaire counts ranged from 11 to 582 for all strategies and displayed a significant decline over time ($R^2 = 0.72$, $P < 0.01$). All taxa on the STE questionnaire were sighted over the 8-year survey (100%).

Preliminary treatment and analyses

Many diving parameters resulted significantly in all dive sites during various years for both taxa datasets ($P < 0.05$) (SI Ch. 2, Table S2). Nevertheless, many of the p-values were borderline and the RELATE Rho was rarely above 0.3, suggesting minimal effects of diving parameters on community structure.

Spatial-temporal analysis

Years (YE) and management strategies (MA), for Relative Abundance and Sighting Frequency data sets showed significant differences among groups (PERMANOVA, RA and SF%: $P < 0.005$) without significant interaction between the two factors (P values of 0.42 and 0.39 respectively) (Table 2.1). Both factors remained significantly correlated with the variation in community structure even after accounting for the influence of the covariates, implying a genuine association between the observed community structure and the two factors. More than 50 percent of the variation was attributed to Management strategy in both taxa datasets (Table 2.1) with temporal variation accounting for most of the remaining percentage.

Table 2.1. PERMANOVA main test results for Relative Abundance and Sighting Frequency (SF%) data, with latitude and seasonality (PY) included as covariates. “Y x MS” represents the interaction between the factors Year and Management Strategy. DF denotes degrees of freedom, SS is the Sum of Squares, and “%ECEV” indicates the percent estimated component of explained variance. The Pseudo-F statistic tests for variation between the specified groups. * Indicates a statistically significant P-value of the test statistic (< 0.05).

	Relative Abundance					Sighting Frequency				
Main Test	DF	SS	Pseudo - F	P-value	% ECEV	DF	SS	Pseudo-F	P-value	% ECEV
Latitude	1	500.34	4.4021	0.0063*	4.4	1	433.41	5.8728	0.0002*	6
PY	1	166.09	1.4613	0.1843	0.6	1	136.83	1.8541	0.083	1
Management Strategy	2	1906.3	8.386	0.0001*	71.1	2	1136.1	7.6975	0.0001*	60.4
Years	7	2450.9	3.0804	0.0001*	23	7	2090.4	4.0465	0.0001*	31.4
Y x MS	14	1638.3	1.0296	0.4158	1	14	1075.4	1.0408	0.3934	1.3

The CAP analyses of both RA and SF% (Figs. 2.2 a and b) revealed separate groupings of dive sites among the three management strategies, while the post-hoc Permanova Pairwise test underscored the significance of the differences between groups (SI Ch.2, Table S3). The five most representative taxa for RA variation among management strategies were Map Angel (*Pomacanthus maculosus*), Plating Acropora (*Acropora spp.*), Other Bony Fish, Lionfish (*Pterois spp.*), and Soft Tree Coral (*Dendronephthya spp.*) (BioEnv BEST test) (Fig. 2.2a). For SF% data, Map Angel, Soft Tree Coral, Leather Coral (*Sarcophyton spp.*), Sohal Surgeonfish (*Acanthurus sohal*), and Other Sea Urchins, were the most representative taxa of community variance between management strategies (Fig. 2.2b). The species trajectories on the CAP plots show impacted sites having a higher representation of Lionfish and Sea Urchins, whereas the protected and intermediate sites are characterized by increased sightings and abundances of the remaining Best taxa (Figs. 2.2a and b).

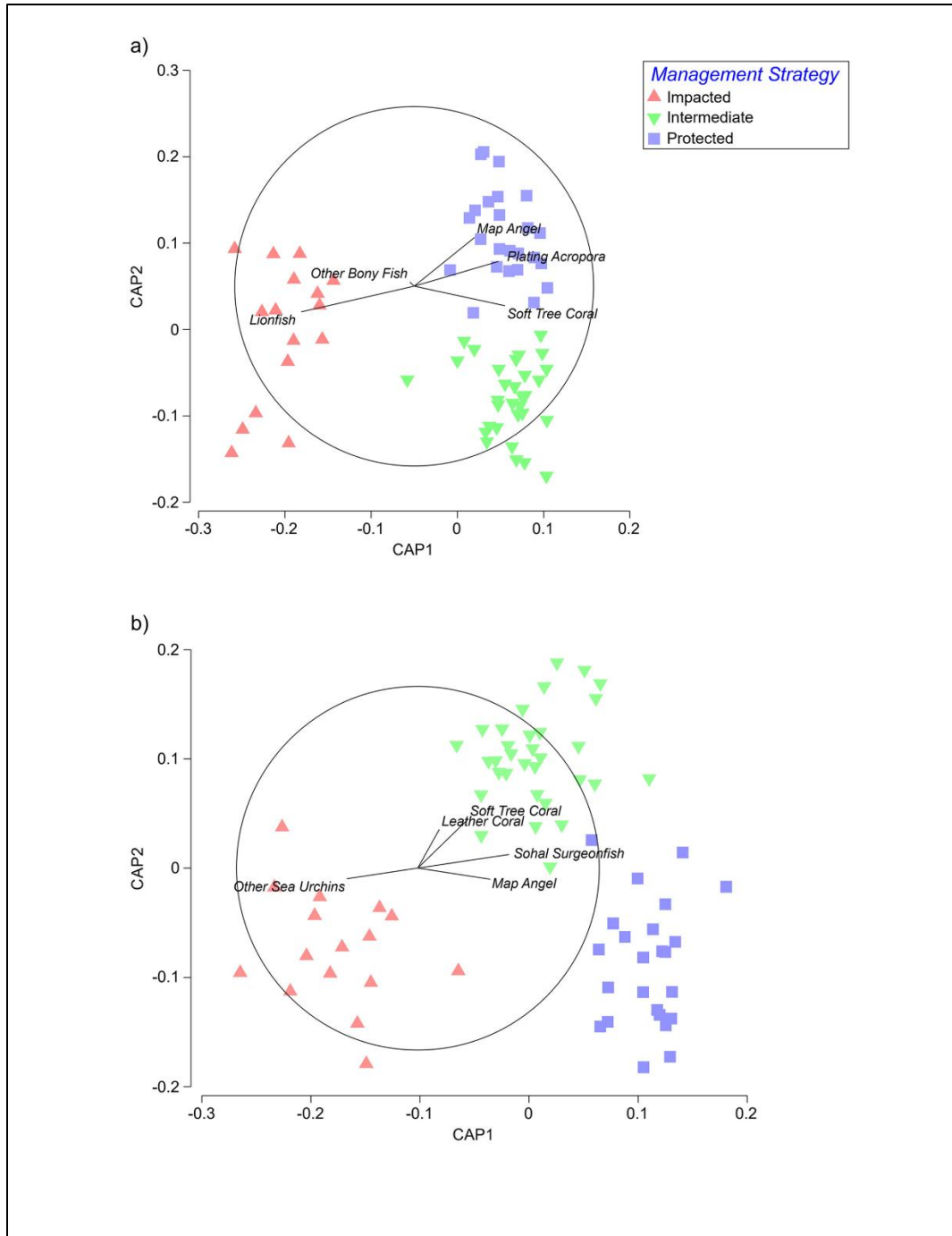


Figure 2.2. CAP plots of taxa community structure variance among the three levels of land-based management strategies considering (a) Relative Abundance data set and (b) Sighting Frequency data sets showing clear separations between groups with Best taxa trajectories in black. Each point represents different years of each dive site while land-based management strategies are indicated by the shape/colour: Impacted (red), Intermediate (green), and Protected (blue).

To visually explore community change over time a non-metric MDS showed variations in community structure centroids among different years for both datasets (Figs. 2.3a and b), with similar conditions observed from 2007 to 2012, and different conditions in 2013 and 2014 for the RA dataset. In the SF% dataset, similar

conditions were found from 2007 to 2011 and different conditions in 2012, 2013, and 2014. The BioEnv BEST test identified the five most representative taxa of community variance between years for each dataset: Sea Cucumber, Groupers, Sea Whips, Pearl Red Star (*Fromia spp.*), and Goatfishes for RA, and Caranxes, Other Sea Urchins, Map Angel, Lionfish, and Plating Acropora for SF% (Figs. 2.3a and b). Figure S1 (SI Ch.2) shows the individual temporal evolutions of Best taxa abundance and frequency at which they were sighted throughout the study (respectively), corroborating the yearly changes in the community structure variance of Figure 2.3. Temporal trends in most observed taxa were similar across management strategies, with an increase in both Relative Abundance (RA) and Sighting Frequency (SF%) of most taxa during the years 2013 and 2014 (Fig. 2.4).

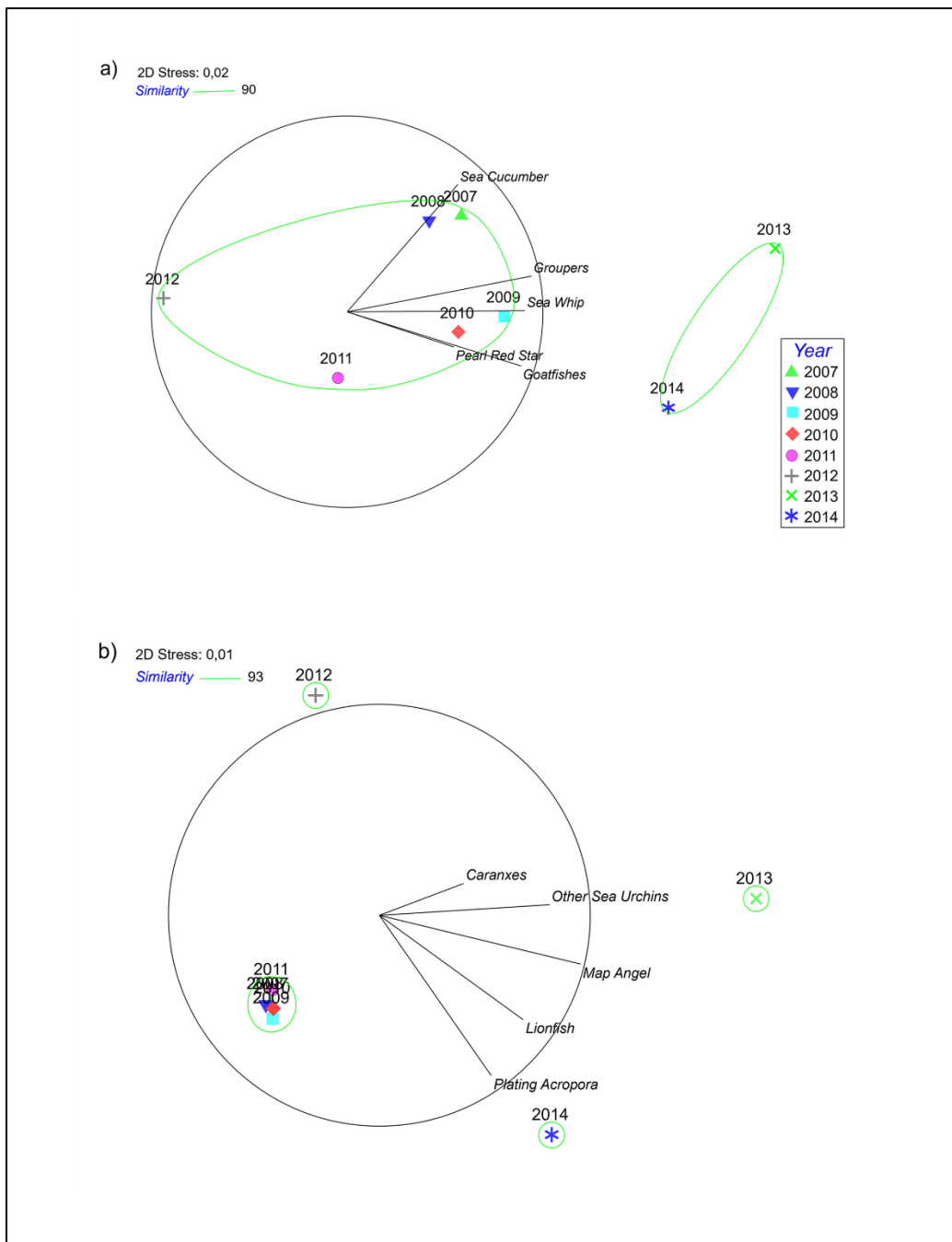


Figure 2.3. MDS plots of taxa community structure change over time from 2007-2014 (a) Relative Abundance data set and (b) Sighting Frequency data sets showing a general increase of Best taxa (trajectories in black) in later years. In both panels, different community structures can be found after 2012. Each point represents the average community location of each year indicated by different shapes/colours. Green groupings represent clusters of similar community structure variance.

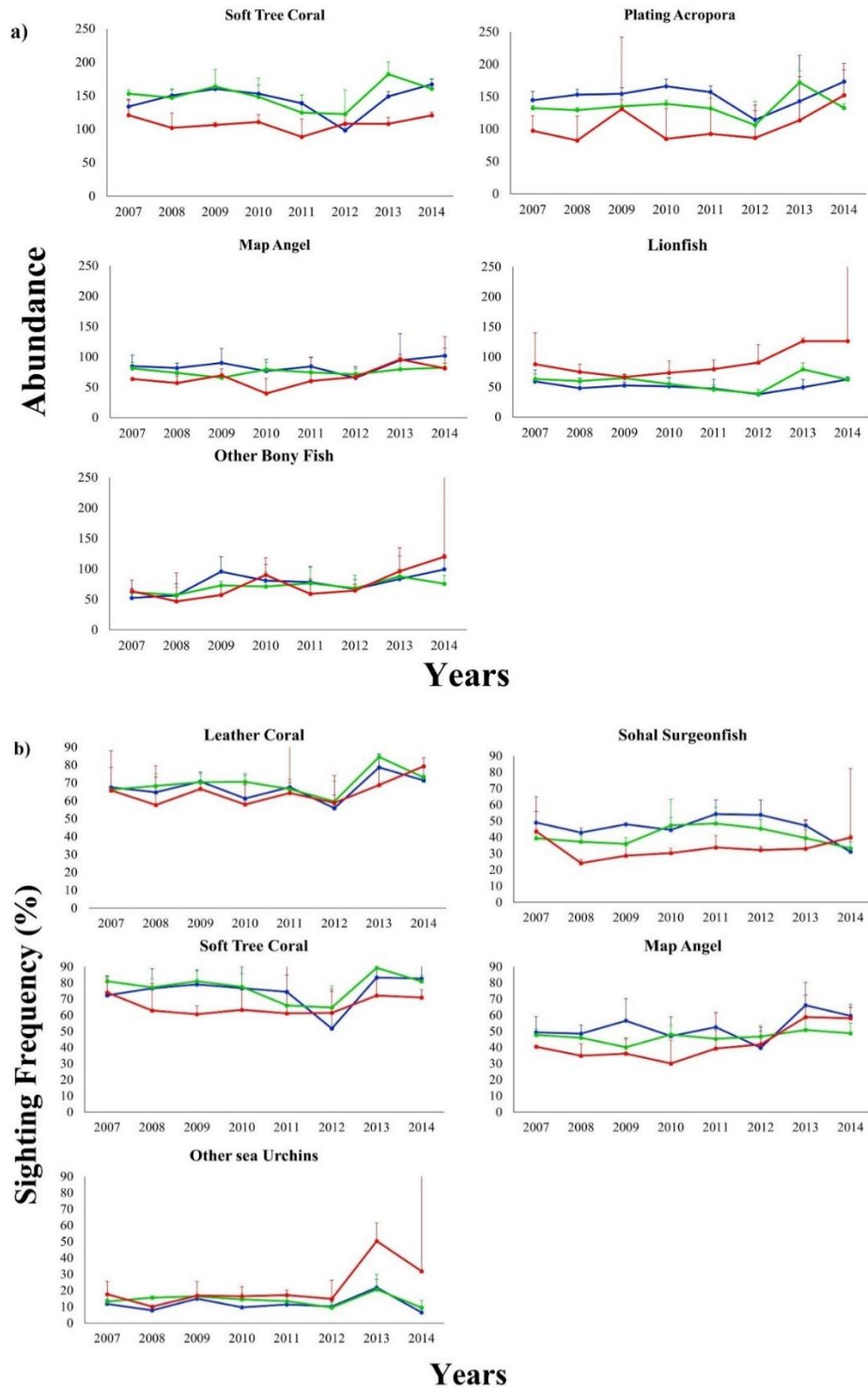


Figure 2.4. Yearly trend of the average values of relative abundance (a) and sighting frequency (b) for species resulting from BEST analysis. Different colors of the lines indicate different management strategies: Protected (blue), Intermediate (Green), Impacted (red). Bars indicate the Confidence Interval.

The correlations between Plating Acropora and all other hard coral taxa were highly significant ($P < 0.001$); however, the strength of these relationships was moderate with R^2 (Pearson's correlation) ranging from 0.2 to 0.5 (Figs 2.5 and 2.6). Overall, these results suggest that Plating Acropora is positively associated with other hard coral species, and its relative abundance and sighting frequency trends may serve as general indicator for broader coral cover patterns. The coefficients of determination (R^2) were notably higher when the relationships were tested based on relative abundance rather than sighting frequency, suggesting that abundance better explains the variance in other hard coral taxa.

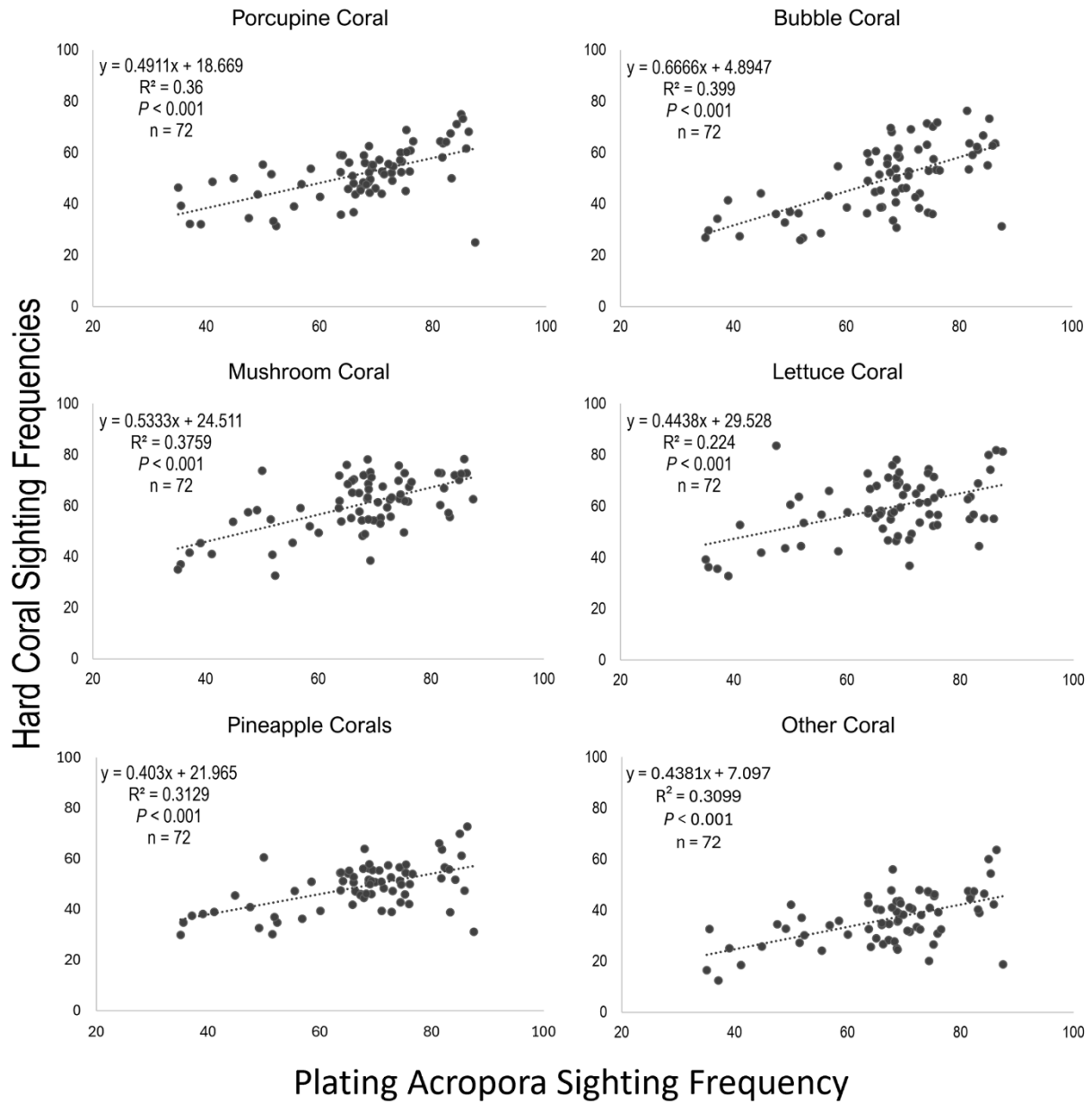


Figure 2.5. Scatter plots of the relationship between sighting frequencies of various hard coral taxa and Plating Acropora including all dive sites throughout all years. Each plot includes the best-fit line, the relationship strength

(R^2), the significance level (P-value), and sample size (n). All panels display highly significant positive correlations with Plating Acropora presence.

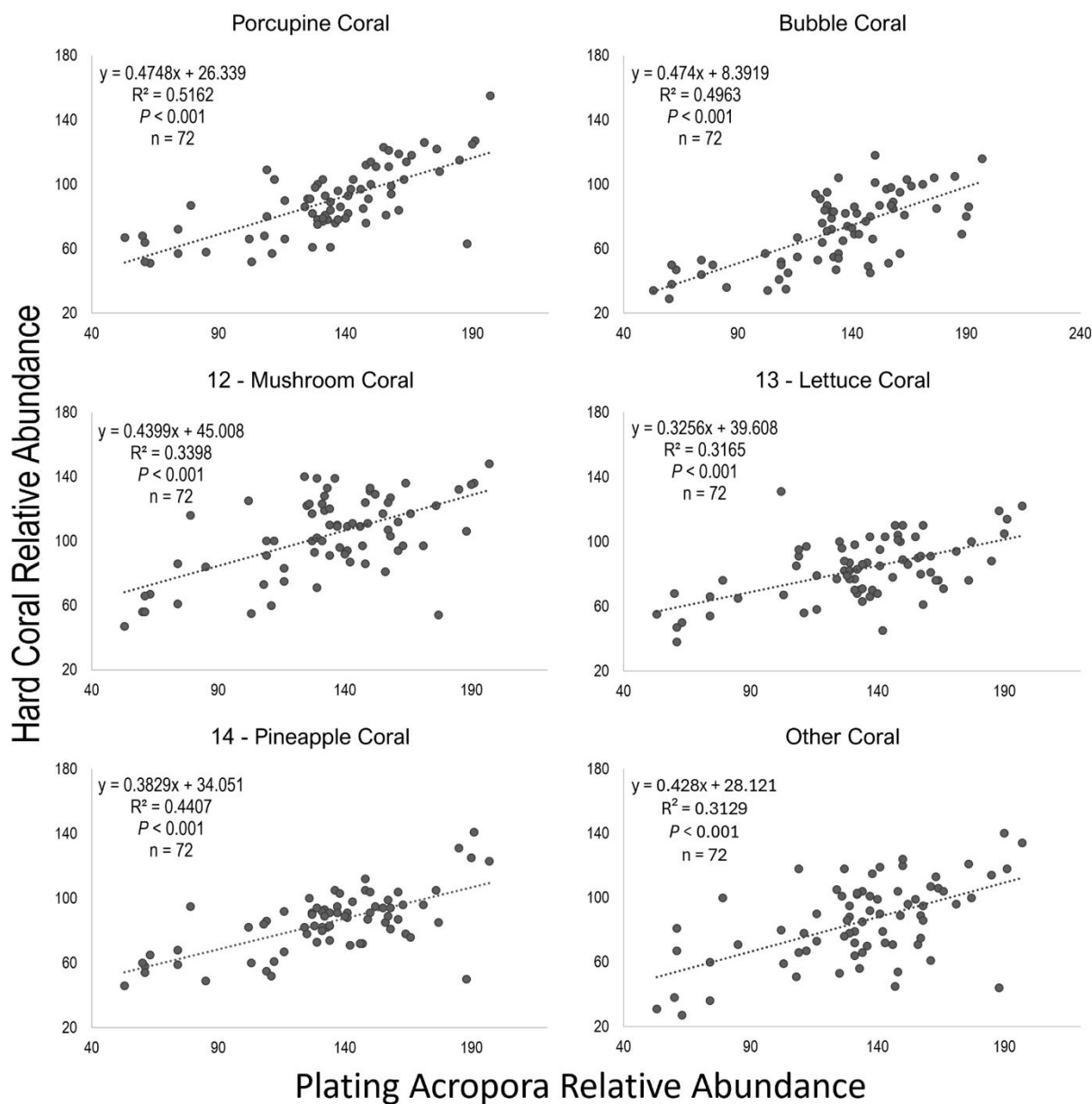


Figure 2.6. Scatter plots of the relationship between the relative abundance of various hard coral taxa and Plating Acropora including all dive sites throughout all years. Each plot includes the best-fit line, the relationship strength (R^2), the significance level (P-value), and sample size (n). All panels display highly significant positive correlations with Plating Acropora relative abundance.

Discussion

This study investigated the influence of land-based management on biological communities of coral reefs within the marine protected zone of Ras Mohammed National Park, utilizing 8 years of citizen-science collected data.

The significant reduction in completed questionnaires over the survey period ($R^2 = 0.72$, $P < 0.01$) is likely due to the political instability triggered by the Arab Spring in 2011, which had lasting effects on tourism in Egypt (Wendt 2019). Tourism declined sharply in the following years, as noted by Former Egyptian Minister of Tourism Hisham Zazou, who reported 2013 as the worst year on record for the industry (Honoltz L, 2014; Yahoo! Finance, 2014). Economic studies further corroborate this, showing that tourism began recovering only around 2014–2015 (Ecchevaria and Garzia-Enriquez, 2019; Groizard et al., 2021). As a result, the reduced number of questionnaires likely reflects a decline in the number of tourists during this period. While lower tourist participation rates could also explain the decline in questionnaire completion, community outreach programs were conducted in 2006, 2007, and 2011–2014 to promote project awareness and encourage participation, making this explanation less probable. Additionally, since each participant received a standardized briefing on species identification and safe diving practices before their survey, the reliability of responses should have remained consistent over time (Branchini et al. 2015a).

The reduction in tourism may have indirectly influenced the observed changes in biological communities across all sites in the later years of the study (Fig. 2.3a and b). With fewer divers and less anthropogenic stress, biological communities may have experienced reduced disturbance, potentially leading to increased abundance and sightings of certain taxa. Notably, indicator taxa driving temporal variation showed marked increases in 2013, while in 2014, the community structure seemed to trend back toward the earlier baseline. Similar patterns emerged in taxa associated with different management strategies (Fig. 2.4). Although studies on how reduction in human activities impact coral communities are limited, the COVID-19 pandemic provided a unique opportunity for researchers worldwide to investigate this phenomenon. Research on the effects of reduced human activity on coral communities is limited, but the COVID-19 pandemic provided a unique natural experiment. For example, in French Polynesia, lockdowns from February to December 2021 led to a 2.7-fold increase in adult reef fish density at eco-tourism sites, suggesting rapid responses to decreased human presence (Gairen et al. 2024). Comparable results have been reported globally: in the Gulf of Mannar, India (Patterson Edward et al. 2021), Cabo Pulmo, Mexico (Olán-González et al. 2023), and the Gulf of Aqaba, Israel, where fish evenness increased (China et al. 2021), all highlighting swift ecological responses to reduced tourism and human activity. Further, a study in Moorea, French Polynesia, observed not only increased adult fish density during the lockdown but also sustained higher fish numbers even after human activity resumed (Bertucci et al. 2022). These findings support our hypothesis that the observed temporal variations in coral reef communities in the later years of this study were likely influenced by reduced tourism due to political upheaval, similar to the patterns seen globally during the

pandemic. This study further underlines that, under reduced human pressure, coral reef communities can quickly shift, increasing biodiversity and abundance among a variety of taxa—not only fish taxa such as groupers, caranxes, map angels, lionfish, and goatfish, but also other marine organisms like sea stars, sea cucumbers, and sea whips (Fig 2.3a and b).

Considering diving parameters, some correlations were observed, however, they generally exhibited weak strength ($Rho < 0.2$) and minimal influence on the collected community data (SI Ch. 2, Table S2). Regarding average depth, the observed differences throughout both years and management strategy were not biologically relevant to influencing the community. Indeed, the average values of depth in Impacted, Intermediate, and Protected sites were 13.85, 13.89, and 14.26 respectively, while the greatest range in average yearly depth was 6.7 meters which hardly reflects a significant difference in the taxa being observed, especially when considering visibility in these areas generally exceed 20 meters (personal observation of author). Yearly average water temperature (T) differences are also negligible. In the impacted area, Temple records an average temperature of 27.2°C, deviating only 0.66°C and 1.34°C from the mean. Jackson Reef, in the intermediate zone, shows an average temperature of 26.82°C, deviating by 0.52° to 0.6°C. In the Protected area, Shark and Yolanda Reef sees minimal deviations from the average temperature of 27°C, with mean temperature deviations ranging from 0.37 to 0.88. It is also important to note that different models of dive computers can contribute to recorded temperature fluctuations (Marlowe et al. 2022).

As far as Latitude and seasonality (PY), which were included as covariates in the statistical model, only latitude resulted as a significant co-variable to the variance in community structure; seasonality did not appear to be a significant confounding factor. However, as latitude remained explained less than 7% of community variance in both analyses, its apparent role in driving community variation is marginal. Additionally, the management strategy levels do not follow a latitudinal gradient, as the northernmost sites were classified as Intermediate, followed by Impacted sites below, and finally, Protected sites in the south. Ultimately, management strategy accounts for more than half of the estimated components of taxa community variation in both the RA and SF% data, highlighting its robustness as a driving factor and suggesting that, relative to other factors, variation in coastal development had the greatest explanatory power for the observed differences in coral communities.

Both RA and SF% were significantly different among years and all three levels of land-based management strategies but without significant interactions between these two factors. This lack of interaction suggests that while the influence of management strategies is crucial in shaping these communities, they also exhibit temporal variation based on natural and/or other anthropogenic factors. Additionally, management strategy seems to influence community structure consistently throughout the eight years however the observed temporal changes are not dependent on these management strategies. As can be observed in the CAPs (Fig. 2.2), there were clear groupings according to the three management strategies in both taxa RA and SF%, with Protected and

Intermediate zones showing more stability and site aggregation than the Impacted zone. Increased coastal disturbance appears to be a pivotal factor driving variations in reef community structure. Notably, the communities of Intermediate sites were more similar to the Protected sites than the Impacted sites, suggesting that their distance from the disturbed coastline likely contributes to geographic protection from sedimentation and coastal disturbance. Numerous studies have highlighted the significant impact of farming and deforestation on coral health, primarily due to increased sedimentation (McClanahan and Obura 1997; Syvitsky et al. 2005; Roberts et al. 2017). The role of tree roots in retaining sediment is pivotal, and agricultural activities often result in considerable soil disturbance (Roberts et al. 2017). Interestingly, one study highlights that seemingly minor changes in the landscape, such as the introduction of dirt roads, can result in substantial sedimentation release (Oleson et al. 2017). Due to severely low annual rainfall, the Senai peninsula has limited vegetation, however, the region remains susceptible to flash flooding during certain seasons (Morsey et al. 2017), and lower vegetation cover tends to increase erosion rates (Mateos-Molina et al. 2015). Even in the absence of flash floods and higher sedimentation rates, human activities can significantly increase organic pollutants, phosphorous, heavy metals, and pathogens in coastal reef systems (McClanahan and Obura 1997; Oleson et al. 2017). Various factors likely contribute to the disparity in coral reef communities between Impacted sites and those under Intermediate and Protected land-based management. Given the lower susceptibility of corals in the northern Red Sea to coral bleaching (Osman et al. 2017), prioritizing their protection and minimizing stress is crucial. The challenge lies in identifying the most influential factors and determining their specific impacts on different parts of the community. These aspects merit further exploration in future studies within the Red Sea, considering the paramount importance of safeguarding these reef systems as coral refuges. Future research should analyse these communities under gradients of various potential driving factors, such as turbidity and/or organic pollutants. Given the nature of citizen science, which aims to remain accessible and simplified to encourage higher participation rates, including these parameters was not feasible in the current study.

The BEST analysis highlighted the five most representative taxa driving community variations between management areas. Notably, the taxa best explaining variation within impacted sights were Lionfish and Other Sea Urchins (for RA and SF% respectively). The Red Sea hosts different species of both native and alien Lionfish but in general, this taxon is widely considered a generalist predator (Ritger et al. 2020). Moreover, biotic interactions such as habitat competition and depredation between Lionfishes and their predators (for example Grouper species) may contribute and play a significant role in Lionfish distribution (Bos et al. 2018). Increased nutrient loads can raise the prevalence of macroalgae which degrades habitat quality and could subsequently affect Grouper numbers due to habitat choice (Wenger et al. 2015). In Japan, Groupers have been shown to have significantly positive microhabitat associations with *Acropora* species (Nanami et al. 2013), which was, coincidentally, more abundant in the protected sites. It has also been shown that increased sediment, whether

suspended or settled, can impact visual and chemical cues of reef fishes, thereby disrupting herbivorous fish feeding, and even predator-prey interactions (Wenger et al. 2015). This may be also at play for Lionfish in Impacted sites, providing an advantage to an already highly successful predator. In 2013 and 2014 there was an evident increase in Lionfish, which could be related to the lower stability of the area allowing this opportunistic taxon to benefit during years characterized by lower touristic pressure.

Regarding higher sea urchin prevalence in the Impacted sites, it has been shown in Kenyan reef studies, that more exploited areas had increased sea urchin densities, most likely due to the top-down effect of trophic levels within the coral reef community (McClanahan and Shafir 1990). Additionally, negative correlations were identified between coral cover and topographic complexity, with sea urchin density (McClanahan and Shafir 1990). Despite limited exploitation at the sites in this study (where only the Bedouin community is permitted fishing during specific seasons; www.iucngreenlist.org), increased coastal sedimentation is shown to have a detrimental impact on coral fish communities at higher trophic levels, potentially leading to increased sea urchin sightings (Wenger et al. 2011; Roberts et al. 2017). From a bottom-up perspective, heightened nutrient levels resulting from urban runoff in developed areas stimulate algal growth, which smothers hard corals, leading to stress and mortality (De'ath and Fabricius 2010). The synergistic effects of both phenomena are likely at play in the Impacted sites. Elevated sea urchin numbers may serve as an indicator of algal abundance along the developed coastline. However, non-target herbivores like sea urchins have also been shown to play an increasingly crucial role in coral resilience by removing smothering algae (Dang Viet et al. 2020), highlighting the importance of monitoring them as part of management strategies in the Red Sea.

Map Angel and Plating Acropora characterized sites managed under the Protected strategy, and to a slightly lesser extent, the Intermediate strategy, showing consistently high relative abundance and sighting frequency over time (Figs. 2.2a and b). This stability may reflect the resilience and health of coral reef communities near protected coastlines (or far from a developed coastline in the case of the Intermediate sites), potentially due to increased habitat complexity in these areas. While Plating Acropora showed some explanatory power in relation to the other hard coral presence and abundance the moderate R^2 values indicate that additional factors are likely influencing the hard coral community at Protected and Intermediate sites, which could, in turn, be influencing the broader biological community structure. These moderate associations most likely reflect shared environmental factors that favour these communities rather than a direct effect of coral cover by Plating Acropora. In any case, the higher presence of plating Acropora at these sites may suggest a greater abundance of other hard corals and, consequently, a generally higher overall coral cover. This extrapolation is subject to limitations however, as the STE project did not directly account for coral cover in the surveys, underscoring the need for more targeted investigations to confirm these observations.

Literature on the ecology of *Pomacanthus maculosus* (Map Angel) is limited, but recent research on angelfish bioecology in the coastal areas of Pangkep (South Sulawesi) provides some insights. This study found that while the overall amount of live coral did not significantly impact *Pomacanthus spp.* presence, certain coral growth forms, specifically branching, submassive, and massive coral fissures, were positively correlated with their occurrence (Kasmi M et al. 2024). This suggests that the increased prevalence of Map Angel in protected and intermediate sites could be linked to the presence of specific coral structures, such as the branching *Acropora spp.*, which may offer favourable habitats. *Acanthurus sohal* (the Sohal Surgeonfish), another key taxon explaining community variation at Protected and Intermediate sites, has been shown to have an inverse relationship with their abundance and the number of divers and snorkelers frequenting reefs around the Hurghada (Red Sea) islands (Shaban W. et al. 2020). Our findings align with this pattern, as the Impacted sites are located along a coastline developed with resorts, making the reefs more accessible to snorkelers and shore divers. This accessibility likely results in significantly higher numbers of divers and snorkelers in those areas, potentially contributing to the reduced abundance of the Sohal Surgeonfish at these sites.

Although all study dive sites fall within a no-take marine reserve, the results emphasize the value of incorporating land-based elements into marine conservation planning. While the “Impacted” areas are less stable and host higher numbers of lionfish and sea urchins, the sites in this area still boast higher biodiversity than other Red Sea regions, like Hurgada, most likely due to the positive influence of RMNP (Branchini et al. 2015b). This study successfully evaluated the impacts of integrating terrestrial components into marine management on coral reef communities with data collected through citizen science. Furthermore, by broadening the focus to all trophic levels within the coral reef community rather than isolated species or taxa this study addressed key data gaps and contributed to ongoing discussions regarding MPA management approaches, while shedding light on further areas for investigation within the Red Sea. RMNP exemplifies successful terrestrial integration of management efforts, benefiting both conservation and Egypt's tourism, emphasizing the global need for tailored marine protection strategies that integrate holistically terrestrial components, especially when considering ecosystems adjacent to coastlines.

Supplementary Information for Chapter 2

Table S1. Questionnaire counts by dive site and year.

Intermediate									
Dive Site	2007	2008	2009	2010	2011	2012	2013	2014	Total
Jackson Reef	318	483	253	272	395	243	78	52	2094
Woodhouse Reef	218	223	232	232	155	100	59	56	1275
Thomas Reef	106	124	70	126	165	106	77	39	813
Gordon Reef	83	199	99	399	402	97	22	38	1339
Total	725	1029	654	1029	1117	546	236	185	5521
Impacted									
Dive Site	2007	2008	2009	2010	2011	2012	2013	2014	Total
Near and Middle Garden	44	61	16	22	32	33	25	13	246
Temple	55	202	136	128	146	135	38	11	851
Total	99	263	152	150	178	168	63	24	1097
Protected									
Dive Site	2007	2008	2009	2010	2011	2012	2013	2014	Total
Ras Za'Atar	151	133	78	114	191	81	46	36	830
Jackfish Alley	119	222	152	227	273	86	54	20	1153
Shark and Yolanda Reef	422	582	268	374	362	167	116	77	2368
Total	692	937	498	715	826	334	216	133	4351
Grand Total	1516	2229	1304	1894	2121	1048	515	342	10969

Table S2. Yearly sample statistics of the Relate test between taxa data sets (abundance and presence/absence) and diving parameters of all dive sites included in this study. * Indicates significant P-value (< 0.05) of the Rho test statistic.

Near and Middle Garden			Temple		Gordon Reef		Thomas Reef		Jackson Reef	
Year	Rho	P-value	Rho	P-value	Rho	P-value	Rho	P-value	Rho	P-value
Abundance										
2007	0.391	0.001*	0.241	0.001*	0.079	0.13	-0.003	0.52	0.007	0.42
2008	0.28	0.001*	0.042	0.14	0.135	0.001*	0.05	0.12	0.105	0.001*
2009	-0.076	0.73	0.049	0.15	0.096	0.03*	0.006	0.44	0.06	0.07
2010	-0.193	0.91	0.106	0.02*	0.212	0.001*	-0.016	0.61	0.108	0.003*
2011	0.114	0.11	0.175	0.001*	-0.01	0.63	0.152	0.001*	0.112	0.001*
2012	0.104	0.12	0.339	0.001*	0.096	0.02*	0.203	0.001*	0.2	0.001*
2013	0.348	0.01*	0.208	0.02*	0.389	0.01*	0.099	0.09	0.033	0.3
2014	0.231	0.14	0.137	0.27	0.148	0.08	0.255	0.003*	0.043	0.26
Presence/Absence										
2007	0.372	0.001*	0.244	0.003*	0.066	0.16	-0.018	0.62	-0.003	0.5
2008	0.286	0.001*	0.012	0.39	0.161	0.001*	0.017	0.38	0.106	0.001*
2009	0.015	0.47	0.040	0.22	0.083	0.03*	-0.023	0.6	0.054	0.1
2010	-0.196	0.89	0.098	0.02*	0.201	0.001*	-0.050	0.84	0.098	0.003*
2011	0.146	0.047*	0.162	0.001*	-0.009	0.61	0.123	0.005*	0.115	0.001*
2012	0.101	0.13	0.330	0.001*	0.072	0.06	0.188	0.001*	0.179	0.001*
2013	0.296	0.019*	0.180	0.03*	0.431	0.001*	0.092	0.12	0.055	0.21
2014	0.318	0.08	0.093	0.34	0.145	0.08	0.252	0.004*	0.025	0.34
Woodhouse Reef			Jackfish Alley		Ras Za'Atar		Shark and Yolanda Reef			
Year	Rho	P-value	Rho	P-value	Rho	P-value	Rho	P-value		
Abundance										
2007	0.039	0.18	0.215	0.001*	0.22	0.001*	0.124	0.001		
2008	0.024	0.29	0.136	0.001*	0.059	0.16	0.037	0.08		
2009	0.196	0.001*	0.177	0.001*	0.217	0.001*	0.046	0.12		
2010	0.062	0.048*	0.242	0.001*	0.04	0.21	0.151	0.001*		
2011	0.215	0.001*	0.152	0.001*	0.189	0.001*	0.211	0.001*		
2012	0.137	0.03*	0.127	0.01*	0.156	0.001*	0.271	0.001*		
2013	0.355	0.001*	-0.075	0.83	0.095	0.12	0.073	0.11		
2014	0.354	0.001*	-0.031	0.56	0.079	0.20	0.278	0.001*		
Presence/Absence										
2007	0.039	0.18	0.224	0.001*	0.217	0.001*	0.115	0.001*		
2008	0.024	0.29	0.137	0.003*	0.073	0.09	0.028	0.19		
2009	0.196	0.001*	0.200	0.001*	0.162	0.01*	0.018	0.31		
2010	0.062	0.048*	0.246	0.001*	0.023	0.34	0.157	0.001*		
2011	0.215	0.001*	0.156	0.001*	0.180	0.001*	0.21	0.001*		
2012	0.137	0.03*	0.106	0.04*	0.112	0.04*	0.262	0.001*		
2013	0.355	0.001*	-0.103	0.92	0.065	0.25	0.071	0.13		
2014	0.354	0.001*	-0.107	0.73	0.086	0.19	0.261	0.001*		

Table S3. PERMANOVA main test and pair-wise test results using taxa RA and SF% data. * Indicates significant P-value (<0.05) of the T-statistic (T).

Pairwise Test: Management Strategy				
Groups	Relative Abundance		Sighting Frequency	
	T	P-value	T	P-value
Impacted, Intermediate	3.33	0.001*	2.9	0.001*
Impacted, Protected	2.74	0.001*	2.71	0.001*
Intermediate, Protected	2.19	0.003*	2.6	0.001*
Pairwise Test: Years				
Groups	Relative Abundance		Sighting Frequency	
	T	P-value	T	P-value
2007, 2008	1.078	0.297	1.3	0.081
2007, 2009	1.305	0.111	1.31	0.09
2007, 2010	1.112	0.251	0.993	0.416
2007, 2011	1.344	0.105	1.29	0.096
2007, 2012	1.804	0.02*	1.802	0.011*
2007, 2013	1776	0.03*	2.923	0.001*
2007, 2014	1.849	0.003*	1.776	0.001*
2008, 2009	1.896	0.197	1.395	0.087
2008, 2010	0.941	0.457	1.039	0.35
2008, 2011	1.123	0.258	1.274	0.134
2008, 2012	1.513	0.084	1.382	0.092
2008, 2013	2.069	0.012*	3.631	0.001*
2008, 2014	1.966	0.001*	2.199	0.001*
2009, 2010	0.720	0.818	0.686	0.929
2009, 2011	1.427	0.111	1.299	0.118
2009, 2012	2.02	0.01*	1.986	0.005*
2009, 2013	1.579	0.055	2.769	0.001*
2009, 2014	1.549	0.007*	1.553	0.003*
2010, 2011	0.965	0.427	0.955	0.452
2010, 2012	1.519	0.074	1.551	0.053
2010, 2013	1.633	0.056	2.478	0.001*
2010, 2014	1.526	0.018*	1.531	0.004*
2011, 2012	1.44	0.251	1.249	0.147
2011, 2013	2.017	0.018*	2.871	0.001*
2011, 2014	2.048	0.001*	1.9	0.004*
2012, 2013	2.538	0.003*	3.603	0.001*
2012, 2014	2.487	0.001*	2.493	0.001*
2013, 2014	1.198	0.17	1.687	0.005*

Chapter 3

Influence of anthropic impacts on benthic community structure over time in the Mediterranean Sea

Abstract

The Mediterranean Sea is a global biodiversity hotspot, home to numerous endemic species and unique, endangered habitats. Marine Protected Areas (MPAs) are a cornerstone of marine conservation, but their effectiveness depends on both their management strategies and the coastal contexts in which they are embedded. This study investigates how varying "protection levels"—defined by a composite score of legal MPA regulations, cumulative human impact, and biodiversity indices—influence benthic community structure along the Italian coast. Using data collected by recreational citizen scientists through the Divers for the Environment – Mediterranean Underwater Biodiversity Project (2002–2005), we analyzed abundance ratings of 61 target taxa across dive sites categorized into four protection levels: very high, high, medium, and low. Our results revealed that Very High protection areas were characterized by greater abundances of ecologically significant taxa such as Precious Red Coral, Yellow Cluster Anemone, Dotted Sea Slug, and Dusky Grouper, while lower protection sites featured higher abundances of Neptune Grass, Mermaid's Wine Glass, Anglerfish, Stony Sponge, and Other Echinoids. Medium protection sites were dominated by generalist taxa such as Other Fish, Other Vegetals, Other Sea Stars, and Rainbow Wrasse. Beyond these species-level patterns, overall community structure varied among protection levels: Very High protection sites were generally more distinct, whereas High, Medium, and Low sites exhibited greater similarity. By integrating legal, human impact, and biodiversity metrics, this study provides a detailed evaluation of protection effectiveness and underscores the need for adaptive monitoring that considers the broader coastal context to ensure the conservation of Mediterranean marine biodiversity.

Introduction

The Mediterranean Sea is considered a global marine biodiversity hotspot due to its large number of unique features which stem from its transitional character between different biophysical conditions from surrounding marine environments (Atlantic Ocean to the west, the Black Sea to the north-east and the Indian Ocean to the south-east) (Rodríguez-Rodríguez et al. 2016). Coastal areas and continental shelves tend to have higher biodiversity, and many Mediterranean marine species are endemic (Coll et al. 2010). Moreover, there are several unique and endangered habitats such as seagrass meadows of the endemic *Posidonia oceanica* as well as coralligenous assemblages (Coll et al. 2010). At present, habitat loss and degradation, followed by fishing impacts, pollution, climate change, eutrophication, and the establishment of alien species are the main threats to Mediterranean ecosystems and affect the greatest number of taxonomic groups (Coll et al. 2010). Conservation efforts and assessing the success of their objectives is vital within the Mediterranean at ecological, political, and administrative scales (Rodríguez-Rodríguez et al. 2016).

MPAs are the leading global strategy for the conservation of marine biodiversity and protection of biological and environmental standards (Davis and Tisdell 1995; Rodríguez-Rodríguez et al. 2016). The International Union for the Conservation of Nature (IUCN 1988) defines an MPA as: “a clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values” that includes some marine area, (Allison et al. 1998). The Commission’s Demonstration Program on Integrated Coastal Zone Management (ICZM) of the European Union has looked at the many interrelated biological, physical, and anthropic challenges presently facing coastal areas (European Commission, 2000). The ICZM aims to promote planning and management within coastal zones through the integration of both civil society and authorities. This new approach could be a complement to the marine Special Areas of Conservation (MSACs), promoted by the EU’s Habitats Directive as part of the Natura 2000 network, where the process of a co-operation platform is provided by Member States across Europe to stakeholders and managers is (Francour et al. 2001; European Commission, 2000).

In the Mediterranean region, MPAs can cover a wide variety of situations, which may differ according to the options chosen or imposed at the time of their planning and establishment such as level of protection and zonation usage, their plans of management, their administrative jurisdictions, and the resources available to manage them (Francour et al. 2001). The literature is unclear regarding the question of whether a single large, protected area or several smaller ones (e.g., MPA network) would be more effective. For reserves designed to protect biodiversity hot spots in areas of high human impact it is more important to isolate the hot spot from threats and therefore it may be more appropriate to have a single large reserve to reduce interactions with heavily used areas (Allison et al. 1998). MPA networks are, however, being established worldwide and have been

supported by numerous research in their ability to protect marine biodiversity and associated ecosystem services (Francour et al. 2001; McLeod et al. 2009). To design MPA networks it is necessary to consider not only the recommendations on MPA size, shape, and spacing but also the threats represented by climate change. Identification and inclusion of key refuges, pathways of connectivity that link them to damaged areas, and measures to build redundancy into networks should also be included in MPA network planning (McLeod et al. 2009). These recommendations combined with the other knowledge allow managers to design and tailor site and regionally specific MPA networks that are more likely to succeed.

The zonation of MPAs within the Mediterranean region is a common practice (www.mase.gov.it). They are commonly set up with at least 3 (sometimes more) levels, with the distinction of a central (core) zone, where human activities and uses are prohibited; an intermediate (buffer) zone, in which only some uses are forbidden (e.g., spear fishing or sometimes scuba diving) or subject to limitation by the authorities; and a transitional area where the regulations are generally less restrictive (Francour et al. 2001).

Extensive literature has illustrated that MPAs have the potential to foster greater biodiversity, increased abundance, and the presence of larger organisms compared to unprotected areas (Fraschetti et al. 2002). However, it is crucial to consistently monitor the progress and success of MPA's to ensure that their efforts align with the original goals set forth. Government agencies are often under-funded, and many cannot afford large-scale monitoring (Goffredo et al. 2010). Indeed, collecting scientific data to investigate the health of ecosystems requires intensive training and high costs (Branchini et al. 2015b). These constraints can be overcome using the skills of non-specialist volunteer researchers (Darwall and Dulvy 1996; Fore et al. 2001; Bell 2007; Bhattacharjee 2005; Greenwood 2007; Cohn 2008; Goffredo et al. 2010). Marine scientists have started to approach Citizen Science as an innovative monitoring technique in which researchers involve divers as volunteers to collect large-scale biodiversity data with substantial cost cutting and important awareness-raising actions towards volunteers (Evans et al. 2000; Goffredo et al. 2004; Goffredo et al. 2010; Branchini et al. 2015b).

Community-based management and community-based monitoring are the two main approaches to citizen science. The first methodology implies a direct participation of citizens and stakeholders in management decisions, while the latter involves citizens and stakeholders collaborating to collect, monitor, and track data (Whitelaw et al. 2003; Keough and Blahna 2006). Citizen science has long been recognized as an essential tool in supporting scientific research and conservation biology (Darwall and Dulvy 1996; Fore et al. 2001; Goffredo et al. 2004; Bell 2007; Delaney et al. 2008; Goffredo et al. 2010), and the "Divers for the Environment: Mediterranean Underwater Biodiversity Project" (Divers for the Environment; Goffredo et al. 2010) represents a prime example of a community-based monitoring initiative carried out in the Mediterranean Sea. The goals of Divers for the Environment were: including large numbers of people in biodiversity monitoring, validating this new approach and comparing results with those from professional investigations, creating a volunteer sightings-

based index model to gauge the health of the marine environment, and to make knowledge available to the whole community (Goffredo et al. 2010). During the dives, each participant observed the abundances of 61 different target taxa, which included plants, invertebrates, and fishes. The questionnaires were filled out by volunteers after completion of the dive and the assistance of scientific divers provided a key element of the survey protocol to control data quality (Goffredo et al. 2004; Goffredo et al. 2010).

The aim of this study was to investigate how varying levels of protection—defined inclusively to account for both management strategies and local coastal environmental conditions—affect benthic community structure in the Mediterranean Sea. This research was conducted over four years of monitoring using recreational citizen science data, specifically a subset of data collected through the Divers for the Environment project.

Materials and methods

Data collection and Isolation

Data Data were collected during the Divers for the Environment – Mediterranean Underwater Biodiversity Project (Divers for the Environment; www.progettosubambiente.org). This project utilized recreational citizen science which implies that the divers did not change their behavior during the dive. From 2002 to 2005, recreational divers completed post-dive questionnaires, recording the abundances of 61 different animal and plant target taxa that were seen during the entirety of the dive. (Goffredo et al. 2010). Volunteers also recorded various personal information (surname, name, address, and diving certification), details about the dive site (dive center, province, and closest populated area), environment type (sandy bottom, rocky bottom or other), and 4 different diving parameters (date of dive, average depth, actual bottom time, and start time of the dive) (Goffredo et al. 2010; www.progettosubambiente.org). The 61 target taxa (4 plant and 57 animal taxa) were selected for their widespread presence across the Mediterranean, ease of recognition, and comprehensive representation of the ecosystem's trophic levels (Goffredo et al. 2010).

For each taxon, the scale of abundance was defined as rare, frequent, or abundant based on the frequency at which the taxon is normally encountered (Goffredo et al. 2010). Individual taxa scales were estimated using scientific databases, literature, and personal observations. As an example, sighting 1–4 Rainbow Wrasse specimens was classified as rare, 5–10 as frequent, and more than 10 as abundant. These scaled abundances were input into the database as 1 (rare), 2 (frequent), and 3 (abundant).

For this study, questionnaires from rocky habitats were aggregated by dive site. Only dive sites with at least 10 valid questionnaires per year were selected for the analysis; those that failed to reach this quorum were not included (Goffredo et al. 2010). Dive sites were then classified according to cumulative rank scores of management strategy, biodiversity index (Goffredo et al. 2010), and cumulative human impact score (CHIS) (Micheli et al. 2013) (Table 3.1). Because these three measures represent complementary aspects of conservation

effectiveness, we combined them into an integrated protection level index to provide a practical gradient for comparing sites.

Table 3.1. Dive sites selected for the analysis and their subdivisions into the four classes based on their cumulative ranks (lower rank indicates a lower protection level) of management strategy, biodiversity index, and CHIR. Protection levels are low protection; medium protection; high protection; and very high protection.

Dive Site	Abbreviation	General Location	Management Strategy rank	Biodiversity Index rank	CHIS rank	Total rank	Protection level
Calafuria	CL	Livorno	1	3	4	8	Low Protection
Secca di Punta Secca	PSG	Giannutri Island	1	3	4	8	Low Protection
Scole	SC	Giglio Island	2	3	3	8	Low Protection
Cala Grande	CG	Argentario	2	3	4	9	Low Protection
Civitata	CV	Capraia Island	2	3	4	9	Low Protection
Formiche della Zanca	FZ	Elba Island	3	3	3	9	Low Protection
Cala dei turchi	CT	Tremiti Islands	4	2	4	10	Medium Protection
Cannelle	CN	Elba Island	3	3	4	10	Medium Protection
Fetovaia	FT	Elba Island	3	3	4	10	Medium Protection
Picchi di Pablo	PB	Elba Island	3	3	4	10	Medium Protection
Scoglio del Remoiolo	SR	Elba Island	3	3	4	10	Medium Protection
Secca di Fonza	SZ	Elba Island	3	3	4	10	Medium Protection
Dragone	DG	Portofino	4	3	4	11	High Protection
Punta Secca	PSC	Tremiti Islands	4	3	4	11	High Protection
Secca delle Vedove	SV	Tremiti Islands	4	3	4	11	High Protection
Enfola	EN	Elba Island	4	3	4	11	High Protection
Scoglione	SCNE	Capraia Island	4	3	4	11	High Protection
Scoglietto	SCTO	Elba Island	4	3	4	11	High Protection
Altare	AL	Portofino	4	3	5	12	Very High Protection
Raviolo	RV	Portofino	4	3	5	12	Very High Protection
Testa del Leone	TL	Portofino	4	3	5	12	Very High Protection

Management strategy levels range from rank 5 (integral zone) to rank 1 (no protected area) (Table 3.1). Rank 5 was not represented among our selected dive sites because only scientific research and service activities

are allowed within the integral zone (www.mase.gov.it). In rank 4, 4 dive sites were in zone B of the Portofino marine protected area; 2 dive sites were off the coast of Elba Island; 3 dive sites were in the Tremiti Islands; and 1 dive site was within the MPA of Capraia Island (Fig. 3.1). In these areas (rank 4), marine regulations are strict: no dive classes or night dives, no water discharges, and pleasure craft are limited to sail, oars, electric propulsion, or motorized vessels meeting eco-compatibility standards. Mooring is permitted only at designated sites, anchoring and towage are prohibited, and both sport and small-scale professional fishing are forbidden to non-residents (Parco Nazionale Arcipelago Toscano, 2025; Portofino Marine Protected Area, 2023; Ministero dell'Ambiente, 2023). Rank 3 sites were located around Elba Island (Fig. 3.1). In this rank, water discharges are banned, diving and fishing with specific tools are forbidden without authorization, and motorized navigation with big vehicles and close to the coast is not allowed (www.isoleditoscanamabunesco.it). Rank 2 dive sites were located near Capraia Island, Argentario, and Giglio Island (Fig. 3.1). Capraia and Argentario fall within the Parco Nazionale Arcipelago Toscano (PNAT), while the marine area around Giglio is not formally included but follows similar restrictions through national fisheries rules and EU Natura 2000 protections. In these areas, water discharges are prohibited, sport and professional fishing require authorization, and bottom trawling is forbidden (Parco Nazionale Arcipelago Toscano, 2025; Consiglio Regionale Toscana, 2009; Presidente della Repubblica, 1996). The dive sites “Secca di Punta Secca” in Giannutri Island (Tuscan Archipelago) and Calafuria, located near the port area of Livorno, were not subject to marine protection regulations during the study period and were therefore classified as rank 1 (Parco Nazionale Arcipelago Toscano, 2025; Regione Toscana, 2020) (Fig. 3.1).

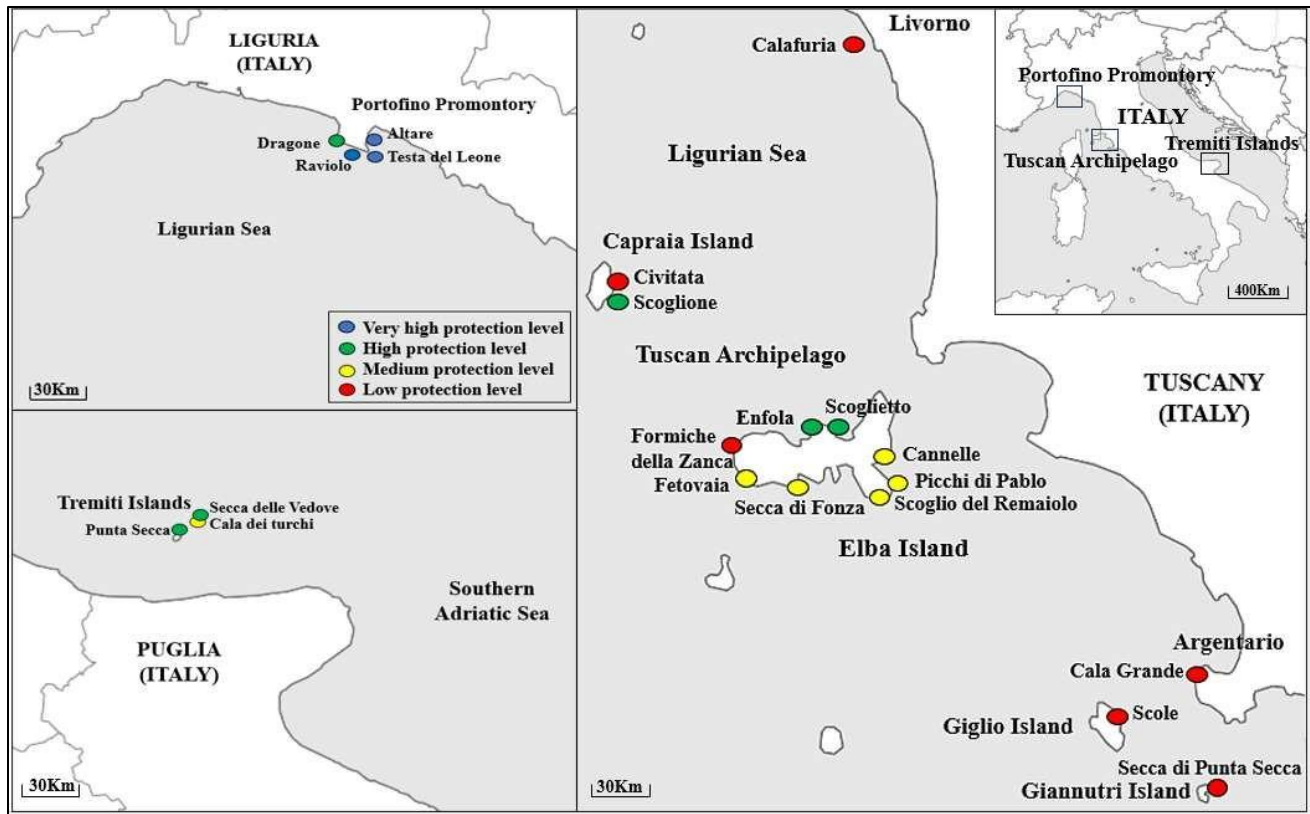


Figure 3.1. Map of dive sites selected for the survey: very high protection (blue), high protection (green), medium protection (yellow) and low protection (red) according to different protection levels.

The biodiversity index was calculated for each individual station and categorized into five classes: very good, good, mediocre, low, and very low (see Goffredo et al. 2010 for details). In Table 1 the “very good” biodiversity class coincides with rank 5, while the “very low” biodiversity class coincides with rank 1. The cumulative human impact scores (CHIS) were scaled as: very high; high; medium high; medium and low impact (see Micheli et al. 2013 for details) (Table 3.1). The CHIS was calculated based on four main categories of drivers: climatic (temperature and UV increase, and acidification), land- based (nutrient input, organic pollution, urban runoff, risk of hypoxia and coastal population density), sea-based (commercial shipping, invasive species, oil spills and oil rigs), and fishing (all fishing gears and types) (Micheli et al. 2013). These drivers differ in their distribution across the Mediterranean Sea; however, climatic drivers had the greatest contribution to the average cumulative human impact score. The scores were then converted to ranks for the purposes of this study. In this case our rank 5 coincides with a “low” cumulative human impact score while rank 1 coincides with the “very high” cumulative human impact score. The “high” (rank 2) and “very high” (rank 1) scores of cumulative human impact described in the paper were not represented among our selected dive sites. The “very low” CHIS was not included in setting the ranking list as it did not appear within the Mediterranean Sea at all.

The dive sites were then divided into the four different protection levels based on the cumulative ranks of the 3 previously described sub-ranks: very high protection; high protection; medium protection and low protection (Fig. 3.1).

Preliminary Analysis and Treatment

Reliability of data collected by volunteers participating in the DUE project was analyzed and confirmed in a previous study, which found that volunteer diver observations had sufficient accuracy and consistency (Goffredo et al. 2010). Data for each dive site were divided by year and used for preliminary analyses. Three datasets for each year of each dive site (252 in total) were considered: taxa abundance dataset, taxa presence/absence (p/a) dataset, and an environmental dataset (diving parameters). The abundance datasets contained abundance ratings for each taxon sighted during the dives while the environmental datasets were composed of four diving parameters (percentage of year, depth of greater permanence, diving time, and fraction of day). Environmental data were normalized, and a Euclidean distance matrix was generated. For a general picture of the community structure and to recognize potential outliers, figures were created using non-metric multidimensional scaling of Bray-Curtis (Shepard 1962; Kruskal 1964) resemblance matrices from both taxa datasets for every year at every site. Another dataset was elaborated by combining the average of diving parameters (percentage of year, depth of longer permanence, diving time, fraction of day) per year of each dive site. To check if diving parameters changed significantly between years or protection levels, a Main PERMANOVA (McArdle and Anderson 2001) test was performed on Euclidean distance resemblance matrices created from data. The normalized longitude and latitude coordinate values (retrieved from Goffredo et al. 2010 Appendix D) were added as covariates to the statistical model to uncover any spatial confounding.

In this study, we did not account for fine-scale habitat variability using an environmental matching framework. The citizen science approach adopted here focuses on standardized surveys conducted by recreational divers but does not include protocols for measuring site-specific structural or biophysical features such as slope, substrate complexity, or exposure. Importantly, the monitored sites were selected to represent rocky reef habitats, and sandy bottoms were avoided, reducing potential confounding effects linked to broad habitat differences. Nonetheless, the lack of detailed measurements of microhabitat features (e.g., rugosity, slope angle, or small-scale topography) may limit our ability to fully resolve fine-scale habitat-driven differences in benthic community structure.

Analysis

Yearly Relative Abundances (RA) and Sighting Frequencies (SF%) of all the taxon were calculated for each dive site as such (Branchini et al. 2015a):

$$SF\% = \left(\frac{\text{Number of Sighting per taxon}}{\text{Number of Dives}} \right) \times 100$$

$$RA = (SF\%)(Average\ Sighting\ Abundance)$$

Two different datasets were elaborated using SF% (relating to presence/absence) values and RA (relating to abundance) values for each taxon which included every year of all dive sites. For each dataset, two factors were considered for the analysis: Years (with 4 levels: 2002-2005) and Stewardship levels (with four levels: very high protection, high protection, medium protection, and low protection). To investigate differences between protection levels and across years, both main and pairwise PERMANOVA (McArdle and Anderson 2001) tests were conducted using Bray-Curtis resemblance matrices generated from the datasets. The normalized longitude and latitude values (retrieved from Goffredo et al. 2010 Appendix D) for each site were also included as covariates in this statistical model to account for potential spatial confounding. This approach removes variation attributable to spatial autocorrelation, allowing the variation associated with protection levels to be more accurately detected (Anderson et al. 2008).

A Canonical analysis of principal coordinates (CAP) (Anderson and Willis 2003) was performed on both datasets to visualize separate groupings of the four different protection levels. Two BVSTEP “BEST” tests were run to identify the main taxa driving variation between both factors (protection levels and years) (Clarke and Ainsworth 1993). The vectors of the taxa resulting from the BVSTEP “BEST” (correlation index $\rho > 0.85$) analysis were superimposed on both graphs to visualize their distribution among the factor levels. Mean values of relative abundance and sighting frequency of each year were calculated for the respective subsets of each “best” taxa and used for temporal variation analyses to investigate the yearly relative abundance of individual taxa within the four different protection levels for both the RA and SF% datasets.

In addition to the composite protection-level PERMANOVA described above, we performed a supplementary PERMANOVA using Management rank, Biodiversity index rank, and Cumulative Human Impact Score (CHIS) rank as separate fixed factors with the RA data. This test was conducted to disentangle the relative contributions of these three components, as they are partially correlated (sites with higher protection levels often coincide with lower human impacts and higher biodiversity). To further assess the role of CHIS, we also repeated the protection-level PERMANOVA after excluding the CHIS component, retaining only management and biodiversity indices. This allowed us to evaluate whether community-level differences remained when human impacts were not explicitly incorporated into protection categorization.

All the analyses were performed with PRIMER-E 6 with PERMANOVA+ version 1 software (PRIMER-E, Ltd., Ivybridge, UK).

Results

Data collection and isolation

A total of 3620 questionnaires were collected from 2002 to 2005 and divided as follows: 999 classified as low protection, 1003 classified as medium protection, 1286 classified as high protection, and 332 classified as very high protection (Table 3.2).

Table 3.2. Yearly counts of analyzed questionnaires by Protection level.

Protection levels	2002	2003	2004	2005	Total
Low Protection	309	279	231	180	999
Medium Protection	102	280	381	240	1003
High Protection	232	331	369	354	1286
Very High Protection	70	81	114	67	332
Total	713	971	1095	841	3620

Preliminary Treatment and Analysis

The main test of diving parameters (percentage of year, depth of longer permanence, diving time, fraction of day) was performed on Euclidean distance resemblance matrices created from the datasets (McArdle & Anderson 2001). Diving parameters had no significant effect on the community data variance as per the PERMANOVA results according to either years or protection levels (Table 3.3).

Table 3.3. Permutational multivariate analysis of variance (PERMANOVA) Main Test results of diving parameters data according to both the Years factor, and the Protection Level factor with covariates added. Df, degrees of freedom; SS, sum of squares; MS, mean squares; P(permutation) significance of permutational analysis.

Source	df	SS	MS	Pseudo-F	P(permutation)
Latitude	1	4.4619	4.4619	1.1357	0.342
Longitude	1	4.4628	4.4628	1.1359	0.356
Years	3	9.7421	3.2474	0.83177	0.583
Protection Levels	3	18.068	6.0228	1.5427	0.1
Years x Protection Levels	9	41.41	4.6011	1.1785	0.212

Analysis

Both Years and Protection Levels (PL) showed significant differences ($P < 0.05$) in community structure for both the SF% and RA datasets in the main PERMANOVA test (Table 3.4). No interaction was detected between the two factors, indicating that their effects on community structure are independent. This suggests that while both

factors significantly influence community variance, the effects of protection levels remain consistent over time, even as communities may change through the years. Spatial covariates (Longitude and Latitude) also significantly influenced community structure variance and accounted for the largest proportion of variation. However, even after accounting for spatial effects, protection levels still explained a substantial portion of the variance, underscoring their importance in shaping community structure. In contrast, Years explained only 7.6% and 5.4% of the variance for SF% and RA datasets, respectively (Table 3.4), highlighting its relatively minor role compared to spatial and protection factors while also indicating that any changes through time in the community structure would be slight.

All pairwise tests between protection levels were also significant ($P < 0.05$) (Table 3.5). For both datasets the Pairwise tests for the factor Years resulted significantly different in only in some groups. Specifically, 2002 was significantly different ($P < 0.05$) than 2005 in the sighting frequency dataset, while 2004 and 2005 were significantly different from each other in the both datasets (Table 3.5).

Table 3.4. PERMANOVA Main Test results using SF% and RA data set with longitude and latitude included as covariates. DF is degrees of freedom, SS is sum of squares, Y x PL is the interaction between the factors Year and Protection Level, and %ECEV is the percent estimated component of explained variation. * Indicate significant differences ($P < 0.05$).

	Sighting Frequency					Relative Abundance				
Main Test	DF	SS	Pseudo – F	P-value	% ECEV	DF	SS	Pseudo-F	P-value	% ECEV
Latitude	1	4570.2	13.834	0.0001*	28.33	1	7206.9	17.836	0.0001*	27
Longitude	1	3345.5	10.127	0.0001*	37.7	1	5435.5	13.45	0.0001*	37.4
Year	3	1845.2	1.862	0.0022*	7.6	3	2237.4	1.8457	0.0027*	5.4
Protection Level	3	3239.4	3.269	0.0001*	25.7	3	5277.2	4.3534	0.0001*	27.7
Y x PL	9	2906.8	0.978	0.5538	0	9	3989.9	1.0972	0.2384	2.5

Table 3.5. PERMANOVA Pairwise Test results of both the SF% and RA data sets with covariates included.

*Indicates significant differences ($P < 0.05$)

Pairwise Tests Protection Levels				
	Sighting Frequency		Relative Abundance	
Groups	T	P	T	P
Low, Medium	1.9662	0.0002*	2.5241	0.0001*
Low, High	1.6523	0.005*	1.6852	0.0026*
Low, Very High	2.2042	0.0001*	2.4645	0.0001*
Medium, High	2.2523	0.0001*	2.3633	0.0001*
Medium, Very High	1.4872	0.0165*	1.4035	0.0386*
High, Very High	1.2308	0.1398	1.4315	0.0513
Pairwise Tests Years				
	T	P	T	P
2002, 2003	1.006	0.4172	0.91258	0.6151
2002, 2004	1.4485	0.0194*	1.4456	0.0221*
2002, 2005	1.4789	0.0141*	1.5093	0.0091*
2003, 2004	1.2704	0.0735	1.2695	0.09
2003, 2005	1.2817	0.0797	1.2245	0.1243
2004, 005	1.7071	0.0016*	1.7394	0.0033*

The CAP analysis (Anderson and Wills 2003) highlighted the different groupings of protection level (four levels: very high protection, high protection, medium protection, and low protection). Eight taxa were found to be the most responsible for variation among protection levels of RA data: Precious Red Coral (*Corallium rubrum*), Salema (*Sarpa salpa*), Mediterranean Fan Worms (*Sabella spallanzanii*), Other Fish, Other Echinoids, Stony Sponge (*Petrosia ficiformis*), Neptune Grass (*Posidonia oceanica*) and Anglerfish (*Lophius piscatorius*) (BVSTEP “BEST” test; correlation index $\rho > 0.85$) (Clarke and Ainsworth 1993) (Fig. 3.2). The vectors on the graph indicated that precious Red Corals were exceptionally abundant in sites with “very high” protection while they drastically decreased at lower protection levels, where the Stony Sponges and Other Echinoids were more abundant. Other Fish were more abundant within “medium” protection sites, while Anglerfish and Neptune Grass were more abundant in sites with “low” protection (Fig. 3.2; Table 3.6).

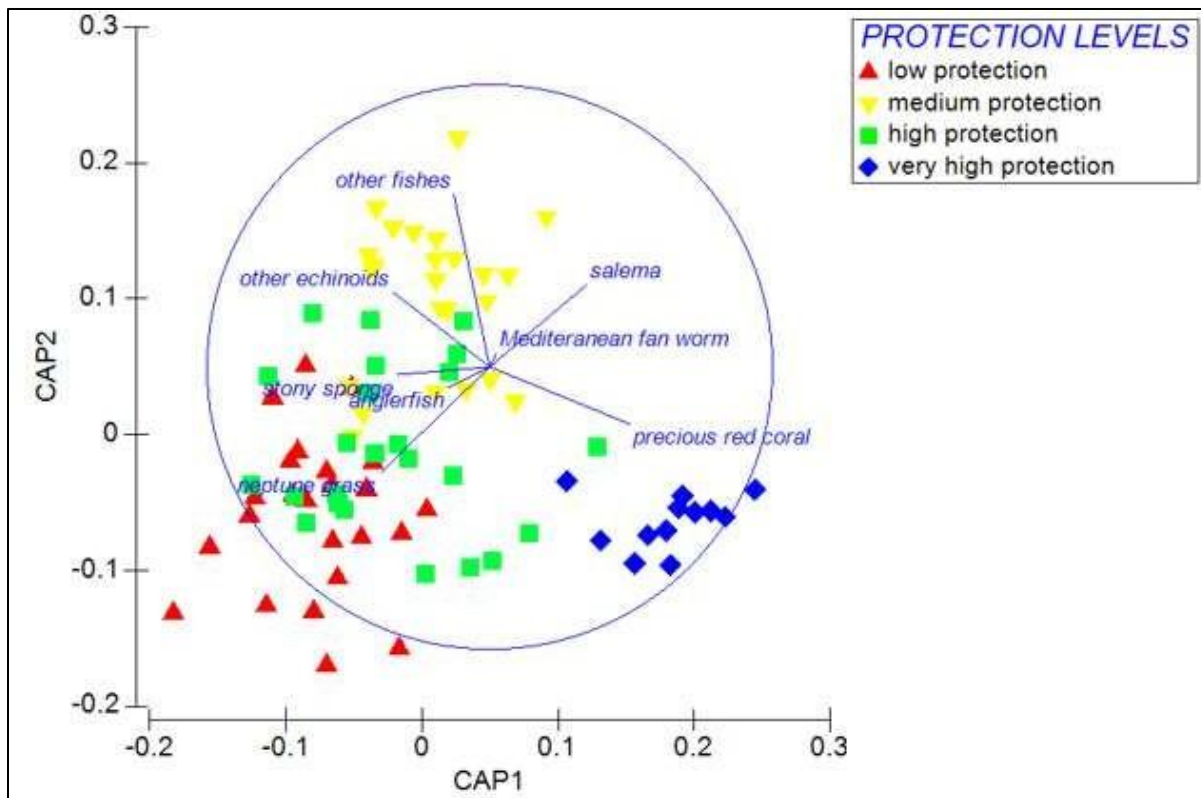


Figure 3.2. CAP plot of taxa community structure variance of the four different protection levels considering RA. Each point represents yearly values of each dive site. Different shape/colors indicate different protection levels: very high protection (blue, diamond), high protection (green, square), medium protection (yellow, inverse triangle) and low protection (red, triangle). Blue vectors indicate the trajectory of taxa from BVSTEP “BEST” test.

Table 3.6. Average RA of best taxa by protection level.

Taxon	Low	Medium	High	Very High
Neptune Grass	152 ± 12	70 ± 10	96 ± 15	70 ± 7
Precious Red Coral	20 ± 9	22 ± 8	27 ± 10	91 ± 11
Other Echinoids	74 ± 11	79 ± 9	61 ± 9	29 ± 5
Salema	106 ± 7	154 ± 10	136 ± 12	155 ± 10
Other Fishes	131 ± 10	176 ± 7	137 ± 10	112 ± 10
Mediterranean Fanworm	157 ± 10	167 ± 11	146 ± 12	162 ± 13
Anglerfish	12 ± 4	6 ± 1	5 ± 1	5 ± 3
Stony Sponge	92 ± 7	83 ± 11	128 ± 10	57 ± 6

The same test performed using SF% data obtained nine different taxa: Dotted Dea Slug (*Peltodoris atromaculata*), Yellow Cluster Anemone (*Parazoanthus axinellae*), Red Lance Urchin (*Stylocidaris affinis*), Dusky Grouper (*Epinephelus marginatus*), Mermaid’s Wine Glass (*Acetabularia acetabulum*), Other Sea Stars, Other Vegetals, Rainbow Wrasse (*Coris julis*), Feather Star (*Antedon mediterranea*) (Fig. 3.3). In this case the

vectors on the graph indicated that Yellow Cluster Anemone and Dusky Grouper were more observed in sites with “very high” protection while their SF% decreased at lower protection levels with an opposite trend of the Mermaid’s Wine Glass. In dive sites with “medium” protection, Other Vegetals, Feather Stars, Other Sea Stars, and Rainbow Wrasses were more sighted. Sites with “low” protection had the highest SF% of Mermaid’s Wine Glass and the lowest of Dotted Sea Slug (Fig. 3.3). Real SF% values can be observed in Table 3.7.

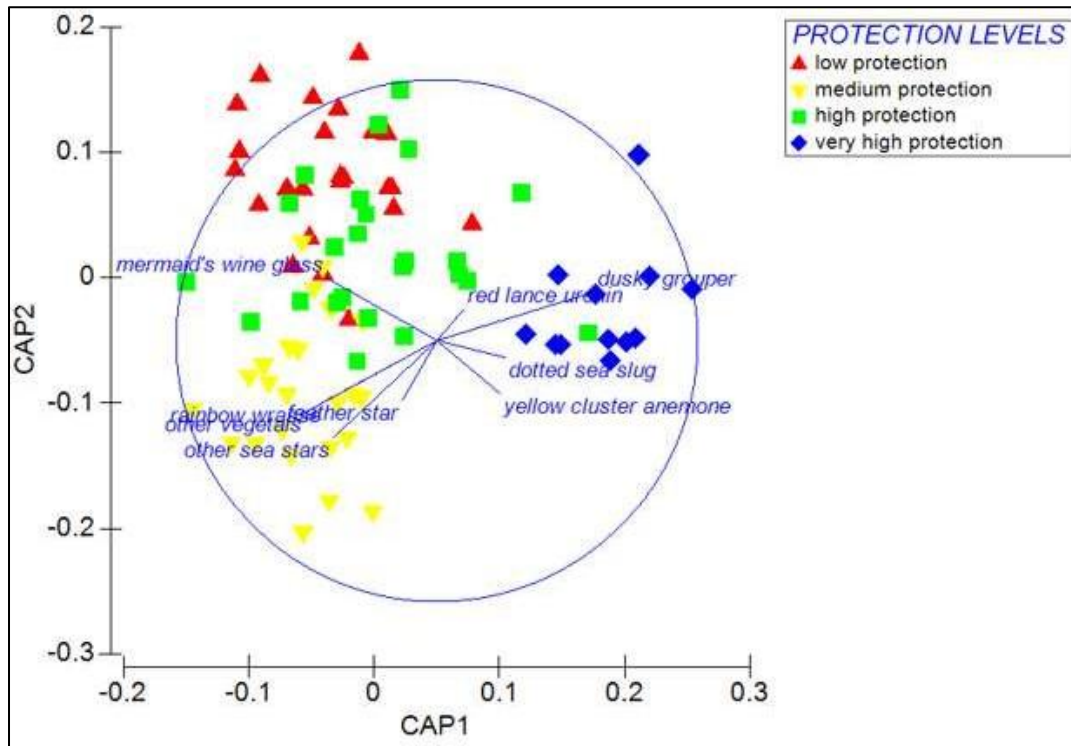


Figure 3.3. CAP plot of taxa community structure variance of the four different protection levels considering SF% data set. Each point represents yearly values of each dive site. Different shape/colors indicate different protection levels: very high protection (blue, diamond), high protection (green, square), medium protection (yellow, inverse triangle) and low protection (red, triangle). Blue vectors indicate the trajectory of taxa from BVSTEP “BEST” test.

Table 3.7. Average SF% of best taxa by protection level.

Taxon	Low	Medium	High	Very High
Mermaid’s Wine Glass	63 ± 3	52 ± 4	51 ± 3	33 ± 7
Other Vegetals	37 ± 4	49 ± 5	28 ± 3	15 ± 3
Yellow Cluster Anemone	44 ± 4	52 ± 5	46 ± 4	64 ± 4
Dotted Sea Slug	23 ± 4	28 ± 5	40 ± 6	40 ± 4
Feather Star	7 ± 3	16 ± 4	7 ± 1	5 ± 1
Other Sea Stars	34 ± 4	49 ± 4	33 ± 3	28 ± 3
Red Lance Urchin	5 ± 2	4 ± 1	5 ± 2	3 ± 1
Dusky Grouper	34 ± 4	23 ± 4	48 ± 5	69 ± 4
Rainbow Wrasse	72 ± 2	81 ± 2	75 ± 3	64 ± 6

The supplementary PERMANOVA with Management rank, Biodiversity index rank, and CHIS rank as fixed factors showed that all three contributed significantly to differences in community structure. Pairwise comparisons revealed that CHIS levels consistently separated communities, while several intermediate Management levels were not significantly different from each other. Estimates of components of explained variation indicated that Biodiversity rank (ECEV $\approx$ 42) and CHIS (ECEV $\approx$ 32) accounted for the largest portions of explained variation, whereas Management rank contributed the least (ECEV $\approx$ 12) (Table 3.8). Interactions involving Biodiversity could not be tested due to the unbalanced distribution of biodiversity scores, with nearly all sites ranked as 3 and only a single site ranked as 2. This uneven distribution likely amplifies the apparent effect of biodiversity rank and limits interpretation of its significance. The PERMANOVA test excluding CHIS from the protection-level PERMANOVA was also significant overall ($P < 0.05$), but pairwise comparisons revealed fewer distinctions among protection levels, underscoring the importance of including CHIS when evaluating community differences.

Table 3.8. PERMANOVA results of partitioned ranks as individual factors. MS is management strategy, BD is biodiversity, CHIS is cumulative human impact score, and ECEV is estimated component of explained variation. * Indicates significant differences among levels of factor detected.

Main Test	DF	SS	Pseudo-F	P-value	ECEV
MS Rank	3	3468.2	2.4087	0.0001*	12
BD Rank	1	1724.8	3.5935	0.0015*	42
CHIS Rank	2	4857.4	5.0601	0.0001*	32
MSxBD	0	0	No test	NA	NA
MSxCHIS	1	850.04	1.771	0.065	14
BDxCHIS	0	0	No test	NA	NA
MAxBDxCHIS	0	0	No test	NA	NA

Discussion

This study investigated how protection levels, estimated through management strategy, biodiversity index, and cumulative human impact (CHIS), affected Mediterranean benthic community structure from 2002 to 2005. The stable number of questionnaires over the years (Table 3.2) reflects the high levels of diving tourism in the study areas (Francour et al. 2001; Vacchi et al. 2013). By leveraging data collected through a large-scale citizen science

initiative, we demonstrate how recreational divers can provide valuable information for marine biodiversity surveys while significantly reducing financial and time costs (Goffredo et al. 2010). Importantly, this citizen science approach also enabled the detection of community structure differences across varying protection levels along the Italian coast, highlighting its scalability and cost-effectiveness for long-term monitoring and conservation planning.

Diving parameters showed no significant variation across years or protection levels, confirming they did not influence community structure differences. Both Relative Abundance (RA) and Sightings Frequency (SF%) data were significantly different among all protection levels. CAP plots (Figs. 3.2 and 3.3) showed clear groupings by protection level, with the greatest differences observed between “very high” protection sites (e.g., Portofino AMP) and lower protection levels (Table 3.5). These results suggest that even minimal protection leads to measurable shifts in community structure, while the highest protection levels support distinct ecological compositions, including greater abundances of habitat-forming species like Precious Red Coral.

The findings from the supplementary PERMANOVA emphasize that cumulative human Impacts (CHIS) and biodiversity index captured most of the detectable variation in benthic community structure, while management strategy alone explained comparatively less variation. The strong effect of CHIS is robust and ecologically interpretable, reflecting the role of nutrient inputs, shipping, coastal development, etc. in shaping communities beyond formal MPA designation. The contribution of biodiversity rank, however, is more problematic: because almost all sites were classified as rank 3, with only one site at rank 2, the analysis may overstate its importance. In other words, the “significance” of biodiversity rank here reflects a very limited contrast, rather than a gradient across multiple biodiversity states. Thus, while CHIS provides consistent and meaningful separation, the effect of biodiversity rank should be viewed cautiously, and future analyses would benefit from broader representation of sites across biodiversity levels. Importantly, the separate-factor analyses confirmed that each component of the protection level rank was significant on its own, supporting the validity of merging them into a composite ranking. Thus, the integrated protection level ranking can be considered both conceptually appropriate and statistically sound, while the partitioned analyses clarify the relative contributions of each component.

Precious Red Coral and Yellow Cluster Anemone were key taxa characterizing community structure in “very high” protection sites, showing higher abundance and sighting frequency, respectively (Figs. 3.2 and 3.3). These species contribute to vertical habitat structure, enhancing habitat heterogeneity and biodiversity (Sala and Knowlton 2006; Cerrano and Bavestrello 2008). Precious Red Coral has long been an important fishery in the Mediterranean, valued for its use in jewelry and ornaments. However, this economic importance has historically made it challenging to protect, leading to overharvesting and population declines (Bavestrello et al. 2015). From the 1950s to the late 1970s, Ligurian red coral banks were heavily exploited, further exacerbating habitat

degradation (Bavestrello et al. 2015). Compounding these pressures, Mediterranean benthic communities have faced mass mortalities of corals, sponges, and other taxa due to climatic anomalies, diseases, and environmental stressors (Cerrano et al. 2000; Coma et al. 2006). These events, particularly severe in the late 1990s and early 2000s, further reduced habitat complexity and biodiversity (Cerrano and Bavestrello 2008). The establishment of the Portofino Marine Protected Area (MPA) in 1999 marked a turning point. Protection measures have enabled the recovery of Precious Red Coral populations to levels comparable to those 50 years ago (Bavestrello et al. 2015). Our findings support this, showing higher abundances of Precious Red Coral and Yellow Cluster Anemone in “very high” protection areas. Despite historical mass mortalities (Linares et al. 2005), these taxa remained stable throughout the study period, underscoring the effectiveness of MPA management in safeguarding marine biodiversity. These results align with observations of undisturbed Red Coral populations in other NW Mediterranean MPAs (Linares et al. 2010, 2012).

The Dusky Grouper was also more frequently sighted in “very high” protection sites (Fig. 3.3; Table 6). As a key species in Mediterranean rocky coastal habitats, its recovery highlights the success of conservation efforts (Tunesi and Molinari 2007). Before the establishment of the Portofino MPA, fishing pressure had drastically reduced its populations, with no specimens recorded in visual census surveys during the 1990s (Tunesi and Molinari 2007). Since the MPA’s creation, fishing has been prohibited in Zone A, while Zones B and C allow only local professional fishermen using traditional methods. By 2002, just three years after the MPA’s establishment, Dusky Grouper populations showed positive recovery, with no significant differences in abundance across the MPA’s management zones (Tunesi and Molinari 2007).

Neptune Grass and Mermaid’s Wine Glass were the key species characterizing “low” protection dive sites (e.g., Calafuria and islands of the Tuscan Archipelago, such as Giannutri, Elba, Giglio, and Capraia). Neptune Grass, an endemic and keystone species, is a vital ecosystem engineer in the Mediterranean, providing critical habitat and ecosystem services (De Battisti et al. 2021; Tursi et al. 2022). However, Mediterranean seagrass beds have declined due to coastal development, pollution, anchoring, invasive species, and climate change (Pazzaglia et al. 2020; Tursi et al. 2022). Recognized as a priority for conservation under the EU Habitat Directive (92/43/EEC) and a bioindicator under the EU Marine Strategy Framework Directive (2008/56/EC), Neptune Grass is highly sensitive to environmental changes (Boudouresque et al. 2004; Montefalcone et al. 2010; Tursi et al. 2022). Despite these pressures, some areas, such as Elba Island, maintain healthy seagrass beds even under “low” and “medium” protection levels, highlighting the importance of local management (Piazzi et al. 2000). Since 2007, the Tuscany region has expanded marine protected areas (SPAs) around the Tuscan Archipelago, including sites of community importance (SICs) to protect Neptune Grass habitats (Mare et al. 2009). These efforts align with our findings of high Neptune Grass abundance in these areas from 2002 to 2004 (Fig. 3.2). Although meadow extent is lower on rocky substrates like Giglio Island (Gobert et al. 2009), recent conservation

measures, such as the designation of Calafuria as a site of community importance in 2020, aim to preserve these critical habitats (Regione Toscana, 2020).

The lower extension of *P. oceanica* in Ligurian dive sites (Table 5) could be explained through intense urban and industrial coastal development and through an increase of tourism since the beginning of the 1960s (Vacchi et al. 2013). The establishment of MPAs (such as Portofino MPA) has allowed the Ligurian region to adopt measures to preserve the coast with a particular focus on the protection and valorization of its underwater geological heritage (Vacchi et al. 2013). The substrate type could also have an influence on Neptune Grass development (De Battisti et al. 2021). The questionnaires analyzed in our research involved only rocky habitats but Neptune Grass forms extensive meadows on a variety of substrates (e.g., sand and rock) up to 45 meters in depth (De Battisti et al. 2021). In the Ligurian Sea (specifically Livorno, Italy) no difference of Neptune grass number was shown between substrates, however the number of standing leaves were higher on rock than on sand (De Battisti et al. 2021). Several works conducted mostly in the NW Mediterranean Sea highlighted the importance of preserving Neptune Grass meadows, promoting the creation of better management tools (De Battisti et al. 2021).

The high presence of Mermaid's Wine Glass (*Acetabularia acetabulum*) within the “low” protection areas may be related to a lack of fishes and sea urchins. Indeed, sea urchins are the most important herbivores on shallow subtidal rocky reefs in the Mediterranean Sea (Bulleri et al. 2002). On the other hand, the abundance of this taxon is associated with many factors such as substrate, depth, and the individual features of each island (Roveta et al. 2022).

Fluctuations in the abundances of taxa such as Dotted Sea Slug, Other Fish, Other Vegetals, Other Sea Stars, and Rainbow Wrasse (Figs. 3.2 and 3.3; Tables 3.5 and 3.6) may be influenced by habitat characteristics and prey availability. For example, Nudibranch richness is often linked to sponge abundance since many species, including Dotted Sea Slug, are closely associated with sponge-encrusted substrates (Adiwijaya et al. 2021). Similarly, the composition and structure of fish assemblages are frequently related to habitat type: rocky algal reefs and *P. oceanica* meadows tend to support higher fish diversity and abundances than sandy bottoms, likely due to differences in food and shelter availability (La Mesa et al. 2010). Rainbow Wrasse, for instance, is a known generalist that occupies both rocky algal reefs and *P. oceanica* meadows (La Mesa et al. 2010), while Other Sea Stars are often associated with rocky substrates due to their feeding behavior on corals and mussels (Glynn and Krupp 1986; Gooding and Harley 2015). However, while these ecological relationships provide potential explanations, the observed fluctuations are likely driven by multiple interacting factors, many of which could not be identified in this study.

The analysis of fish community structure revealed significant variation driven by protective (Protection Levels), spatial (Longitude and Latitude), and temporal (Years) factors. While spatial gradients played a key role,

with Longitude and Latitude exhibiting the highest estimated components of variation the influence of Management Strategy remained prevalent and significant (Table 4). This underscores the effectiveness of protection levels in shaping community composition, particularly in “very high” protection sites like the Porto Fino AMP, where ecologically important taxa such as precious red coral were more abundant. Interestingly, the previous study which published results from Divers for the Environment found that the biodiversity index increased with decreasing latitude along the Italian coast (Goffredo et al. 2010), likely due to enhanced environmental conditions in southern coastal regions (Goffredo et al. 2010). However, our findings reveal a more complex pattern, with habitat-building and biodiversity-enhancing species like precious red coral and yellow cluster anemone being more abundant at higher latitudes, potentially reflecting the success of management efforts in these regions. These results highlight the value of protection strategies in fostering ecologically significant species, even within the spatial limitations inherent to citizen science data collection. Temporal changes (Years) showed limited influence, indicating that protection effects remained consistent over time. Together, these results demonstrate that while spatial variation is an important consideration, management strategy and coastal human impact exert a clear and significant influence on community structure, providing critical insights for conservation planning and the interpretation of citizen science datasets.

In conclusion, this study revealed clear differences in benthic community structure across four protection levels, as monitored by recreational citizen scientists. The Protection Level factor, integrating management strategy, biodiversity indices, and cumulative human impacts, was a significant driver of these differences, underscoring both the effectiveness of well-implemented MPAs and the necessity of accounting for anthropogenic pressures. While MPAs remain limited in extent and enforcement (Vacchi et al. 2013), our findings highlight that conservation outcomes depend not only on protection status but also on the broader coastal pressures that strongly shape community structure.

Chapter 4 – Long-term trends in coral reef fish community structure at Little Cayman Island

Abstract

Coral reef fish communities play essential roles in maintaining ecosystem function, yet their responses to long-term environmental change remain poorly resolved, particularly in the Caribbean. This study examines 18 years (2007–2024) of fish community composition around Little Cayman Island, using a multi-metric approach that integrates biomass, relative abundance (RA), and sighting frequency (SF) data across sites within and outside marine protected areas (MPAs). Temporal change emerged as the dominant driver of community variation, while management strategy exerted an additional, independent influence. Despite the occurrence of a severe bleaching event in 2023 that caused widespread coral mortality, fish community composition in 2024 showed no abrupt reorganization and instead clustered with assemblages from recent years. This suggests a delayed response to benthic disturbance, likely mediated by the persistence of structural habitat following coral death. Across datasets, key taxa exhibited distinct trajectories, including a long-term decline of the Yellowtail Snapper (*Ocyurus chrysurus*), and an increase in Bar Jack (*Caranx ruber*). Shifts in parrotfish assemblages and changes in trophic structure further reflect broader ecological reorganization. MPAs supported higher biomass and abundance of several target species, though outcomes varied most likely due to spillover, competition, and predation dynamics. Together, these findings highlight that reef fish communities in the Caribbean are undergoing continuous, long-term change shaped by both climate-driven disturbances and local management, emphasizing the importance of extended monitoring for detecting and interpreting ecological responses in a rapidly changing ocean.

Introduction

Coral reefs are among the most valuable and productive ecosystems on Earth (Bellwood and Hughes, 2001; McAllister et al. 1994) and providing essential ecosystem services, including coastal protection, nutrient cycling, fisheries, and tourism, with an estimated global economic value in the trillions of dollars annually (Costanza et al. 1997; Woodhead et al. 2019). Yet these ecosystems are undergoing rapid and unprecedented change due to the combined impacts of climate change, overfishing, and coastal development (Hughes et al. 2003; Fine et al. 2019), driving widespread coral loss and altering the structure and functioning of reef ecosystems worldwide.

Among the most critical components of these systems are their fish communities. Reef-associated fishes underpin global and local fisheries, provide food security, and sustain coastal livelihoods (Barnes-Mauthe et al. 2013). Ecologically, they perform vital functions that maintain reef health and resilience. Herbivores regulate algal growth and prevent competitive overgrowth on corals (Robinson et al. 2020; Adam et al. 2015), planktivores transfer energy across trophic levels, and predators structure communities through top-down control (Mumby et al. 2006; Brandl et al. 2019). Many species also depend on coral reefs for nursery habitat, feeding grounds, and shelter, linking benthic condition and fish community structure (Graham and Nash 2013). Disruptions to these assemblages can destabilize trophic interactions, compromise resilience, and reduce the socio-economic services reefs provide, making it critical to understand how fish communities respond to accelerating environmental and anthropogenic change.

The Caribbean offers an especially important setting for understanding how reef fish communities change over time. The region has seen some of the steepest losses of live coral cover in the world, which has in turn reduced structural complexity and reshaped the habitats fish depend on (Alvarez-Filip et al. 2009; Jackson et al. 2014). Because so many reef fish are closely tied to this structure for food, shelter, and breeding, changes in the reef's physical landscape often lead to shifts in which species are present, how they interact, and the roles they play in the ecosystem (Graham and Nash 2013; Cramer et al. 2021). Yet despite how important these processes are, most research has focused either on individual species or short-term changes. Much less attention has been given to how whole fish communities reorganize over decades. Long-term studies like this one are key to filling that gap and helping us untangle how large-scale pressures and local management decisions shape communities over time.

Little Cayman Island offers a particularly valuable case study for looking at these long-term changes. It is one of the least developed islands in the Caribbean, with minimal coastal infrastructure and a small permanent population, and much of its terrestrial and marine environment is formally protected (McCoy and Shaw 2010). Because of this low level of local disturbance, changes in fish community composition here are more likely to reflect broader ecological processes, like habitat shifts, biological interactions, and long-term

environmental change, rather than direct human impacts. This makes it an ideal place to study how reef communities evolve over time and how management strategies such as Marine Protected Areas (MPAs) influence that trajectory. The Central Caribbean Marine Institute (CCMI), based on Little Cayman, has coordinated ecological surveys around the island for over two decades, producing one of the longest continuous datasets on Caribbean reef fish communities. These data provide a rare opportunity to look beyond short-term fluctuations and investigate how fish assemblages are shaped by both large-scale pressures and local protection efforts over 18 years.

Building on this unique setting, the present study uses more than two decades of ecological monitoring to investigate how coral reef fish communities around Little Cayman have changed through time and how local management has influenced those patterns. By integrating multiple measures of community structure, including biomass, relative abundance, and sighting frequency, we aim to capture a comprehensive picture of how reef assemblages shift across spatial and temporal scales and which species or groups are most indicative of those changes. This long-term perspective is particularly valuable considering recent events, such as the unprecedented 2023 marine heatwave, which caused widespread coral bleaching and mortality across the Caribbean (Doherty et al. 2025). Understanding how fish communities respond to such large-scale disturbances, and how those responses differ inside and outside Marine Protected Areas, is crucial for assessing the resilience of these ecosystems and informing future conservation strategies.

Materials and Methods

Data collection and isolation

Fish community data were collected following the Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocol (Lang et al., 2015). This protocol involves visual fish censuses conducted by trained operators to ensure consistency and reliability. At each reef site, 30 m × 2 m transects were laid, and all selected fish species observed within the 2 m segment directly ahead of the diver and within the water column above, were identified, counted, and assigned to 10 cm size classes. A minimum of six transects were recorded per site during each sampling year. For full methodological details, see (Lang et al., 2015).

The data analyzed in this study originate from standardized ecological monitoring surveys conducted between 1999 and 2024 (years: 1999, 2006–2007, 2009, 2012–2015, and 2017–2024) across 15 coral reef sites surrounding Little Cayman Island. These surveys were designed and coordinated by the Central Caribbean Marine Institute (CCMI) as part of its long-term reef monitoring program, which provides one of the most continuous datasets on Caribbean coral reef communities. Of the monitored sites, six were located within designated Marine Protected Areas (MPAs) and nine were situated outside protected boundaries. For each

transect, metadata including operator, date, site, transect ID, and species composition were recorded, with fish identified to one of 72 reference taxa.

Data were screened for outliers and potential entry errors, which were corrected where appropriate. Of the 15 sites monitored, seven were retained for analysis (three within MPAs and four outside) (Fig. 4.1; Table 4.1). These sites provided the most continuous and balanced time series, with at least six transects per year.

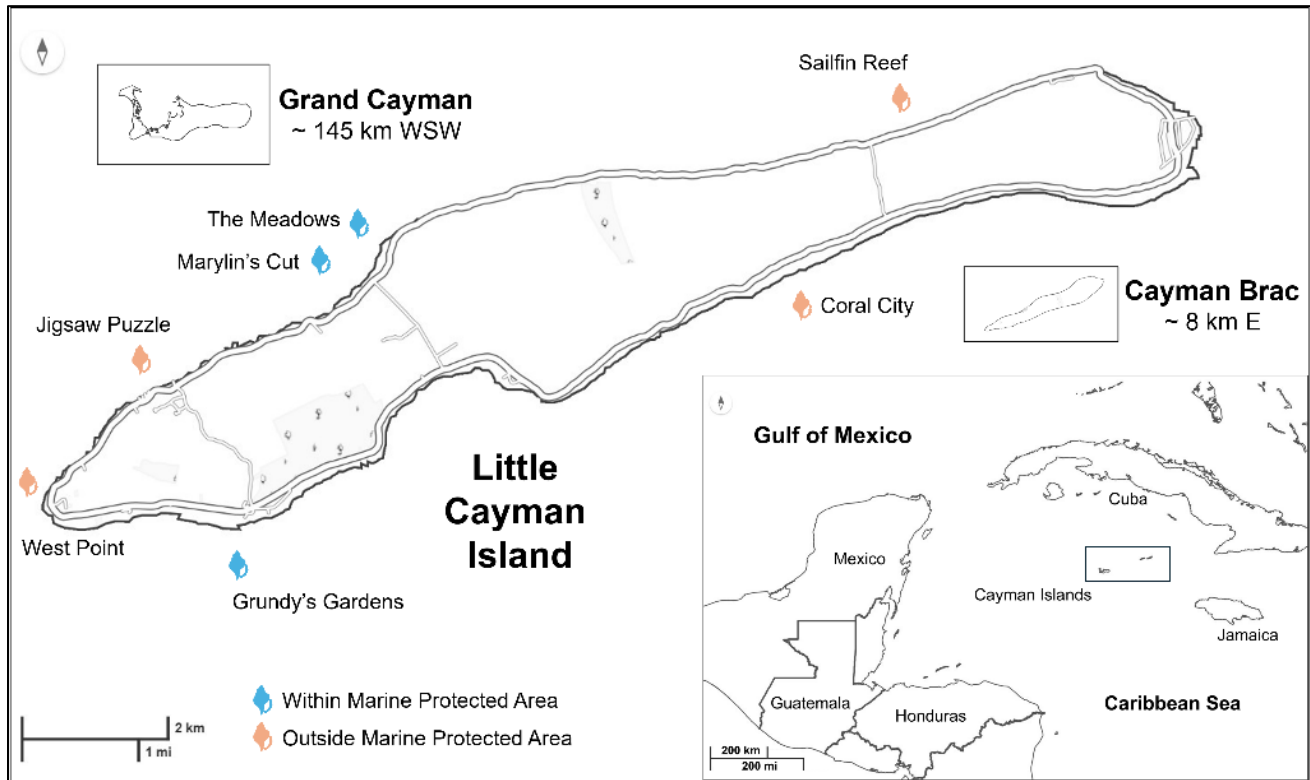


Figure 4.1. The figure shows the geography of Little Cayman Island, indicating the analyzed sites and distinguishing their location as inside a Marine Protected Area (MPA) or outside a MPA (orange).

Table 4.1. The site selected for the analysis and the number of transects for each year.

	Inside MPA			Outside MPA			
	Grundy's Garden	Marilyn's Cut and Mixing Bowl	Meadows	Coral City	Jigsaw	Sailfin	West Point
1999	10	12	14	11	11	12	10
2006	10	10	10	0	10	10	10
2007	0	0	8	10	10	10	10
2009	10	10	10	10	10	10	10
2012	10	10	10	10	10	10	10
2013	10	10	10	10	10	10	10
2014	10	10	11	10	10	10	10
2015	10	10	10	10	10	10	10
2017	10	10	10	10	10	10	10
2018	12	0	10	9	10	0	12
2019	10	10	10	10	10	10	10
2020	13	10	12	11	9	6	8
2021	11	11	12	8	12	12	8
2022	9	9	6	9	6	9	9
2023	11	11	11	10	11	11	8
2024	9	9	9	9	8	9	9

Fish biomass was estimated from visual length data by converting the midpoint of each 10 cm size class to weight using species-specific length–weight relationships ($W = a \times L^b$), compiled for Western Atlantic reef fishes primarily from FishBase and AGRRA (Froese et al., 2025; Lang et al., 2015).

To evaluate potential environmental influences on fish community structure, corresponding sea surface temperature (SST) and chlorophyll-a data were extracted for the Little Cayman region (-80.1407° N, 19.6234° W to -79.9361° N, 19.7566° W) using NASA's Giovanni platform (JPL OurOcean Project, 2010). Because no single dataset covered the entire 1999–2024 period, SST data were combined from two sources: 1999–2002 (Global Modeling and Assimilation Office, 2015) and 2003–2024 (JPL OurOcean Project, 2010). Similarly, chlorophyll-a data were obtained from two datasets: 1999–2001 (Watson Gregg and Rousseaux, 2017) and 2002–2024 (Aqua MODIS Level-3 Global Mapped CHL, 2022). Datasets were selected for their comparable spatial resolution and methodological consistency to minimize potential bias in temporal comparisons.

Analysis

Three datasets were elaborated using average Biomass, Relative Abundance (RA) and Sighting Frequency (SF), for each taxon per site-year combination. SF and RA values were calculated as:

$$SF = \frac{\text{Number of sightings per taxon}}{\text{Number of transects}}$$
$$RA = (SF)(\text{Average Sighting Abundance})$$

All analyses were conducted using PRIMER-E v7 with PERMANOVA+ version 1 software (PRIMER-E, Ltd., Plymouth, UK).

For each dataset, three factors were considered: Years (with 15 levels: 1999, 2006-2007, 2009, 2012-2015, 2017-2024), MPA (Inside; Outside) and Bleaching (Before; After). PERMANOVA (Clarke and Gorley 2015) models were initially designed to test the factors Year, Management Strategy (MPA vs. non-MPA), and Before/After bleaching (1999-2023 vs. 2024). Although the Before/After factor included replication across sites in 2024, it lacked temporal replication, making the design problematic. In practice, the test effectively contrasted a single post-bleaching year against the pooled set of pre-bleaching years, meaning that any observed differences could reflect natural interannual variability rather than bleaching per se. Even when considering individual transects as replicates, this limitation remained and risked inflating Type I error. For this reason, the Before/After factor was not included in the main analyses. Instead, models focused on Year and management status, with longitude and latitude incorporated as covariates to reduce spatial confounding and ensure that the significant variation detected was driven by the design factors rather than underlying geography. These two-factor designs were applied to each dataset (average biomass, relative abundance, and sighting frequency), while the position of 2024 communities relative to previous years was assessed descriptively in ordinations.

RELATE tests were performed between the community resemblance matrices (average biomass, relative abundance, and sighting frequency) and normalized environmental parameter matrices to evaluate whether temporal changes in community structure were significantly related to variation in sea surface temperature and chlorophyll. These tests provided a non-parametric measure of correlation between environmental drivers and community patterns across years.

BVSTEP "BEST" tests (Clarke & Ainsworth, 1993) were performed on the resemblance matrices of Average for the factor "Year." For a visual representation of community variance across the years considered, a canonical analysis of principal coordinates (CAP) (Clarke and Gorley, 2015) was conducted on each of the three datasets (RA, SF, Biomass). Non-metric multidimensional scaling plots (MDS) (Clarke and Gorley, 2015), using Bray-Curtis resemblance matrices of the mean values, were also created to visualize the variance of the fish co structure over time.

BEST (BIO-ENV) procedures were applied to identify the taxa that best explained temporal patterns in community structure, with the resulting variables projected onto the MDS and CAP ordinations. Temporal trends in species identified as key drivers by the BEST test were assessed for relative abundance, biomass, and sighting frequency using linear regression across survey years. For each metric, yearly mean values were plotted and fitted with linear trendlines to evaluate directional change over time.

To investigate management effects, SIMPER analyses were conducted on each dataset (average biomass, relative abundance, and sighting frequency) to identify the taxa contributing most to dissimilarities between MPA and non-MPA sites.

Results

PERMANOVA tests showed significant differences in community structure among years (YE) and between management strategies (MS) across all three datasets: Biomass, Relative Abundance (RA), and Sighting Frequency (SF) ($P < 0.005$ for all; Table 4.2). No significant interaction between the two factors was detected ($P = 0.90, 0.78, \text{ and } 0.92$, respectively), indicating that temporal and management effects acted independently. In all datasets, temporal variation accounted for the largest proportion of explained community variation ($>47\%$), while management strategy explained most of the remaining variation (approximately 27–29%). Both factors remained significant after accounting for geographic covariates (latitude and longitude), confirming a robust link between community structure, time, and management regime.

Table 4.2. PERMANOVA table of results including all datasets. MS is Management strategy, df is degrees of freedom, SS is sum of squares and ECEV % is the percent estimated component of explained variation.

Biomass					
Main Test	df	SS	Pseudo-F	P-Value	ECEV %
Latitude	1	1839.2	2.5003	0.006*	3.365981
Longitude	1	3669.5	4.9886	0.0001*	20.97476
Year	13	24104	2.5206	0.0001*	47.85325
MS	1	4838.7	6.578	0.0001*	27.806
Year x MS	13	8050.7	0.8419	0.9001	0
Relative Abundance					
Main Test	df	SS	Pseudo-F	P-Value	ECEV %
Latitude	1	1949.7	4.5239	0.0003*	4.126543
Longitude	1	3122.3	7.2445	0.0001*	17.1404
Year	13	23276	4.1544	0.0001*	51.81236
MS	1	4890.2	11.346	0.0001*	26.9207
Year x MS	13	5043.1	0.90008	0.779	0
Sighting Frequency					
Main Test	df	SS	Pseudo-F	P-Value	ECEV %
Latitude	1	2232.1	5.1667	0.0001*	3.93887
Longitude	1	4275.6	9.897	0.0001*	19.71348
Year	13	25543	4.5482	0.0001*	47.04625
MS	1	6458.5	14.95	0.0001*	29.3014
Year x MS	13	4727.1	0.84171	0.9155	0

The taxa most representative of the variation in Biomass among the years were Permit (*Trachinotus falcatus*), Puddingwife Wrasse (*Halichoeres radiatus*), Stoplight Parrotfish (*Sparisoma viride*), Threespot Damselfish (*Stegastes planifrons*) and Yellowtail Snapper (*Ocyurus chrysurus*), as identified by the BIO-ENV BEST test (Table 4.3).

Table 4.3. The average Biomass per cluster. The values are the result of the average of each species' contribution for each year.

BIOMASS	Cluster 1	Cluster 2	Cluster 3	2019	2020
Permit	574.115	0.000	0.404	411.305	0.000
Puddingwife Wrasse	0.000	0.000	3.313	148.232	68.791
Stoplight Parrotfish	1271.321	432.805	578.943	1086.177	453.903
Threespot Damselfish	0.000	5.208	27.514	33.404	28.769
Yellowtail Snapper	1125.877	567.623	659.188	2994.008	0.000

The CAP plot revealed three clearly distinct clusters (C1, C2, C3) (Fig 4.2). Cluster C1, characterized primarily by *S. viride*, corresponded to communities with relatively higher parrotfish biomass, while C2 was dominated by Permit and Puddingwife Wrasse, and C3 by Yellowtail Snapper and Threespot Damselfish. The clusters showed minimal overlap, indicating well-defined differences in community biomass structure among years. Species trajectories on the CAP plot suggest that the Stoplight Parrotfish and Yellowtail Snapper were the main taxa driving separation along the first canonical axis, while the Permit and Puddingwife Wrasse contributed most to differentiation along the second axis.

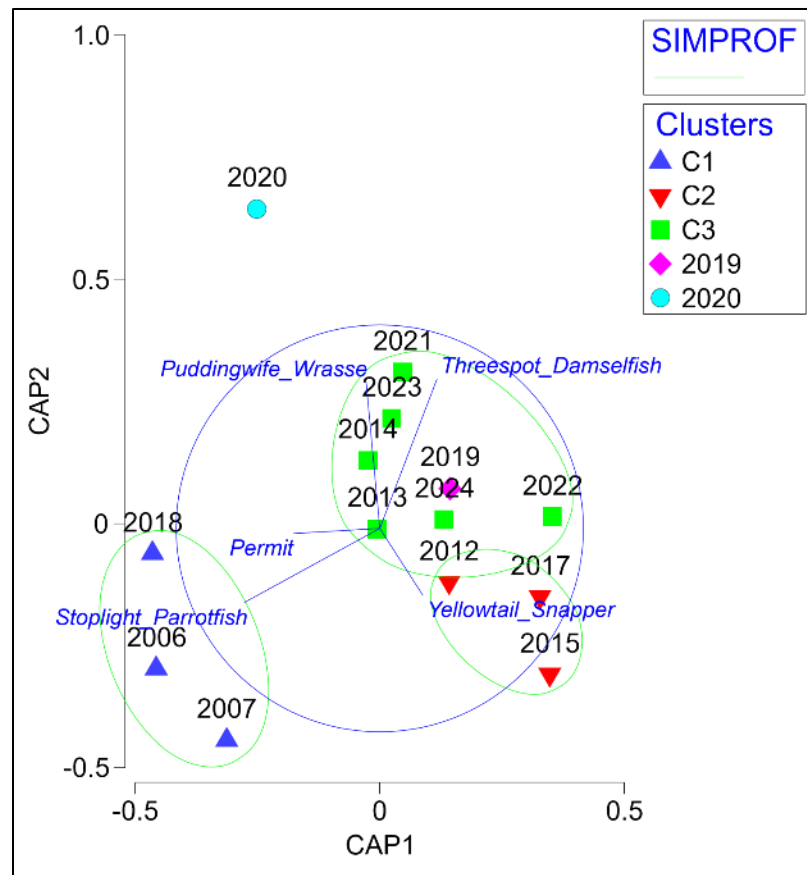


Figure 4.2. CAP plot showing variation of community structure among years based on the Biomass dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

The MDS plot based on yearly average biomass supported these patterns (Fig. 4.3). The three main clusters remained distinct, with limited overlap between C2 and C3. The relative positioning of taxa was consistent with the CAP results, confirming that years grouped within each SIMPROF-defined cluster shared similar biomass compositions. Overall, biomass-based community structure exhibited clear differentiation among clusters, reflecting consistent shifts in dominant taxa across years.

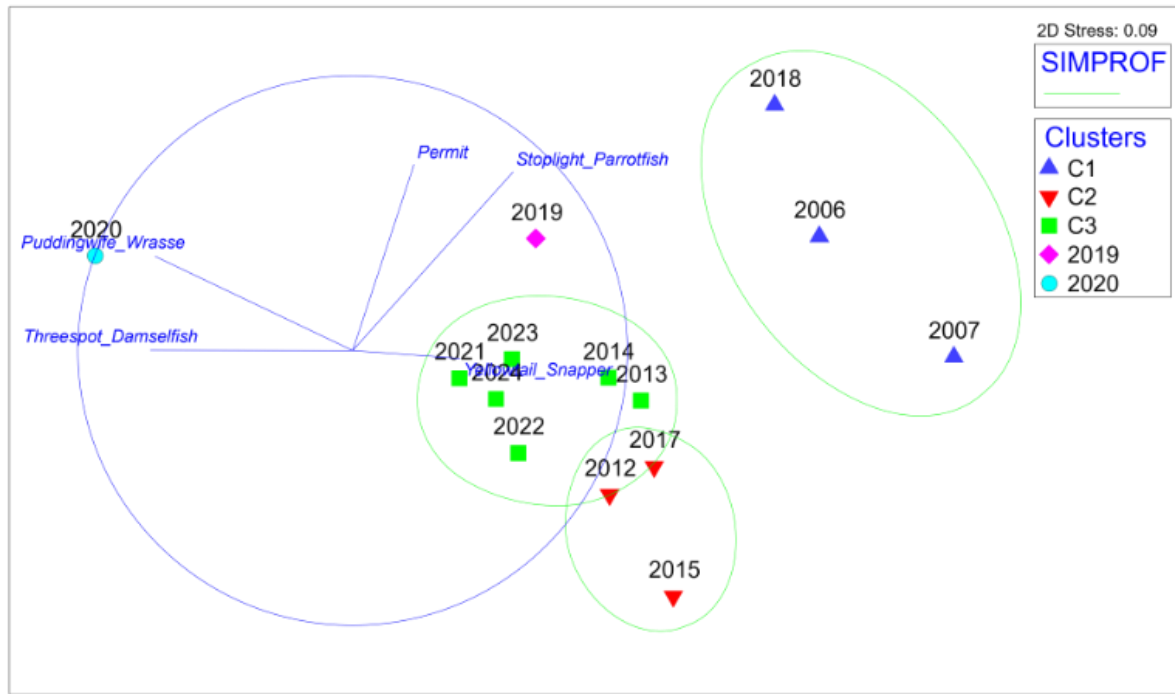


Figure 4.3. MDS plot showing variation in community structure among years based on the Biomass dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

Temporal trends in Biomass of the BIOENV Best taxa are shown in Figure 4.4. Stoplight Parrotfish displayed a significant decline over time ($R^2 = 0.31$, $P < 0.05$), while the Threespot Damselfish increased significantly over the years ($R^2 = 0.55$, $P < 0.05$). No significant directional change was detected for the remaining species.

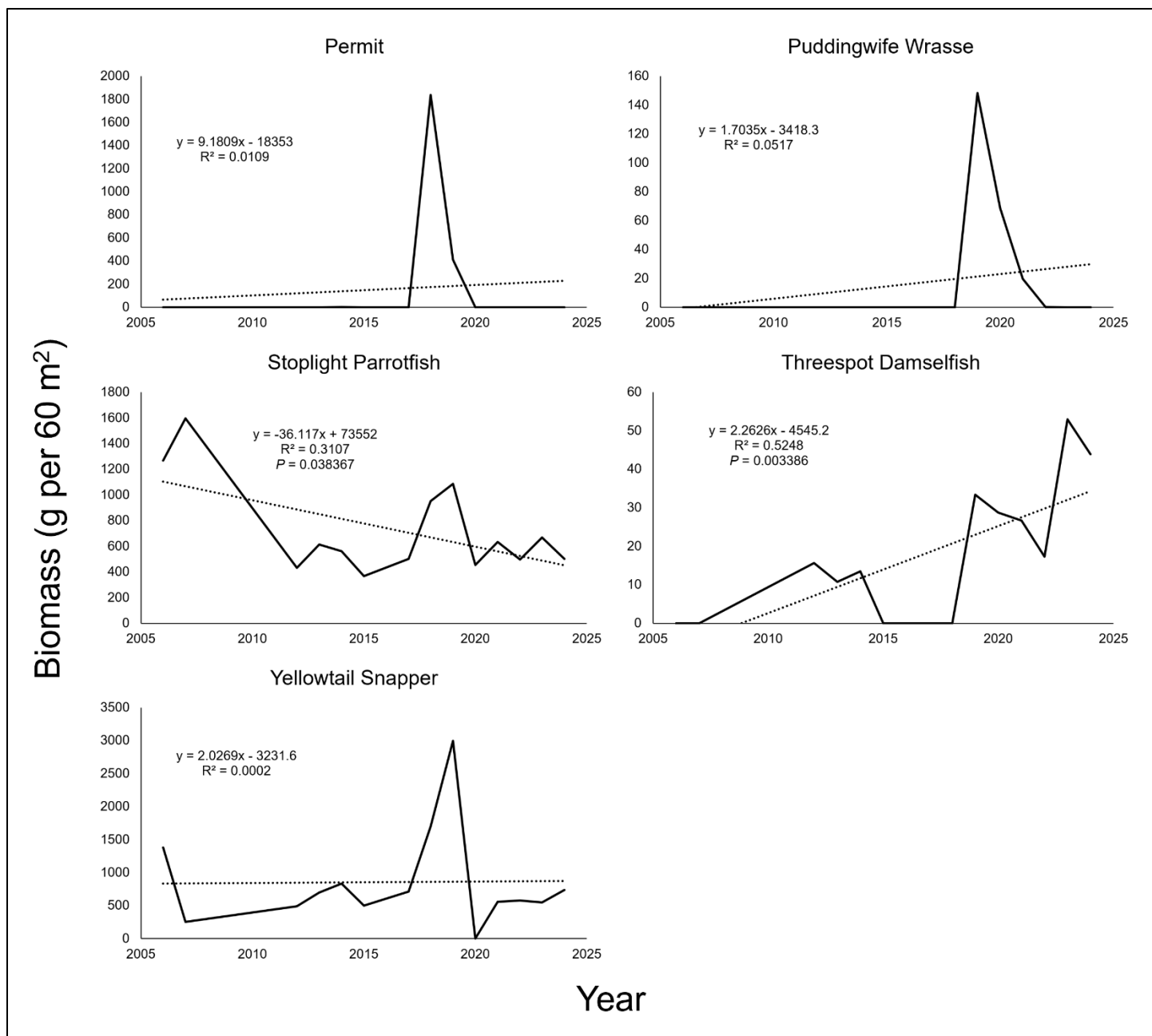


Figure 4.4. Temporal trends in Biomass of BIOENV Best taxa.

The BIO-ENV BEST test identified Bar Jack (*Caranx ruber*), Bluestriped Grunt (*Haemulon sciurus*), Doctorfish (*Acanthurus chirurgus*), Striped Parrotfish (*Scarus iseri*), and Yellowtail Snapper (*Ocyurus chrysurus*) as the taxa most representative of variation in Relative Abundance among years (Table 4.4).

Table 4.4. The average RA per cluster. The values are the result of the average of each species' contribution for each year.

RA	Cluster 1	Cluster 2	Cluster 3	Cluster 4	2019	2020	2021
Bar Jack	0.256	0.236	0.164	1.606	3.986	2.085	0.803
Bluestriped Grunt	1.281	1.495	0.250	0.354	0.805	0.024	0.095
Doctorfish	0.385	0.058	0.021	0.121	0.186	0.581	0.144
Striped Parrotfish	2.223	4.271	2.321	7.089	0.727	5.553	2.594
Yellowtail Snapper	0.539	0.714	0.157	0.817	0.829	0.000	0.201

The CAP plot revealed four clusters (C1–C4) with considerable overlap among groups (Fig. 4.5), suggesting that community structure based on Relative Abundance was less clearly defined than in the Biomass dataset. While the clusters were significant, boundaries were diffuse, indicating that the relative abundances of dominant taxa remained broadly similar among years. The years 2019, 2020, and 2021 appeared as outliers, positioned outside the main clusters, similar to the biomass results (Figs. 4.2 and 4.3), likely reflecting anomalous community structures.

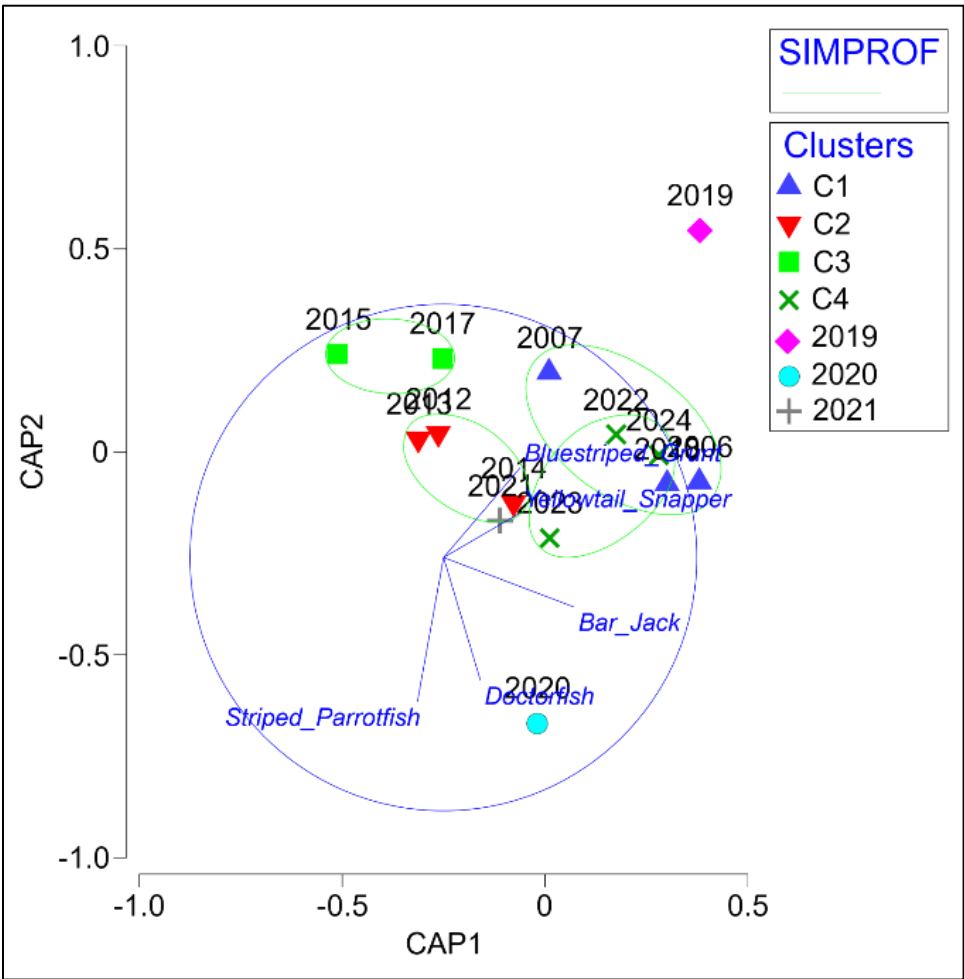


Figure 4.5. CAP plot shows variation of community structure among years based on the RA dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

The non-metric multidimensional scaling (MDS) plot showed greater separation among the four clusters (Fig. 4.6). The most recent four years grouped together in a distinct cluster, consistent with the pattern observed in the Biomass dataset, while earlier years were more dispersed. The Bar Jack, Striped Parrotfish, and Doctorfish contributed most strongly to the recent-year cluster, whereas the blue striped grunt characterized earlier periods. Overall, the Relative Abundance dataset indicated moderate differentiation among years, with clearer segregation emerging in recent years. This pattern parallels the biomass-based results, suggesting a gradual but consistent community shift over time.

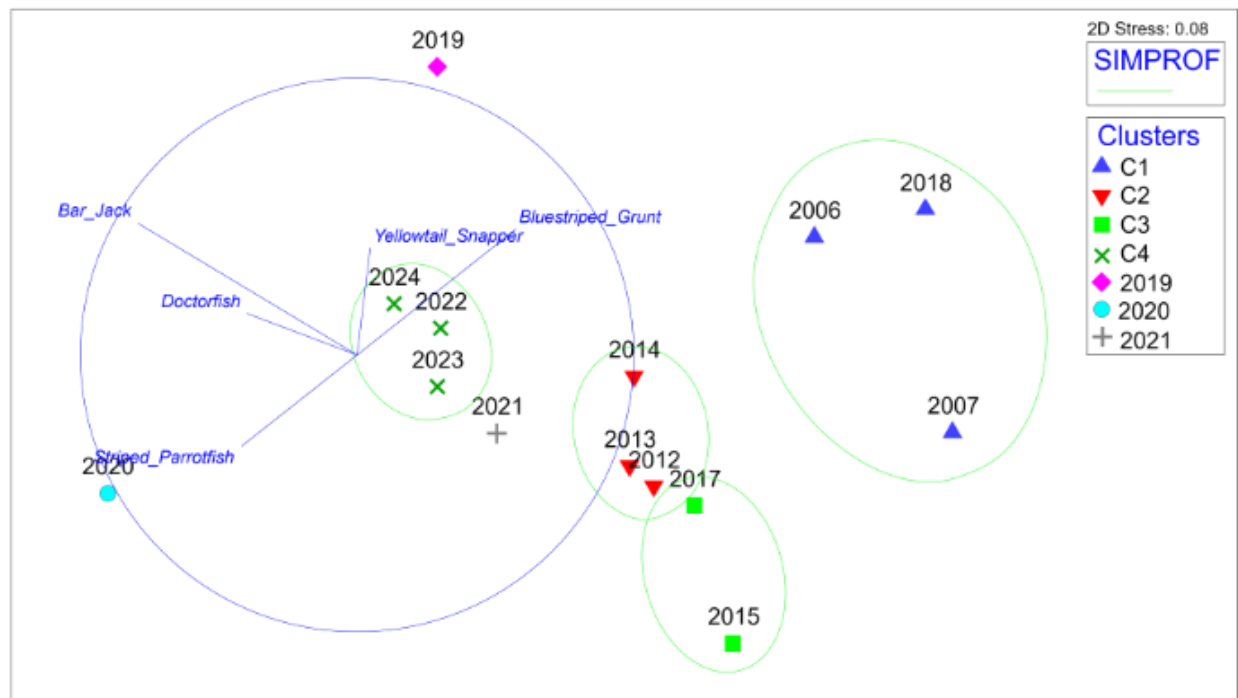


Figure 4.6. MDS plot showing variation in community structure among years based on the RA dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

Of the five BIO-ENV BEST species in the relative abundance dataset, Bar Jack increased significantly through time ($R^2 = 0.40$, $P < 0.05$), while Bluestriped Grunt showed a significant decline ($R^2 = 0.31$, $p < 0.05$) (Fig. 4.7). Doctorfish, Stoplight Parrotfish, and Yellowtail Snapper did not exhibit significant temporal trends.

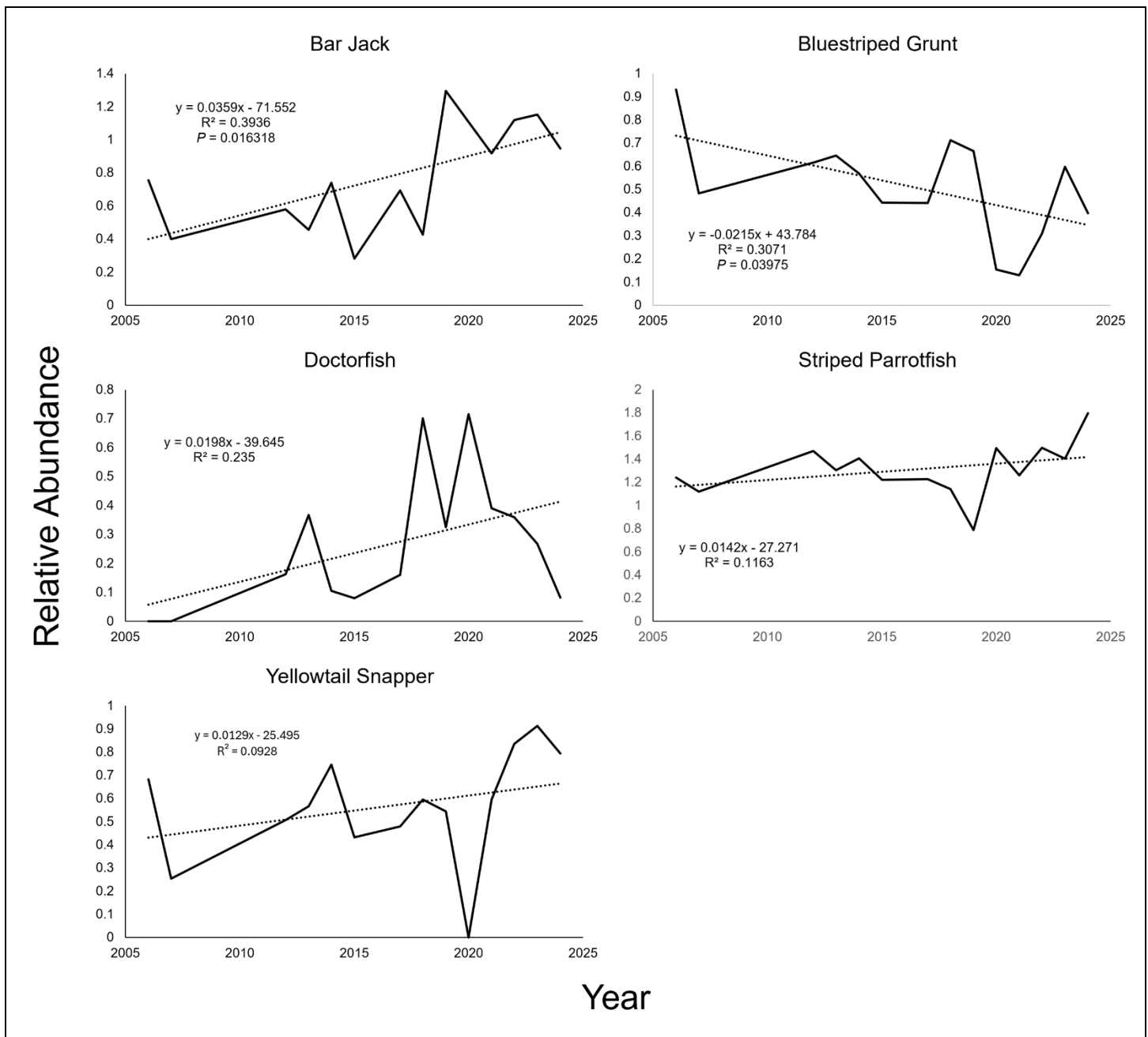


Figure 4.7. Temporal trends in Relative Abundance of BIOENV Best taxa.

The BIO-ENV BEST test identified Bar Jack (*Caranx ruber*), Ocean Surgeonfish (*Acanthurus bahianus*), Queen Parrotfish (*Scarus vetula*), Striped Parrotfish (*Scarus iseri*), and Yellowtail Parrotfish (*Sparisoma rubripinne*) as the taxa most representative of variation in Sighting Frequency among years (Table 4.5).

Table 4.5. The average SF per cluster. The values are the result of the average RA of each species' contribution for each year.

SF	Cluster 1	Cluster 2	Cluster 3	2019	2020
Bar Jack	0.175	0.131	0.516	0.522	0.436
Ocean Surgeonfish	0.691	0.460	0.489	0.271	0.220
Queen Parrotfish	0.277	0.181	0.258	0.222	0.000
Striped Parrotfish	0.641	0.744	0.874	0.365	0.795
Yellowtail Parrotfish	0.091	0.052	0.074	0.057	0.234

The CAP plot revealed four clusters (C1–C4) with considerable overlap among groups (Fig. 4.8), indicating limited differentiation in community structure based on sighting frequency. Despite the lack of clear separation, the clustering pattern remained broadly consistent with those obtained from the biomass and relative abundance datasets, suggesting similar underlying community patterns.

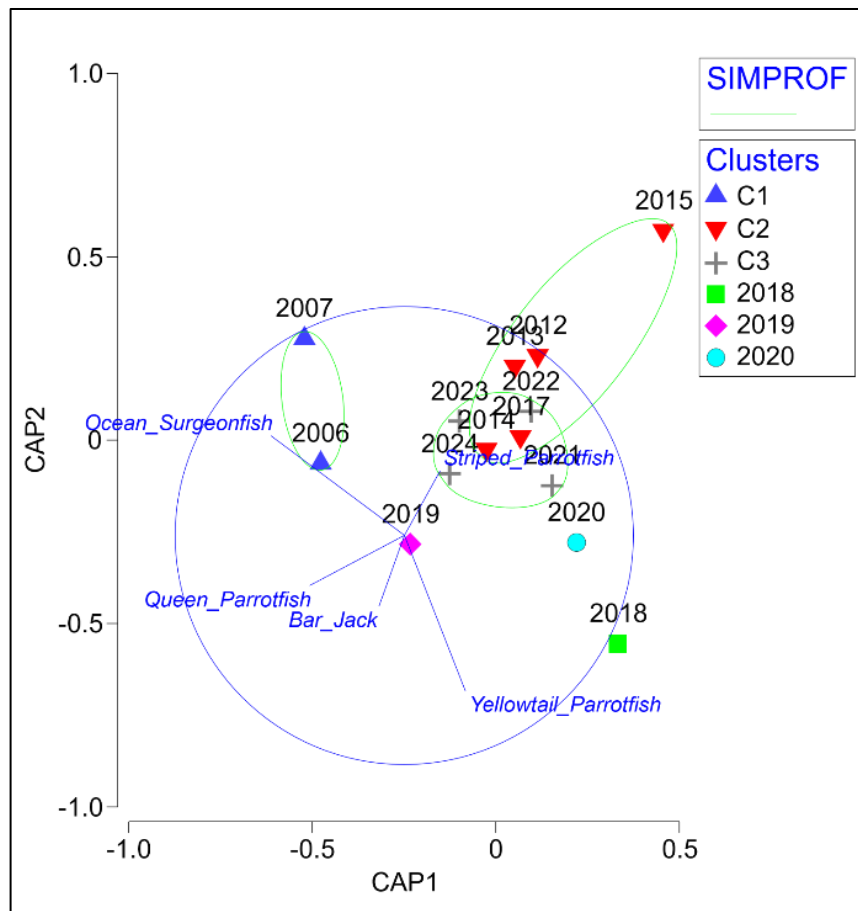


Figure 4.8. CAP plot showing variation of community structure among years based on the SF dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

The MDS plot, however, showed clearer structure (Fig. 4.9). The most recent four years were tightly grouped, reflecting high similarity in sighting frequency patterns and suggesting stabilization or homogenization of community observations in recent years. The years 2019 and 2020 again appeared as outliers, with 2018 also emerging as an additional outlier in this dataset. Overall, the Sighting Frequency dataset revealed weak separation among clusters but a distinct grouping of the most recent years, consistent with patterns observed in biomass and relative abundance. The outlier years indicate transient shifts or anomalies in observation frequency likely driven by short-term ecological or sampling variability.

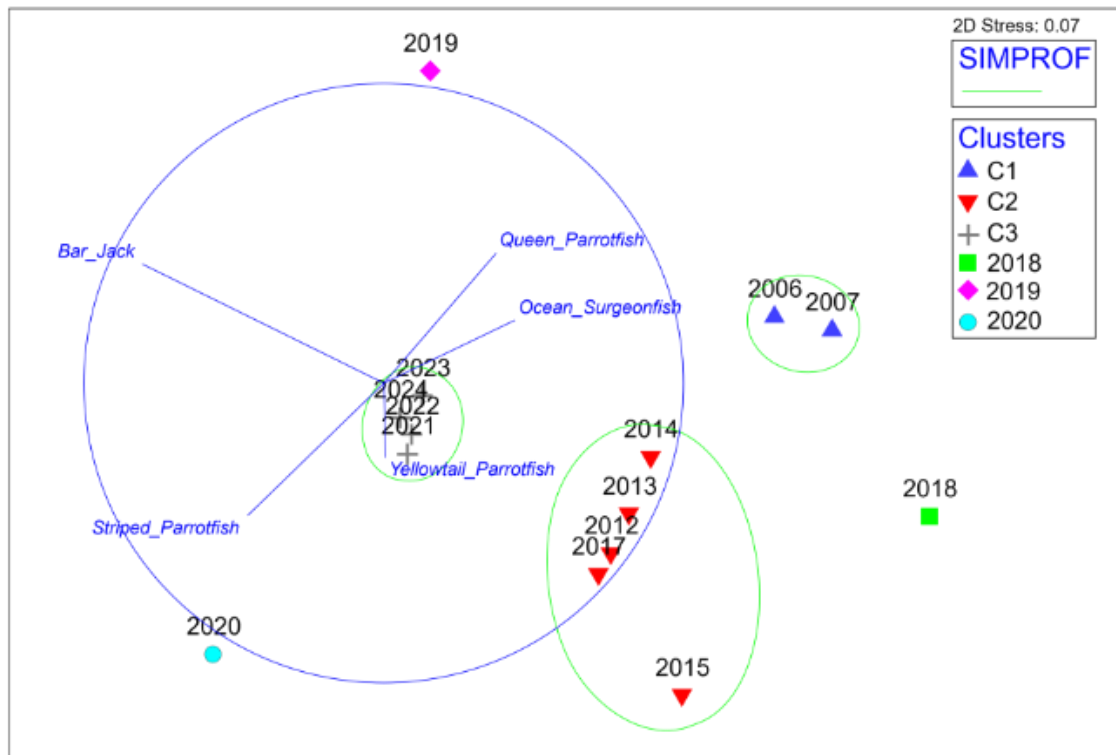


Figure 4.9. MDS plot showing variation in community structure among years based on the Sighting Frequency dataset. Significant clusters identified by the SIMPROF test are outlined in green. Blue vectors represent the taxa selected by the BIO-ENV BEST test that best explain patterns in community structure. Each point represents the centroid of the yearly community resemblance matrix; point shape and color correspond to the different clusters.

Temporal trends in sighting frequency of species identified as key drivers by the BIOENV BEST analysis are presented in Figure 4.10. Bar Jack exhibited a significant increase in sighting frequency across survey years ($R^2 = 0.45$, $P < 0.05$), whereas Ocean Surgeonfish showed a significant decline over time ($R^2 = 0.27$, $P < 0.05$). No significant directional changes were detected for the remaining taxa included in the analysis.

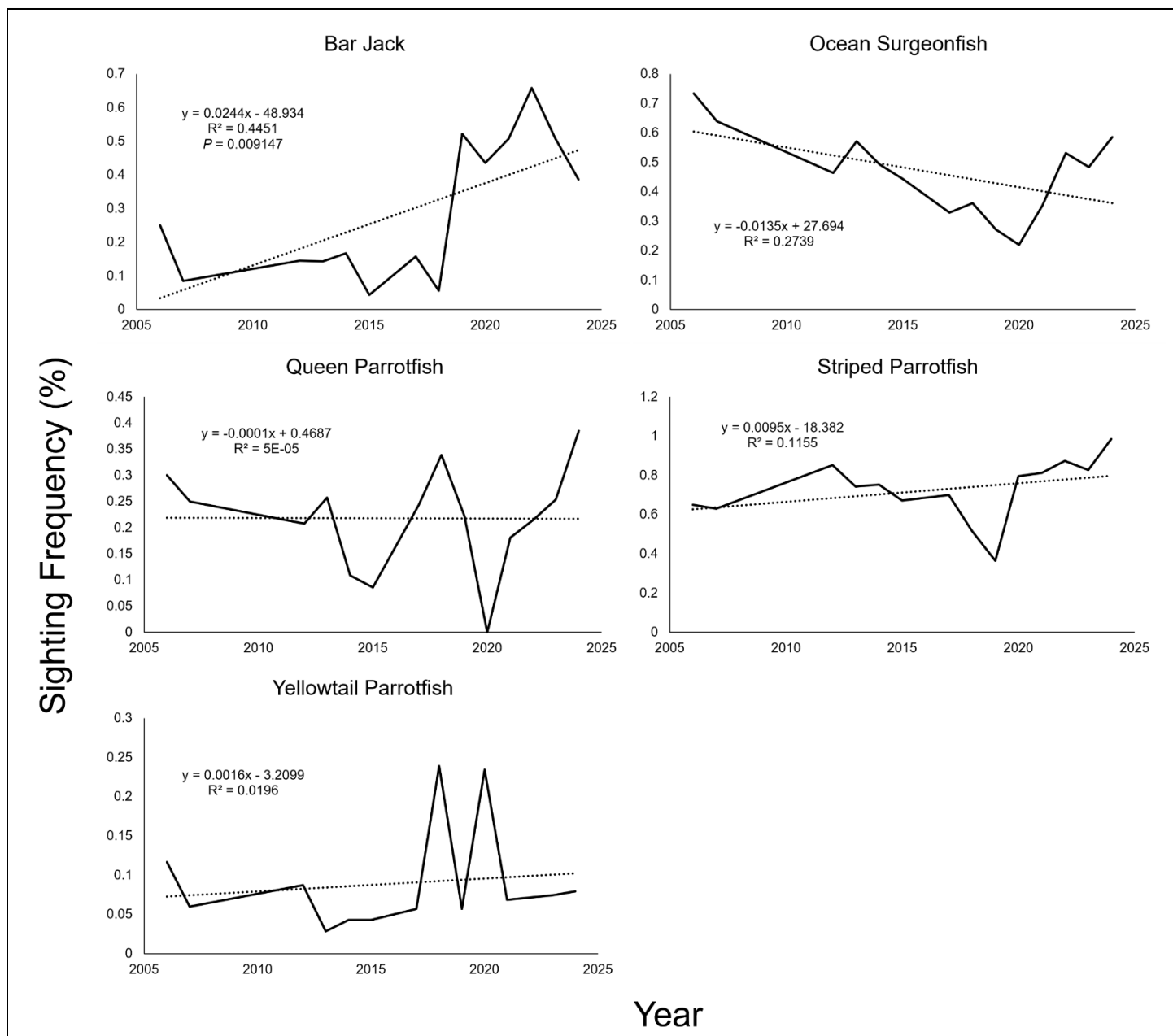


Figure 4.10. Temporal trends in Sighting Frequency of BIOENV Best taxa.

Across all three datasets, Biomass, RA, and SF, consistent temporal patterns were observed. Years 2019 and 2020 consistently appeared as outliers. Several year-pairings remained stable across the datasets (2013–2014, 2015–2017, 2006–2007, and 2021–2024, excluding 2021 in Relative Abundance), indicating similar community composition within those intervals. Common taxa identified by the BIO-ENV BEST tests, including Bar Jack (*Caranx ruber*), Yellowtail Snapper (*Ocyurus chrysurus*), and Striped Parrotfish (*Scarus iseri*), showed consistent trajectories across datasets. Bar Jack has significantly increased in representation in recent years, Yellowtail Snapper, and Striped Parrotfish remained relatively stable. These recurring temporal and taxonomic patterns suggest coherent community trends across all three datasets.

Once the PERMANOVA results were verified, SIMPER analysis (Clarke and Gorley, 2015) allowed for the identification of the taxa that contribute most to the average dissimilarity between samples collected in the sites inside and outside the Marine Protected Area (MPA). For this reason, only the species that occurred in two or more of the datasets are presented in Table 4.6; the SIMPER results of individual datasets are reported in Supplementary Information for Chapter 5 (Tables S1- S3) and include only the species contributing up to 50% of the variation.

Table 4.6. Taxa consistently contributing to dissimilarities between MPA and non-MPA sites across datasets. Values represent percentage contributions from the SIMPER analyses. NA indicates that the taxa were not in the top contributing species while the MPA (-/+) indicates whether the average values of species were higher (+) or lower (-) within the MPA than outside of it.

Species	Biomass %	RA %	SF %	MPA (-/+)
Bermuda or Yellow Chub	4.46	3.35	NA	+
Bluestriped Grunt	3.84	3.51	3.32	+
Caesar Grunt	3.84	3.83	3.11	+
Redtail Parrotfish	3.84	2.42	NA	+
Yellowtail Snapper	3.84	2.75	NA	+
Schoolmaster	3.84	3.19	3.53	+
Sailors Choice	3.84	2.09	NA	+
Mahogany Snapper	3.84	2.3	NA	+
Ocean Surgeonfish	NA	3.34	5.51	-
Yellowhead Wrasse	NA	3.31	5.03	-
French Grunt	3.84	2.45	4.02	-
Threespot Damselfish	3.84	3.05	3.44	+
Yellowtail Damselfish	3.84	2.62	3.27	+
Bar Jack	3.84	2.28	3.18	+
Queen Parrotfish	3.84	2.22	3.05	+

Discussion

This study provides one of the longest-term perspectives on coral reef fish community dynamics in the Cayman Islands, revealing significant shifts over an 18-year period and highlighting the influence of both temporal processes and management interventions. Across all three metrics (Biomass, Relative Abundance (RA), and Sighting Frequency (SF)) community composition varied significantly through time and between areas inside and outside Marine Protected Areas (MPAs). Temporal change emerged as the dominant driver, explaining nearly half of the observed variation, while management strategy accounted for most of the remainder. The lack

of significant interaction between these factors suggests that ecological shifts and management effects operate independently. Despite methodological differences among the three metrics, all showed broadly coherent temporal trajectories and indicated a long-term reorganization of fish assemblages likely driven by a combination of ecological processes, anthropogenic pressures, and management outcomes.

The temporal patterns identified here show that fish assemblages around Little Cayman have undergone substantial reorganization over the past two and a half decades, consistent with broader Caribbean trends (Cramer et al. 2021; Jackson et al. 2014). However, Little Cayman represents a distinctive case because direct anthropogenic pressures remain unusually low: the island is one of the least developed in the region, with fewer than 200 permanent residents, minimal coastal infrastructure, and significant portions of terrestrial habitat, such as the Booby Pond Nature Reserve, formally protected (McCoy and Shaw, 2010). As a result, common local stressors such as sedimentation, nutrient runoff, and land-based pollution are minimal, and observed changes are more likely to reflect ecological processes and broad-scale environmental influences than local human impacts. The absence of a significant relationship between temperature and community variation in the RELATE analysis further suggests that direct climatic forcing alone does not explain the observed patterns. Instead, indirect consequences of climate change, such as coral mortality and habitat restructuring likely play a more decisive role in shaping community dynamics.

Habitat structure is a primary determinant of fish community composition, shaping species associations, behavioral interactions, and niche distribution. Across the Caribbean, declines in live coral cover and reductions in structural complexity are consistently linked to changes in fish assemblages (Cramer et al. 2021; Graham and Nash 2013; Alvarez-Filip et al. 2009). At Little Cayman, these habitat dynamics were profoundly affected by the extreme marine heatwave of 2023, which caused approximately 80% bleaching and 54% mortality by early 2024 (Doherty et al. 2025). Mortality was highest among fast-growing species such as *Agaricia spp.* and *Porites porites*, while framework-building taxa like *Orbicella spp.* bleached but largely survived (Doherty et al. 2025), a selective shift expected to gradually alter benthic composition and, in turn, the structural context for reef fishes.

Despite the magnitude of this disturbance, our analyses show no abrupt reorganization of fish community composition in 2024. Assemblages from that year clustered closely with those from 2021–2023, indicating that fish community responses to severe coral mortality are neither immediate nor linear. This apparent lag likely reflects the persistence of structural habitat even after coral death: the physical framework of reefs can remain intact for years before bioerosion substantially reduces complexity (Carreiro-Silva & McClanahan 2012; Roff et al. 2019). Even when erosion begins within months, it progresses gradually, with fine-scale features disappearing before larger structures (Morais et al. 2022). Such structural retention can buffer fish assemblages against rapid compositional change (Emslie et al. 2014), delaying detectable shifts until

complexity declines below ecological thresholds. Our findings therefore do not suggest resilience per se, but rather a slow trajectory of community restructuring following major habitat loss. This underscores the importance of long-term ecological monitoring, as the full consequences of the 2023 bleaching event, especially in the context of fish community composition, are likely to unfold over extended timescales.

Temporal trajectories in community composition were characterized by gradual but consistent shifts in dominance patterns, indicating long-term ecological change independent of protection status. Across the study period, large-bodied herbivores such as *Scarus vetula* (Queen Parrotfish) and *Sparisoma viride* (Stoplight Parrotfish) declined (Fig. 4.9 and Fig. 4.3 respectively), while smaller-bodied species like *Scarus iseri* (Striped Parrotfish) increased throughout the study period (Figs. 4.7 and 4.9). These contrasting trends likely reflect ecological shifts, as changes in benthic composition and habitat structure following coral decline can alter competitive dynamics within the herbivore guild. Functional roles in parrotfishes are strongly linked to size and morphology: smaller individuals, and species, tend to act as scrapers that feed efficiently on turf algae and proliferate under more algal-dominated or degraded conditions, whereas larger excavators rely on consolidated substrates and are more competitive on structurally complex reefs (Bonaldo & Bellwood 2008; Adam et al. 2015). The increasing prevalence of *Halichoeres radiatus* (Puddingwife Wrasse) (Fig. 4.3) and *Stegastes planifrons* (Threespot Damselfish) (Fig. 4.3) further suggests potential shifts in trophic interactions and microhabitat availability, possibly linked to benthic reorganization following coral mortality events.

It is important to note that the species-level regressions (Figs 4.4, 4.7, and 4.10) represent univariate assessments of linear change through time. Community-level patterns identified in the multivariate ordinations reflect the combined and potentially non-linear responses of multiple taxa occurring simultaneously. Therefore, the absence of a significant temporal trend for a given species does not imply ecological stability, but rather indicates that its contribution to community reorganization may occur through covariance with other taxa. Species identified as key drivers in the BIO-ENV analyses remain ecologically influential even when individual linear trends are weak or non-significant.

Temporal patterns in mid- and apex-predators such as *Ocyurus chrysurus* (Yellowtail Snapper) (Fig. 4.3) were variable rather than consistently directional. Although biomass exhibited a slight negative tendency through time, trends were not statistically significant, and relative abundance showed intermittent increases. This divergence between metrics may reflect shifts in size structure rather than overall occurrence. Given that the Yellowtail Snapper is among the most heavily targeted species both commercially and recreationally across the Caribbean (Mumby et al. 2012), even relatively low but persistent fishing pressure can selectively remove larger individuals, gradually altering population structure without producing strong linear declines in abundance (Jackson et al. 2014; Paddock et al. 2009). Such size-selective exploitation is a hallmark of the “fishing down the food web” process (Pauly et al. 1998) and may contribute to subtle reorganization within predator

assemblages. Concurrent increases in mid-level predators such as *Caranx ruber* (Bar Jack) (Figs. 4.7 and 4.9) are consistent with mesopredator release dynamics (Mumby et al. 2012), suggesting that trophic restructuring may occur even in the absence of strong univariate declines. Together, these patterns indicate that fishing pressure may shape predator assemblages through gradual shifts in size structure and relative dominance rather than abrupt population collapse.

Fish assemblages inside MPAs were dominated by larger-bodied and commercially targeted species, including *Ocyurus chrysurus* (Yellowtail Snapper), grunts (*Haemulon spp.*), and large parrotfishes (*Scarus vetula*, *Sparisoma viride*), reflecting the well-documented effects of reduced fishing mortality (Halpern 2003; Mumby et al. 2006; Juan et al. 2010). In no-take areas, individuals are less likely to be removed before reaching maturity, allowing them to survive longer, grow larger, and reproduce more frequently, demographic changes that result in higher biomass and a greater dominance of large-bodied species. In contrast, non-protected areas were characterized by smaller, opportunistic taxa such as *Acanthurus bahianus* (Ocean Surgeonfish) and *Halichoeres garnoti* (Yellowhead Wrasse), which may benefit from lower predation pressure or competitive release where larger predators are depleted (Mumby et al. 2012; Noble et al. 2013). The consistent prevalence of larger herbivorous parrotfish within reserves further illustrates how MPAs support key functional groups that regulate algal growth and sustain reef resilience (Roff et al. 2019; Jackson et al. 2014). These spatial contrasts mirror and complement the long-term temporal shifts observed in this study, together demonstrating how local management and broader ecological change jointly shape the structure and function of reef fish communities.

By integrating biomass, relative abundance, and sighting frequency, this study offers a more complete perspective on community dynamics than any single metric could provide. Biomass highlights the contribution of larger, often commercially targeted species, while relative abundance captures the influence of smaller and more numerous taxa. Sighting frequency, in turn, reflects behavioral patterns and spatial use of the reef. Together, these complementary approaches reveal both structural and functional dimensions of community change, strengthening the long-term picture of how fish assemblages on Little Cayman's reefs are evolving.

This study shows that reef fish communities on Little Cayman are undergoing long-term shifts driven by environmental change and evolving habitat conditions. By combining extended temporal data with complementary metrics, it reveals subtle but significant transformations in fish community composition that shorter studies could miss, highlighting the importance of sustained, long-term monitoring for understanding and anticipating ecological change on Caribbean reefs.

Supplementary Information for Chapter 4

Table S1. SIMPER results for Biomass dataset.

Species	Group Outside Av. Biomass	Group Inside Av. Biomass	Average Dissimilarity	Diss/SD	Contrib %	Cumm Contrib %
Bermuda or Yellow Chub	3.38	5.86	1.97	1.24	4.46	4.46
Bluestriped Grunt	2.13	4.95	1.69	1.34	3.84	8.3
Caesar Grunt	2.27	4.87	1.67	1.32	3.79	12.09
Nassau Grouper	3.45	5.2	1.5	1.1	3.4	15.49
Tiger Grouper	1.95	3.32	1.4	1.05	3.17	18.66
Redtail Parrotfish	2.44	3.52	1.26	1.23	2.87	21.53
Yellowtail Snapper	3.18	4.43	1.25	1.19	2.83	24.36
Schoolmaster	4.2	6.11	1.21	1.06	2.74	27.1
Yellowtail Parrotfish	2.17	2.45	1.19	1.07	2.71	29.81
French Angelfish	1.82	2	1.15	0.87	2.6	32.42
Sailors Choice	1.21	2.43	1.13	0.88	2.57	34.99
Queen Triggerfish	2.21	1.5	1.12	0.82	2.55	37.54
Great Barracuda	1.23	2.46	1.11	0.93	2.53	40.07
White Grunt	1.87	2.29	1.1	1	2.51	42.58
Mahogany Snapper	1.26	2.39	1.09	0.93	2.48	45.06
Black Durgon	4.03	4.07	1.09	1.14	2.48	47.54
Mutton Snapper	1.55	2.19	1.08	0.89	2.46	50

Table S2. SIMPER results for relative abundance dataset (MPA vs. non-MPA).

Species	Group Outside Av. RA	Group Inside Av. RA	Average Dissimilarity	Diss/SD	Contrib%	Cum Contrib %
Caesar Grunt	0.35	0.85	1.47	1.34	3.83	3.83
Bluestriped Grunt	0.3	0.77	1.35	1.34	3.51	7.34
Bermuda or Yellow Chub	0.41	0.78	1.29	1.36	3.35	10.69
Ocean Surgeonfish	1.16	0.61	1.28	1.31	3.34	14.03
Yellowhead Wrasse	1.07	0.88	1.27	1.21	3.31	17.34
Schoolmaster	0.67	1.06	1.23	1.24	3.19	20.54
Threespot Damselfish	0.52	0.64	1.17	1.23	3.05	23.59
Juvenile Parrotfishes	0.38	0.42	1.13	0.95	2.95	26.54
Yellowtail Snapper	0.47	0.72	1.05	1.28	2.75	29.29
Yellowtail Damselfish	0.51	0.51	1.01	1.25	2.62	31.91
Black Durgon	0.7	0.71	1	1.23	2.61	34.52
French Grunt	0.96	0.83	0.94	1.2	2.45	36.97
Redtail Parrotfish	0.39	0.5	0.93	1.28	2.42	39.39
Mahogany Snapper	0.19	0.38	0.88	0.9	2.3	41.69
Bar Jack	0.75	0.85	0.88	1.16	2.28	43.97
Queen Parrotfish	0.49	0.71	0.85	1.21	2.22	46.19
Rock Beauty Angelfish	0.48	0.2	0.82	1.26	2.13	48.32
Sailors Choice	0.15	0.35	0.8	0.89	2.09	50.41

Table S2. SIMPER results for sighting frequency dataset.

Species	Group Outside Av SF	Group Inside Av SF	Average Dissimilarity	Diss/SD	Contrib%	Cum Contrib %
Ocean_Surgeonfish	0.63	0.22	2.22	1.59	5.51	5.51
Yellowhead_Wrasse	0.63	0.46	2.03	1.39	5.03	10.53
French_Grunt	0.53	0.38	1.62	1.45	4.02	14.55
Black_Durgon	0.29	0.28	1.43	1.22	3.55	18.1
Schoolmaster	0.26	0.41	1.43	1.37	3.53	21.63
Threespot_Damselfish	0.22	0.27	1.39	1.17	3.44	25.07
Graysby_Grouper	0.5	0.5	1.36	1.35	3.37	28.44
Striped_Parrotfish	0.79	0.65	1.34	1.34	3.33	31.77
Bluestriped_Grunt	0.08	0.3	1.34	1.13	3.32	35.09
Yellowtail_Damselfish	0.21	0.22	1.32	1.03	3.27	38.36
Princess_Parrotfish	0.72	0.69	1.3	1.28	3.23	41.59
Bar_Jack	0.27	0.34	1.28	1.32	3.18	44.77
Caesar_Grunt	0.08	0.29	1.26	1.2	3.11	47.88
Queen_Parrotfish	0.14	0.32	1.23	1.26	3.05	50.93

Chapter 5

Over 25 years of monitoring the Eastern Emilia-Romagna watershed reveals environmental effects on freshwater fish communities

Abstract

Freshwater ecosystems are increasingly threatened by anthropogenic pressures and climate change, leading to biodiversity loss and shifts in community dynamics. Long-term monitoring of freshwater fish assemblages is therefore essential to understanding how these systems respond to environmental change. In this study, we analysed fish communities from rivers across the Emilia-Romagna region of northern Italy using a 29-year dataset (1995–2024) encompassing 20 native species. The data were collected by the Freshwater Science Group (University of Bologna) through long-term monitoring efforts and collaborative projects between the Department of Biological, Geological and Environmental Sciences (BiGeA, formerly BES) and the Regional Agency for Environmental Protection of Emilia-Romagna (ARPAE). This biological dataset was integrated with environmental variables derived from NASA satellite data, including temperature, precipitation, and evapotranspiration. The analyses revealed significant temporal changes in community composition, characterised by a decline in cold-water taxa such as the *Salmo trutta* complex and an increase in cyprinid species tolerant to higher temperatures and hydrological variability, including *Telestes muticellus* and *Squalius squalus*. These results point to a climate-driven restructuring of freshwater fish communities and highlight the importance of long-term monitoring as a tool for understanding ecosystem responses and informing conservation strategies.

Introduction

Within the Mediterranean area, inland aquatic ecosystems represent critical biodiversity hotspots, hosting numerous endemic and keystone species (Cuttelod et al. 2009). Fish biodiversity, in particular, plays an essential ecological role acting as consumers across multiple trophic levels and contributing to nutrient dynamics and cycles (Whitfield et al. 2002; Dudgeon et al. 2006). However, it is now well established that climate change, attributed to human-induced alterations, is affecting biodiversity across the entire planet, which is why, in recent decades, the study of changes in freshwater biodiversity has also received considerable attention (Sala et al. 2000; Filipe et al. 2013).

Among the climatic variables undergoing the most pronounced changes, temperature stands out as one of the most affected. Global average air temperature has already increased by more than 1 °C above pre-industrial levels, and current projections indicate that it may exceed the 1.5 °C threshold within the next two decades if present trends continue (IPCC, 2021; 2023). Resulting in global warming and repercussions on average annual inland water temperatures (Caissie D. 2006; Filipe et al. 2013). This will cause critical issues for most aquatic organisms, which, being ectothermic, have specific temperature ranges that they can tolerate for prolonged periods of time, determining their local and regional distribution (Burgmer et al. 2007). One of the consequences of this progressive rise in temperature, is a change and/or reduction in the distribution of species within their range (Walther et al. 2002; Heino et al. 2009). An example of this trend can be found in several studies on various areas of Europe that have reported a decline in salmonid populations in response to rising average water temperatures (Hari et al. 2006; Sánchez-Hernández and Nunn 2016).

Precipitation is another climatic variable undergoing major global changes, both seasonal and interannual, leading to increases in the duration or intensity of extreme weather events such as massive floods or prolonged droughts (Alcamo et al. 2007; Filipe et al. 2013). Within Mediterranean river ecosystems, a decrease in total annual runoff volume is expected, accompanied by a seasonal redistribution of flows, with higher discharge during winter and reduced flow in summer (Boix-Fayos et al. 1998; Arnell et al. 2004). According to model projections, these changes in precipitation are expected to lead to a deterioration in ecological conditions, greater hydrological instability, and a reduction in the connectivity of watercourses associated with prolonged droughts and declining water quality in Mediterranean basins (Xenopoulos et al. 2005; Grill et al. 2019; Reid et al. 2019). An aspect linked to changes in water bodies in the Mediterranean area is its vegetation, which has relatively high evapotranspiration rates. This phenomenon, combined with climate change and in particular rising temperatures, will cause a greater reduction in river flow than would be expected from the decrease in rainfall alone (Tague et al. 2009). This scenario is further aggravated by human-induced alterations to natural water bodies, such as the construction of dams, the introduction and subsequent invasion of non-native species, water pollution and the intensive exploitation of surrounding areas for human activities (Reid et al. 2019; Malmqvist and Rundle 2002).

It is therefore important to study and monitor inland fish communities in order to preserve and maintain their biodiversity and ecological complexity.

With these objectives in mind, namely, to preserve aquatic habitats by assessing their quality and developing management strategies for the conservation of these water bodies, the European Union established the Water Framework Directive (WFD) in 2000. This directive bases the assessment of the ecological status of freshwater systems on biological quality elements such as phytoplankton, macrophytes, benthic invertebrates and fish (Water Framework Directive, 2000). In Italy, the WFD directive has been implemented with the creation of an index (NISECI) which, through the assessment of the qualitative and quantitative composition of fish populations used as bio-indicators, allows us to understand the health status of that basin or stretch of river (Macchio et al. 2017). Building on this framework, Marchi et al. (2023) proposed a substantial refinement of the NISECI index to enhance its regional applicability and ecological accuracy within the eastern basins of the Emilia-Romagna region. To achieve this, the study defined six homogeneous ecological zones based on geomorphological and habitat variables, replacing the course three-zone structure (salmonid, lithophilic, and phytophilic cyprinid zones) previously adopted at the national level.

The river basins of the region Emilia-Romagna, located in northern Italy, have a dense hydrographic network flowing from the Apennines to the Adriatic Sea (Bianco 1995; EEA 2020). Of these basins, those that flow directly into the Adriatic Sea include the Reno, Lamone, Fiumi Uniti, Savio, Bevano, Rubicone, and Marecchia rivers, which together form a continuous longitudinal gradient across the region (Marchi et al. 2023). The Reno River, one of the main fluvial systems of Emilia-Romagna, drains a basin area of approximately 5,965 km², and flows to the Adriatic Sea (Pavanelli et al. 2019). It originates in the Apennine mountains and exhibits a marked morpho-hydrological gradient: its upper reaches are steep and narrow, whereas downstream sections in the lowlands transition into wider channels with lower slopes (Pavanelli et al. 2019). The river network of Emilia-Romagna shows a pronounced altitudinal and geomorphological gradient that underlies ecological zoning and, consequently, reflects transitions in water temperature, substrate composition and flow velocity (Marchi et al. 2023). Fish communities in temperate freshwater systems are spread across altitudinal and spatial gradients, which, in river environments, correspond to longitudinal zoning from mountain springs to lowland plains (Zerunian 2002; Bianco 1995; Marchi et al. 2023). Within these gradients, environmental conditions such as flow rate, water temperature and substrate composition change progressively, resulting in a different distribution of species according to their ecological requirements. TMn this context, fish are divided TMnto well-defined ecological groups that reflect both hydromorphological and climatic factors (Poff et al. 2010; Marchi et al. 2023), from cold-water salmonids of mountain springs to warm-water cyprinids of the plains, reflecting changes in flow, substrate, and temperature (Fortini et al. 2018; Zerunian 2002).

Monitoring freshwater fish communities, especially in the long term, has become essential for understanding their response to climatic and anthropogenic pressures (Daufresne et al. 2004; Reid et al. 2019; Logez and Pont 2013). In fact, previous studies conducted over several decades in various parts of Europe have already highlighted a constant reorganisation of river communities (Pont et al. 2015; Goutte et al. 2020; Friedrichs-Manthey et al. 2024), with the decline of cold-water and rheophilic taxa and the simultaneous expansion of tolerant cyprinids (Daufresne and Boët 2007; Comte and Grenouillet 2013; Carosi et al. 2019; Abbà et al. 2024). In Italy, such data are still limited, but emerging evidence points to similar dynamics. In particular, Abbà et al. (2024) analysed thirty years of fish monitoring in the upper Po River basin, revealing significant spatial and temporal changes in community composition, including the decline of *Salmo trutta* and the increasing dominance of generalist cyprinids. These findings mirror patterns observed in other European rivers, where hydrological alteration and rising temperatures have led to a functional homogenisation of freshwater assemblages (Goutte et al. 2020; Friedrichs-Manthey et al. 2024). On a larger scale, the global loss of free-flowing rivers poses an additional threat to freshwater biodiversity (Grill et al. 2019).

Recognising the lack of long-term studies of this type, particularly in Italy, this research uses over three decades of fish monitoring data to investigate the dynamics of freshwater communities in the eastern Emilia-Romagna watershed and their relationships with environmental and geographical factors. It further examines the ecological patterns and variability that emerge along the river gradient, providing new perspectives on long-term community trajectories and the shifting environmental conditions that shape Mediterranean freshwater ecosystems.

Materials and Methods

Data acquisition

Fish community data were collected by ARPAE along the rivers of Emilia-Romagna over a 29-year period (1995–2024). For each sampling site, information on basin and sub-basin, altitude, and geographical coordinates was recorded. Samples were classified into six ecological zones according to the refined ecological classification proposed by Marchi et al. (2023) for the rivers of Emilia-Romagna, which describes a gradient from mountain headwaters (Zone 1) to lowland plains (Zone 6). Zone 1 was associated with mountain ecosystems, whereas the intermediate zones (2, 3, and 4) were characterized by a gradual increase in rheophilic species. In contrast, the lowland zones (5 and 6) were linked to the presence of rheophilic–limnophilic and limnophilic species (Figure 5.1). During sampling activities, the abundance of all observed species was recorded, forming the basis of the initial dataset.

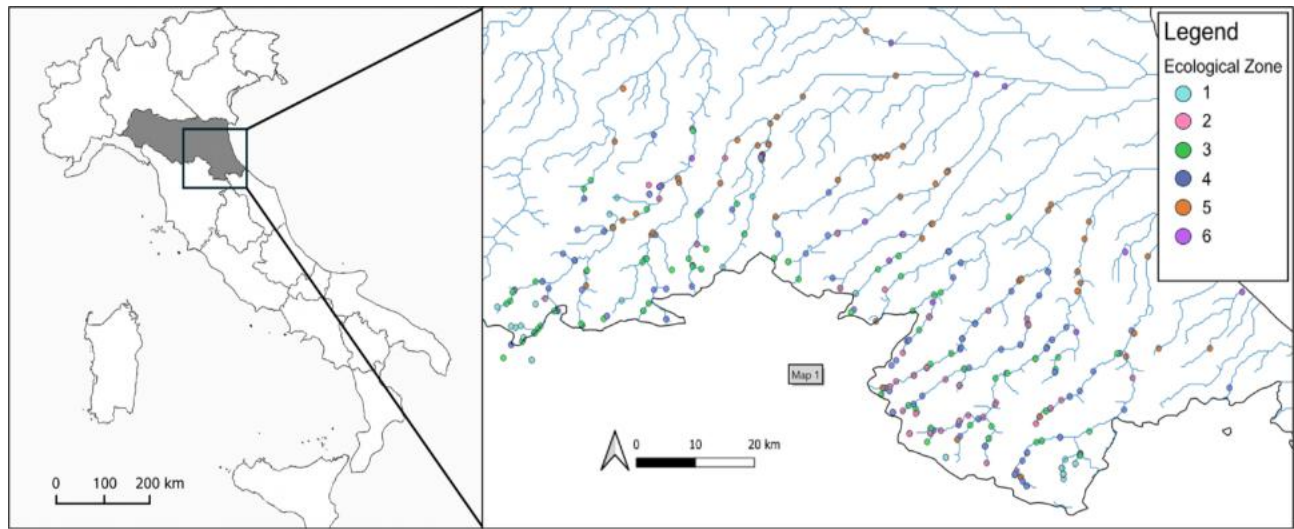


Figure 5.1. Map of study sites included in the ARP AE dataset within the Romagna sub-region of Emilia-Romagna, Italy, with different colours corresponding to different ecological zones.

For data collection by ARP AE, 521 sampling activities were carried out on fish communities and environmental parameters distributed in 84 water bodies across the region. Fish sampling carried out by ARP AE was performed using electrofishing according to the standardized protocols for the NISECI index (Sollazzo et al., 2007; Marchi et al. 2023). Most surveys relied on a single-pass approach, while only a minority employed multiple-pass removal. Captured individuals were provisionally placed in buckets filled with river water, then each individual fish was measured for total length with a ruler (± 1 mm) and weighed with a precision balance (± 0.1 g). For more details see (Marchi et al. 2023). A total of 20 native freshwater fish species were considered for all analyses, including all consistently recorded native taxa throughout the study period, while non-native species were excluded as they are not representative of the natural state of these communities. Each species was assigned an abbreviation used in all figures and statistical results to simplify visualization (Table 5.1).

Table 5.1. List of freshwater fish species considered in the analyses, with their scientific and common names and the corresponding species codes used throughout the text and figures.

Code	Scientific name	Common name
AA	<i>Alburnus alburnella</i>	Italian bleak
AN	<i>Anguilla anguilla</i>	European eel
BP	<i>Barbus plebejus</i>	Italian barbel
BM	<i>Barbus meridionalis</i>	Southern barbel
PG	<i>Liza aurata</i>	Golden grey mullet
CB	<i>Cobitis bilineata</i>	Italian spined loach
CC	<i>Cyprinus carpio</i>	Common carp
SS	<i>Squalius squalus</i>	Italian chub
PB	<i>Padogobius bonelli</i>	Padanian gobi
RB	<i>Romanogobio benacensis</i>	Italian gudgeon
PG	<i>Protochondrostoma genei</i>	Italian nase
MC	<i>Mugil cephalus</i>	Grey mullet
PF	<i>Platichthys flesus</i>	European flounder
PL	<i>Perca fluviatilis</i>	European perch
SH	<i>Scardinius hesperidicus</i>	Italian rudd
CS	<i>Chondrostoma soetta</i>	Soetta nase
CG	<i>Cottus gobio</i>	European bullhead
SX	<i>Salmo cettii</i>	Mediterranean trout
	<i>Salmo trutta</i>	Brown trout
	<i>Ibrido Salmo spp.</i>	Hybrid trout
LU	<i>Leucos aula</i>	Italian bleak
TM	<i>Telestes muticellus</i>	Italian riffle dase

To obtain environmental data corresponding to the sampling years from 1995 to 2024, the NASA Giovanni portal (NASA GES DISC, 2025) was utilized in conjunction with the site coordinates contained within the ARPAE dataset. Spatial extents were created on the map with QGIS version 3.34 (QGIS Development Team, 2023) containing 1 to 3 sampling sites that were close in geographical distance and similar in ecological zone and altitude. These extents were then input into Giovanni for the resulting environmental variables. Three environmental variables were considered as possible indicators of change in the freshwater fish communities: yearly average Surface Air Temperature (°C) obtained from the MERRA-2 reanalysis dataset (GMAO, 2015; NASA GES DISC, accessed April 2, 2025), yearly average Total Evapotranspiration (kg m⁻² s⁻¹) obtained from the FLDAS Noah Land Surface Model (McNally et al., 2018; NASA GES DISC, accessed April 2, 2025) and yearly average Total Precipitation – land bias corrected (kg m⁻² s⁻¹), also obtained from the MERRA-2 reanalysis dataset (GMAO, 2015b; NASA GES DISC, accessed April 2, 2025).

Data treatment and analyses

A combined dataset was constructed by integrating ARPAE data with the environmental variables collected in this study. Zone 6 was excluded from the analyses due to the low number of samples, which would have substantially reduced usable data. The original dataset reported the total linear density of individuals for each species within each sampling event. To ensure comparability between samples with different total catches, the relative abundance of each species was first calculated as follows:

$$RA_{i,t} = \frac{LD_{i,t}}{\sum_{j=1}^S LD_{j,t}}$$

Where $LD_{i,t}$ is the linear density of species i in transect t , and the denominator represents the total linear density of all species (S) recorded in that transect. Relative abundance was calculated at the transect level to retain sampling replication and avoid overweighting zones or years with a higher number of transects. This approach standardizes for differences in total fish density across transects while preserving information on community composition (Clarke et al. 2014).

All statistical analyses were performed using PRIMER V7 with PERMANOVA+ (PRIMER-E, Ltd., Ivybridge, UK). Each analysis was performed on the bray-curtis resemblance matrices of the square-root transformed species data. The transformation helps to reduce the disproportionate weight of dominant taxa while still allowing for the retention of quantitative information of rarer species (Anderson et al. 2008; Clarke and Gorley, 2015). Environmental variables (surface air temperature, total precipitation, and total evapotranspiration) were normalised to remove scale effects and compared with the biological community matrices in subsequent analyses using Euclidean distance as a resemblance measure.

Focusing on zones 1 through 5, an initial PERMANOVA was conducted using the years with the greatest overlap across zones to ensure consistent coverage. This analysis included Ecological Zone and Year as fixed factors, with the following years represented: 1999, 2000, 2004, 2006–2008, and 2010. Subsequently, a zone-by-zone analysis was performed to capture the full temporal variability within each ecological zone. For these analyses, all available years of data were included for each zone (see SI Ch. 5, Table S1 for a full list of samples per year by ecological zone).

A supplementary analysis was also conducted to test whether basins exert a spatial influence on the fish community structure. Only three basins (Fiumi Uniti, Reno and Savio) had sufficiently comparable data, with samples available in ecological zones 3 and 4 for five shared years (1998, 1999, 2000, 2003, and 2005). In this analysis, only the factor Basin was tested. Year was not included as a factor, as restricting both would have reduced sample size and statistical power. Instead, consistent years across basins were selected to maximize sampling strength and isolate the effect of basin identity on community structure.

Ecological zone analysis

To analyse each ecological zone individually a PERMANOVA was performed with the factor Year, followed by a CAP (Anderson et al. 2008) with species projections derived from the Simper analyses (Similarity Percentage Analysis; Clarke 1993) to examine how the community changed over time and which species were primarily responsible for these changes. Subsequently, a RELATE test was performed between biological and environmental resemblance matrices for each zone to detect significant relationships between the environmental parameters and fish community variance. Then a distLM analyses (Anderson et al. 2008) was conducted to ascertain which specific environmental parameters were correlated with the community data for any zone resulting in a significant Relate test. Two dbRDA graphs (Anderson et al. 2008) were created to visualize how the community variance matched environmental variance. One with projections of the statistically significant environmental variables and the other with projections of the species with the highest dbRDA correlation and which also appeared as driving taxa from the SIMPER analyses. DistLM analyses were conducted on the complete sample dataset, whereas dbRDA figures were generated using yearly averages of the environmental distance matrix and the fish community centroid distance matrix (allowing for better visualization).

SIMPER analyses were used to identify the taxa contributing most to the dissimilarities among groups of samples defined by year or ecological zone. These were performed on Bray–Curtis resemblance matrices, using square-root transformed abundance data. For each pair of groups, SIMPER decomposed the average Bray–Curtis dissimilarity into contributions from individual taxa, allowing the identification of those most responsible for differences in community structure. To identify the taxa most responsible for temporal variation in community structure, SIMPER results were ranked by both the average contribution to overall dissimilarity and their frequency of occurrence among pairwise group comparisons. The taxa consistently ranking highly in both criteria were selected for interpretation. These selections were further supported by concordance with BIOENV BEST (Clarke and Gorley 2015) test results which identifies subsets of taxa best explaining the observed patterns in community structure.

Results

The general PERMANOVA based on the years chosen for all five zones, with the criterion of including years for which data was available in all five zones, revealed that both the Year factor and Ecological zone factor had statistically significant differences among levels ($P < 0.05$) (Table 5.2). Moreover, the interaction term (Year $\times$ Ecological Zone) resulted significantly ($P < 0.05$) (Table 5.2), indicating that temporal changes in community composition differed across ecological zones. These results justified performing subsequent analyses at the individual zone level, to disentangle temporal patterns specific to each ecological context. Both latitude and longitude were included as covariates in the primary PERMANOVA with latitude showing a strong correlation

(64.7% ECEV; Table 2) to community variance throughout the years. However, correlation tests showed that latitude was moderately but significantly correlated to temperature ($r = -0.32$, $P < 0.01$) reflecting the distribution of higher altitude, southern sites (primarily zones 1 and 2). Because temperature is explicitly considered in the zone level analyses, latitude was interpreted as a spatial proxy for underlying climatic gradients rather than an independent ecological driver. Importantly, even after accounting for its influence, both Year and Ecological Zone remained highly significant predictors of community structure variation (PERMANOVA, $P < 0.01$; Table 5.2).

Table 5.2. PERMANOVA Main Test of the 5 zones with Year and Ecological Zone as factors, including latitude and longitude as covariates in the model. “%ECEV” is the percent estimated component of explained variance.

All Zones					
Main Test	DF	SS	Pseudo – F	P – value	%ECEV
Longitude	1	14642	11.85	0.0001	2.06
Latitude	1	2.64x10 ⁵	214.01	0.0001	64.7
Year	12	76015	5.13	0.0001	10.78
Ecological Zone	4	45973	9.3	0.0001	9.22
Year X Ecological Zone	48	1.14x10 ⁵	1.93	0.0001	13.3
Zone 1					
Year	16	37868	5.0092	0.0015	-
Zone 2					
Year	17	64300	2.0124	0.0007	-
Zone 3					
Year	22	1.1605E5	3.4909	0.0001	-
Zone 4					
Year	21	1.2524E5	3.7707	0.0001	-
Zone 5					
Year	20	41476	1.599	0.0095	-

The basin-level PERMANOVA revealed no significant differences in community structure among the three basins tested (Fiumi Uniti, Reno, and Savio; $P > 0.05$).

Ecological zone Analysis

Zone 1

The PERMANOVA analysis of ZONE 1 confirmed significant differences between levels of the factor Year ($P < 0.05$, Table 5.2). The CAP analysis of Zone 1 (Fig. 5.2) showed a main cluster that included most of the years, from the earliest to some of the latest, along with a few scattered years outside this group. SIMPER identified SX, TM, CG, PB, BP and SS as the main contributors to temporal community variation (Table 5.3). SX was strongly associated with the cluster C1 as well as with the year 2010, characterizing much of the early and intermediate

years. TM was consistently associated with temporal variation, particularly with cluster C2 and the year 1997. CG was mainly associated with the intermediate years around 2010, while BP was primarily associated with the early years around 1997. PB and SS showed more stable associations across all years, generally at lower relative abundances. Together, these taxa explained the separation between clusters of past, intermediate, and recent years.

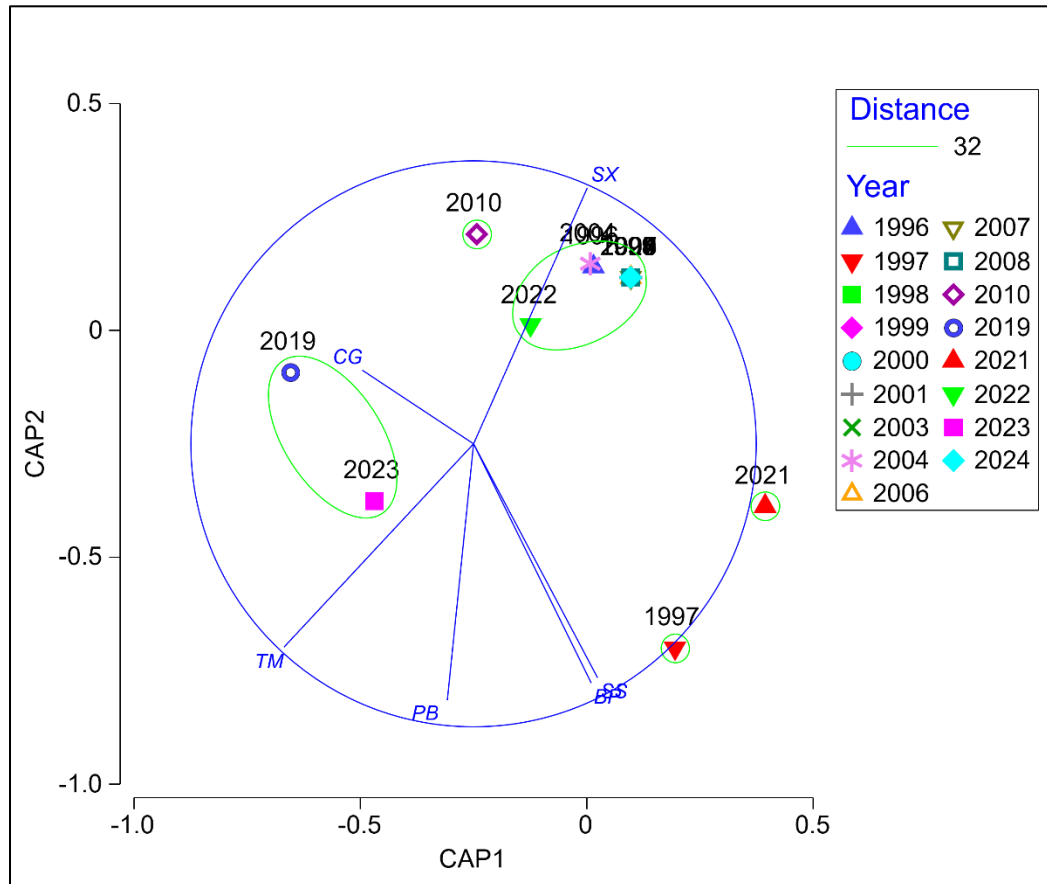


Figure 5.2. Canonical Analysis of Principal Coordinates (CAP) ordination plot of fish community structure in *Ecological Zone 1* based on the centroid resemblance matrix. Each point represents the general location of community structure in that year. Blue vectors indicate the main taxa contributing to differences among years, as identified by SIMPER analysis, while the green circles indicate similar groupings of community structure.

Table 5.3. Average relative abundances by cluster of the main taxa identified by SIMPER analysis as contributors to temporal differences in community composition within *Ecological Zone 1*. Cluster 1 includes years: 1996,1998,1999, 2000, 2001, 2003, 2004, 2006-2008, 2022, 2024; Cluster 2 includes: 2019 and 2023.

	CG	TM	PB	SX	BP	SS
C1	0.027	0.033	0	0.976	0	0
C2	0.079	0.937	0.136	0.242	0	0
1997	0	0.643	0.236	0.045	0.468	0.287
2010	0.655	0	0	0.756	0	0
2021	0	0.184	0.124	0.500	0.344	0.268

The RELATE test between community variation and environmental parameters resulted significantly (Spearman's $Rho = 0.244$, $P < 0.05$), while the distLM showed that only temperature was significantly correlated to zone 1 community variance ($P < 0.05$; SI Ch.5, Table S2) and was strongly correlated ($r=0.96$) with the first principal axis explaining approximately 16% of the total variation (SI Ch. 5, Table S6). These results were obtained from the complete sample dataset, while the dbRDA graphs are based on yearly averaged matrices of environmental variables and community centroids, which were used to improve visualization.

In the dbRDA graphs (Fig. 5.3), the left panel shows the significant environmental variables identified in the sequential test (SI Ch.5, Table S2), while the right panel shows the species most correlated with community structure in relation to environmental parameters. Specifically, TM, PB, and CG, which appeared in both the dbRDA vector projections and the SIMPER (SI Ch.5, Table S10), were the taxa most strongly associated with temperature variation.

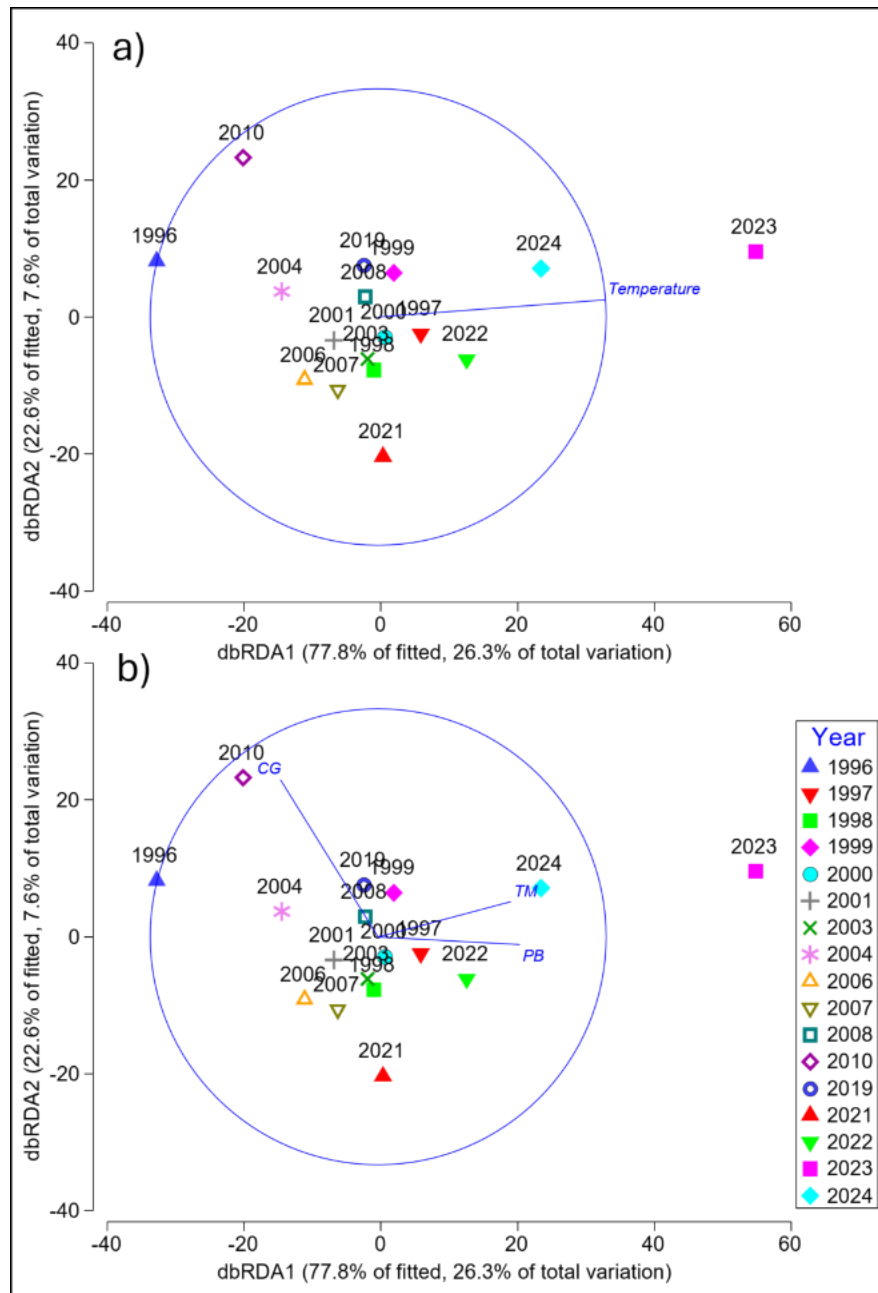


Figure 5.3. Distance-based redundancy analysis (dbRDA) of fish community structure in Ecological Zone 1. The ordination is based on Bray–Curtis resemblance of square-root transformed yearly centroid abundance data, matched to yearly average environmental variables. Plot a) shows the fitted vectors of environmental parameters, while plot b) displays the projections of the most influential species, identified both by strong correlations (CG 70%; TM, PB 50%) with environmental gradients and as top contributors to community change in SIMPER.

Zone 2

The PERMANOVA analysis of Zone 2 also confirmed the significance of the factor Year ($P < 0.05$, Table 2) explaining approximately 18% of community variance. The CAP plot of Zone 2 showed four main clusters of yearly community structure (Fig. 5.4), while the SIMPER identified SX, TM, PG, PB, BP, and SS as the main contributors to temporal community variation (Table 5.4). SX was strongly associated with the small cluster

containing the first two sampling years C3 and to a lesser extent in cluster C1. TM was consistently associated with both the early and late clusters, particularly with clusters C1 and C4. SS was mainly associated with the cluster C2 spanning past to more recent years, and to a lesser extent with the other clusters. PG, BP, and PB showed more stable contributions across all years. Together, these taxa explained the separation between clusters of past, intermediate, and recent years.

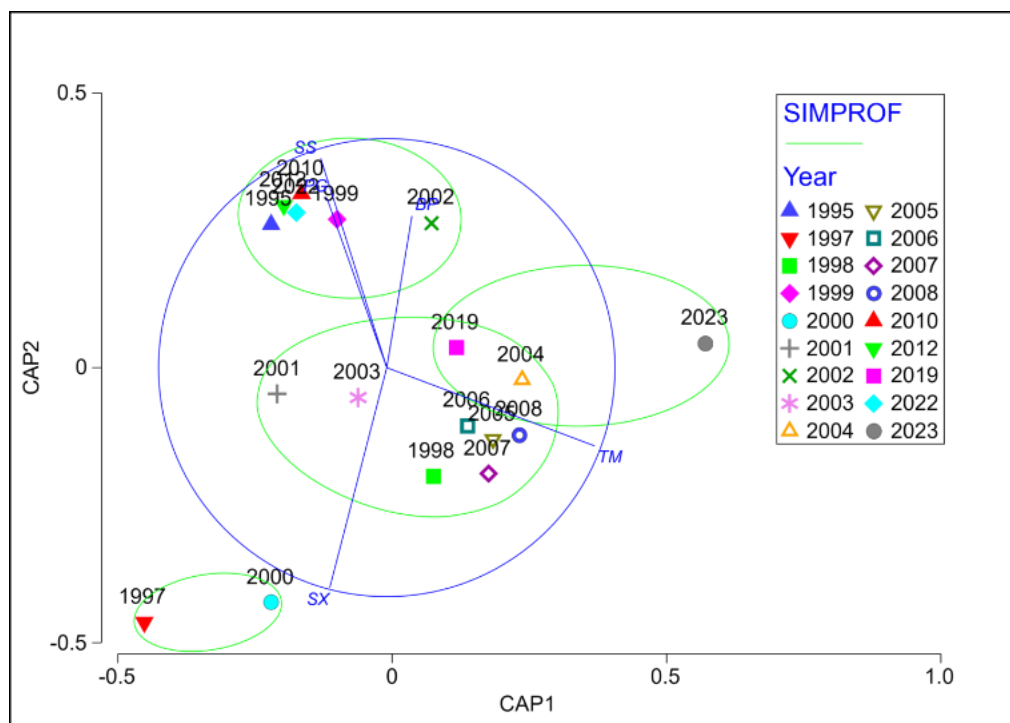


Figure 5.4. Canonical Analysis of Principal Coordinates (CAP) ordination plot of fish community structure in *Ecological Zone 2* based on the centroid resemblance matrix. Each point represents the general location of community structure in that year. Blue vectors indicate the main taxa contributing to differences among years, as identified by SIMPER analysis, while the green circles indicate similar groupings of community structure.

Table 5.4. Average relative abundances by cluster of the main taxa identified by SIMPER analysis as contributors to temporal differences in community composition within *Ecological Zone 2*. Cluster 1 includes year: 1998, 2001, 2003, 2004, 2005, 2006, 2007, 2008. Cluster 2: 1995, 1999, 2002, 2010, 2012, 2022. Cluster 3: 1997, 2000. Cluster 4: 2019, 2023.

	SX	TM	SS	PG	BP	PB
C1	0.414	0.441	0.246	0.100	0.229	0.057
C2	0.007	0.077	0.687	0.335	0.371	0.114
C3	0.932	0.104	0	0	0	0
C4	0.110	0.479	0.195	0.166	0.217	0.212

The RELATE test resulted significantly (Spearman's Rho = 0.134, $p < 0.05$), between environmental variables and the fish community while the distLM showed that temperature and precipitation were statistically significant (SI Ch.5, Table S3). Both temperature and precipitation were negatively correlated with the first axis, while for

the second axis, temperature was negatively correlated, and precipitation was positively correlated. The first axis alone explained 9.3% of the total variance out of 9.7% (SI Ch.5, Table S7), (see also Methods for explanation of differences between numerical results and dbRDA figures based on yearly averages).

In the dbRDA graphs (Fig. 5.5), the left panel shows the significant environmental variables identified in the sequential test (SI Ch.5, Table S3), while the right panel shows the species most correlated with community structure in relation to environmental parameters. Specifically, SX, SS, BP and PG, which appeared in both the dbRDA vector projections and the SIMPER (SI Ch.5, Table S11), were the taxa most strongly associated with temperature and precipitation variations.

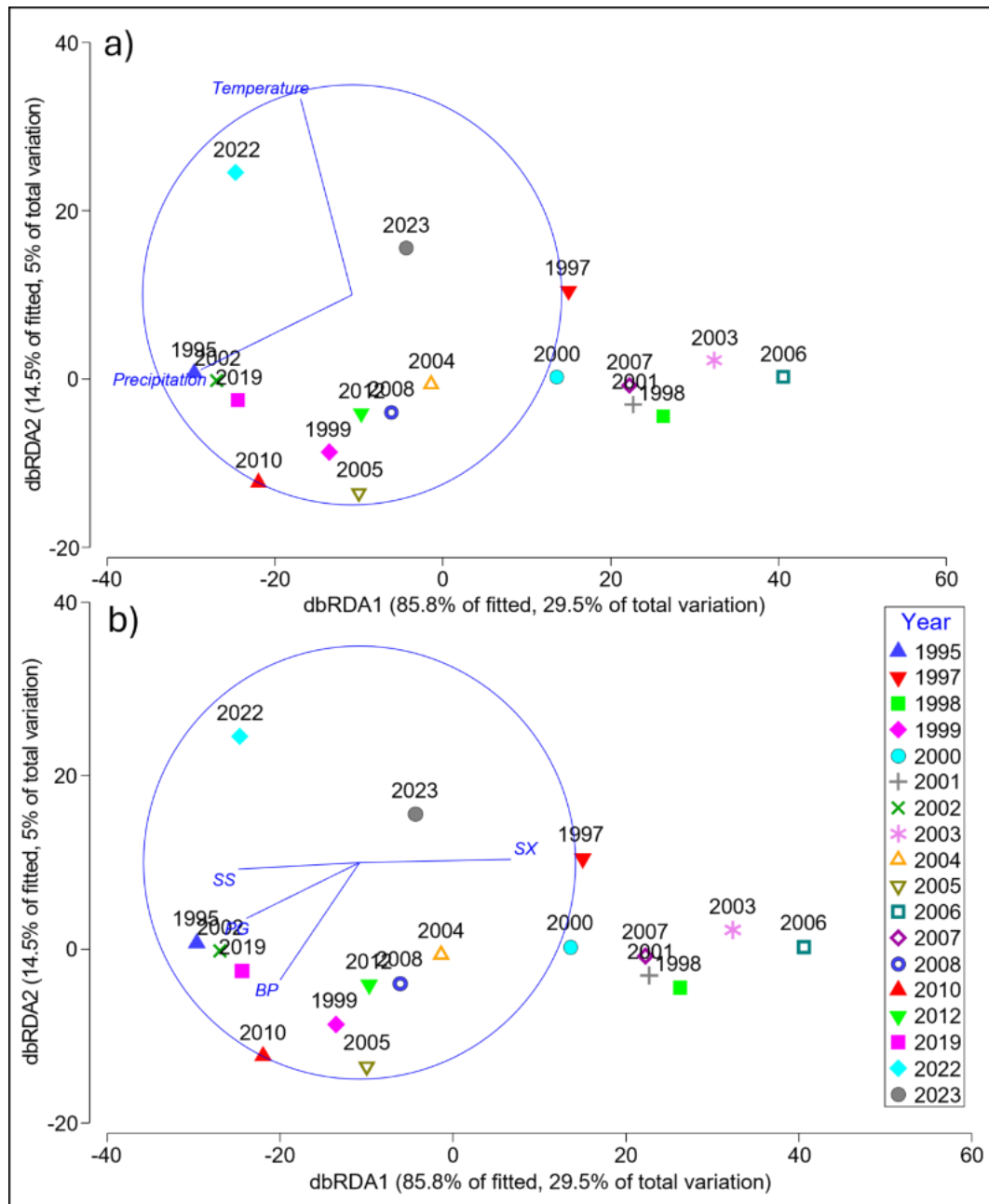


Figure 5.5. Distance-based redundancy analysis (dbRDA) of fish community structure in Ecological Zone 2. The ordination is based on Bray–Curtis resemblance of square-root transformed yearly centroid abundance data, matched to yearly average environmental variables. Plot a) shows the fitted vectors of environmental parameters, while plot b) displays the projection of the most influential species, identified both by strong correlations (SX 70%; PG, SS and BP 50%) with environmental gradients and as top contributors to community change in SIMPER.

Zone 3

In the third ecological zone, the PERMANOVA analysis with the factor Year yielded a statistically significant result ($P < 0.05$, Table 5.2), confirming differences among yearly communities and explaining approximately 43% of the observed variation. The CAP analysis of Zone 3 (Fig. 5.6) showed four main clusters, while SIMPER identified SX, TM, PG, PB, BP, and SS as the main contributors to temporal community variation (Table 5.5). SX

was strongly associated with the small cluster C4 containing two of the early years of sampling. TM was consistently associated with temporal variation, being more abundant in clusters C2 and C3 while SS was mainly associated with the cluster C1 spanning past to more recent years. PG, BP, and PB showed more stable contributions across all years. Together, these taxa explained the separation between clusters of pasts, intermediate, and recent years.

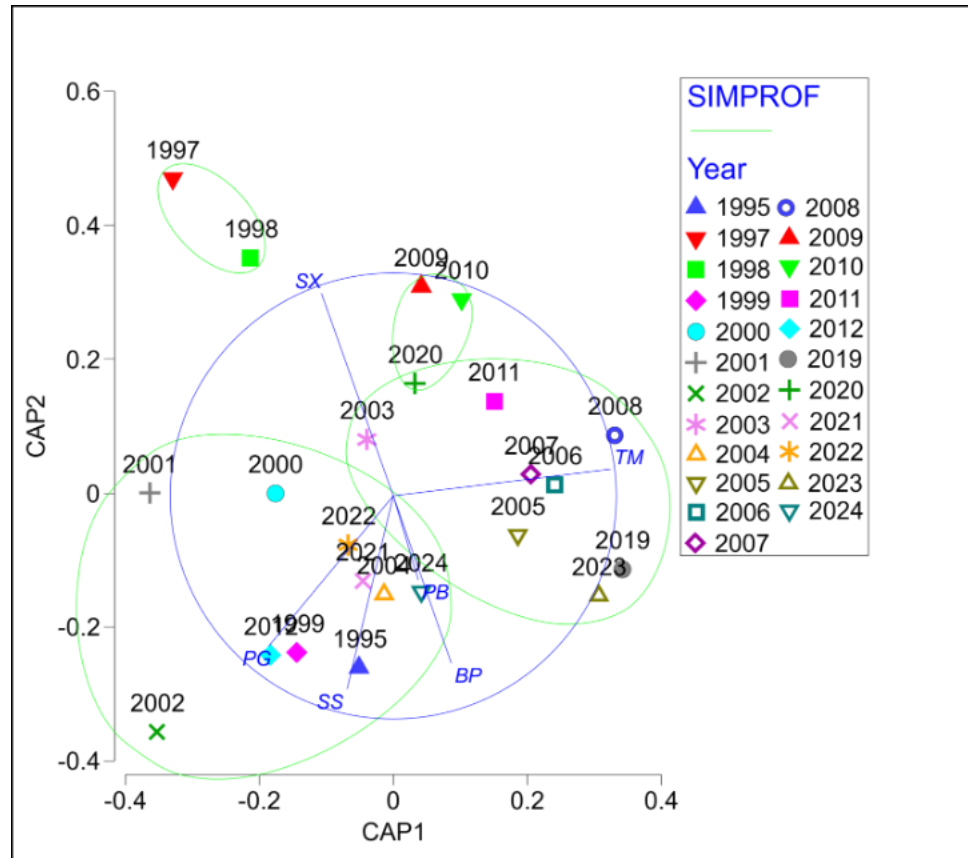


Figure 5.6. Canonical Analysis of Principal Coordinates (CAP) ordination plot of fish community structure in *Ecological Zone 3* based on the centroid resemblance matrix. Each point represents the general location of community structure in that year. Blue vectors indicate the main taxa contributing to differences among years, as identified by SIMPER analysis, while the green circles indicate similar groupings of community structure.

Table 5.5. Average relative abundances of the main taxa identified by SIMPER analysis as contributors to temporal differences in community composition within *Ecological Zone 3*. Cluster 1 includes year: 1995, 1999, 2000, 2001, 2002, 2004, 2012, 2021, 2022, 2024. Cluster 2: 2003, 2005, 2006, 2007, 2008, 2011, 2019, 2023. Cluster 3: 2009, 2010, 2020. Cluster 4: 1997, 1998.

	SX	TM	SS	PG	BP	PB
C1	0.146	0.270	0.494	0.282	0.342	0.149
C2	0.218	0.663	0.273	0.043	0.335	0.112
C3	0.401	0.491	0.100	0	0.131	0.022
C4	0.871	0.207	0.021	0	0.077	0.009

The RELATE test resulted significantly (Spearman's $Rho = 0.11$, $p < 0.05$), while the distLM showed that temperature, evapotranspiration, and precipitation were significantly correlated with community variance throughout the years ($P < 0.05$; SI Ch.5, Table S4). Temperature had a strong negative association with the first axis and, to a lesser extent, with the second axis. Evapotranspiration was highly correlated with the first axis and negatively correlated with the second axis. Finally, precipitation was weakly negatively correlated with the first axis and strongly correlated with the second axis. The first axis alone explained 13.8 of the total variances out of 16.3% (SI Ch.5, Table S8), (see also Methods for explanation of differences between numerical results and dbRDA figures based on yearly averages).

In the dbRDA graphs (Fig. 5.7), the left panel shows the significant environmental variables identified in the sequential test (SI Ch.5, Table S4), while the right panel shows the species most correlated with community structure in relation to environmental parameters. Specifically, SX and PB which appeared in both the dbRDA vector projections and the SIMPER (SI Ch.5, Table S12), were the taxa most strongly associated with temperature, evapotranspiration and precipitation variations.

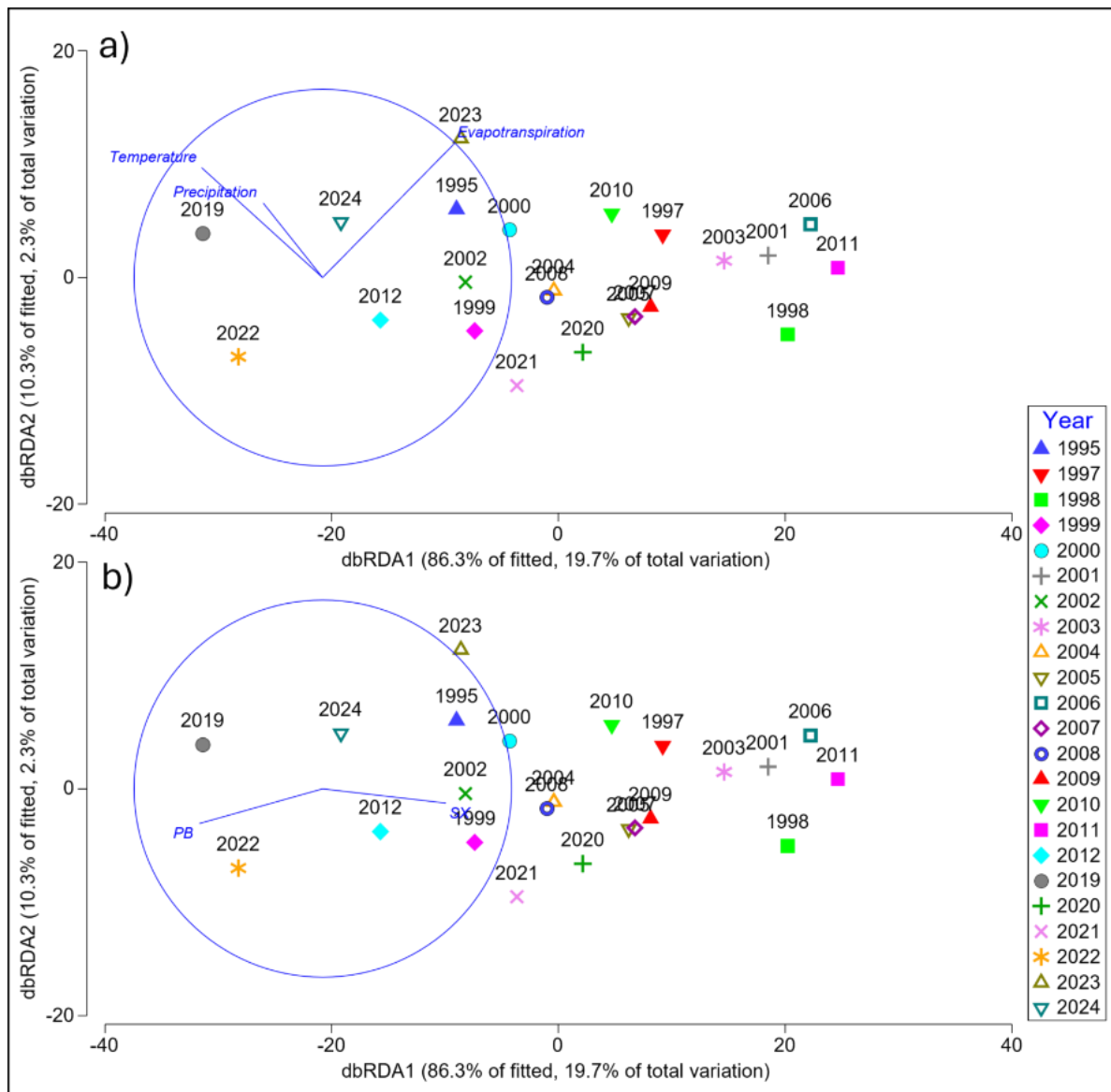


Figure 5.7. Distance-based redundancy analysis (dbRDA) of fish community structure in Ecological Zone 3. The ordination is based on Bray–Curtis resemblance of square-root transformed yearly centroid abundance data, matched to yearly average environmental variables. Panel a) shows the fitted vectors of environmental parameters, while panel b) displays the projection of the most influential species, identified both by strong correlations (SX and PB 50%) with environmental gradients and as top contributors to community change in SIMPER.

Zone 4

In the fourth ecological zone, the PERMANOVA analysis with the factor Year yielded a statistically significant result ($P < 0.05$, Table 5.2), confirming the effect of the year in driving community change and explaining approximately 29% of the observed variation. The CAP analysis of Zone 4 (Fig. 5.8) showed three main clusters, while SIMPER species projections (Table 5.6) identified SX, TM, PG, BP, and SS as the main contributors to temporal community variation. SX was more abundant in the small cluster C3 containing two of the early years of sampling. TM was consistently associated with community variation, with temporal shifts reflected in lower abundances in some clusters and higher abundances in others like C2. SS was mainly associated with clusters C1

and C2, showing higher relative abundance in C1 and moderate values in C2. PG was mainly associated with the biggest cluster C1 and BP showed more stable contributions across all years. Together, these taxa explained the separation between clusters of pasts, intermediate, and recent years.

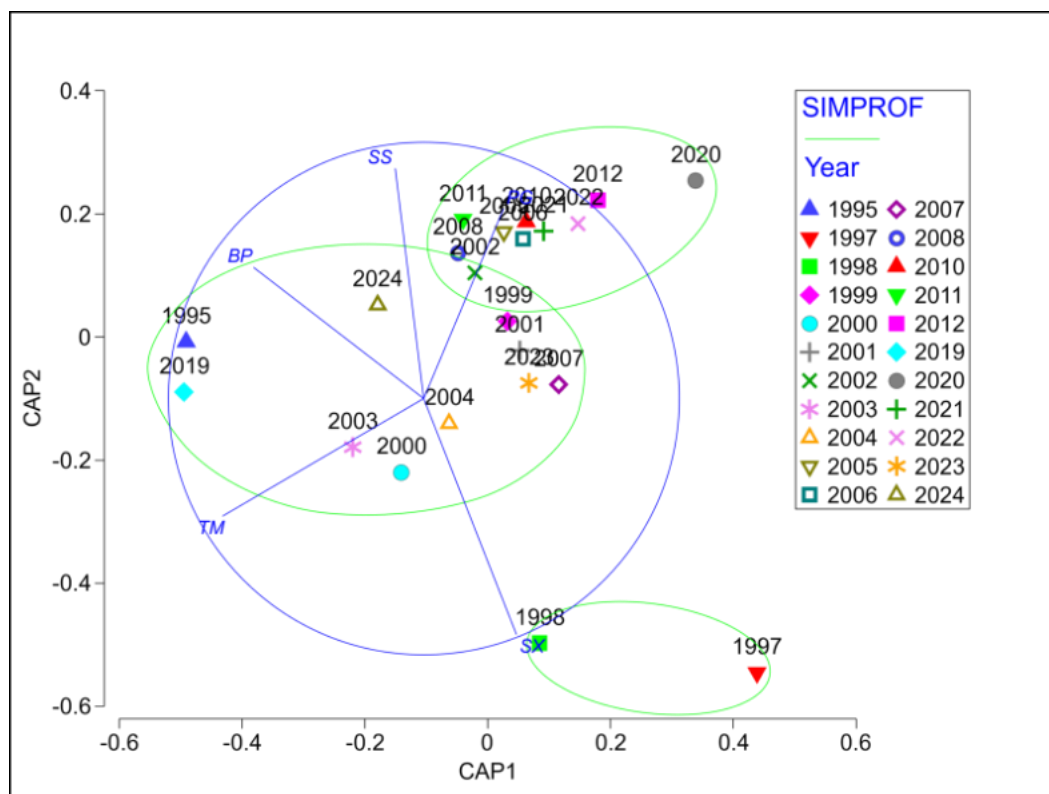


Figure 5.8. Canonical Analysis of Principal Coordinates (CAP) ordination plot of fish community structure in *Ecological Zone 4* based on the centroid resemblance matrix. Each point represents the general location of community structure in that year. Blue vectors indicate the main taxa contributing to differences among years, as identified by SIMPER analysis, while the green circles indicate similar groupings of community structure.

Table 5.6. Average relative abundances by of the main taxa identified by SIMPER analysis as contributors to temporal differences in community composition within *Ecological Zone 4*. Cluster 1 includes year: 2002, 2005, 2006, 2008, 2010, 2011, 2012, 2021, 2022. Cluster 2: 1995, 1999, 2000, 2001, 2003, 2004, 2007, 2019, 2023, 2024. Cluster 3: 1997, 1998.

	TM	SX	SS	PG	BP
C1	0.121	0.020	0.641	0.448	0.360
C2	0.369	0.161	0.459	0.187	0.315
C3	0.222	0.760	0.071	0	0.142

The RELATE test was not statistically significant, indicating no detectable correlation between environmental variables and the fish community; therefore, subsequent distLM and dbRDA analyses were not performed.

Zone 5

The PERMANOVA analysis of Zone 5 confirmed the significance of the factor Year ($P < 0.05$, Table 5.2) and approximately 13% of the variance explained by the annual variation. The CAP analysis of Zone 5 (Fig. 5.9) showed three main clusters, along with a few scattered years outside these groups. SIMPER identified TM, PG, SS, AA, and PB as the main contributors to temporal community variation (Table 5.7). AA was strongly associated with the small cluster containing one year (2002) and showed a lower abundance across all years. SS was strongly associated with all the clusters C1, C2 and C3, while TM was consistently associated with temporal variation, showing lower abundances in the early and late clusters. PG and BP showed more stable contributions across all years. Together, these taxa explained the separation between clusters of pasts, intermediate, and recent years.

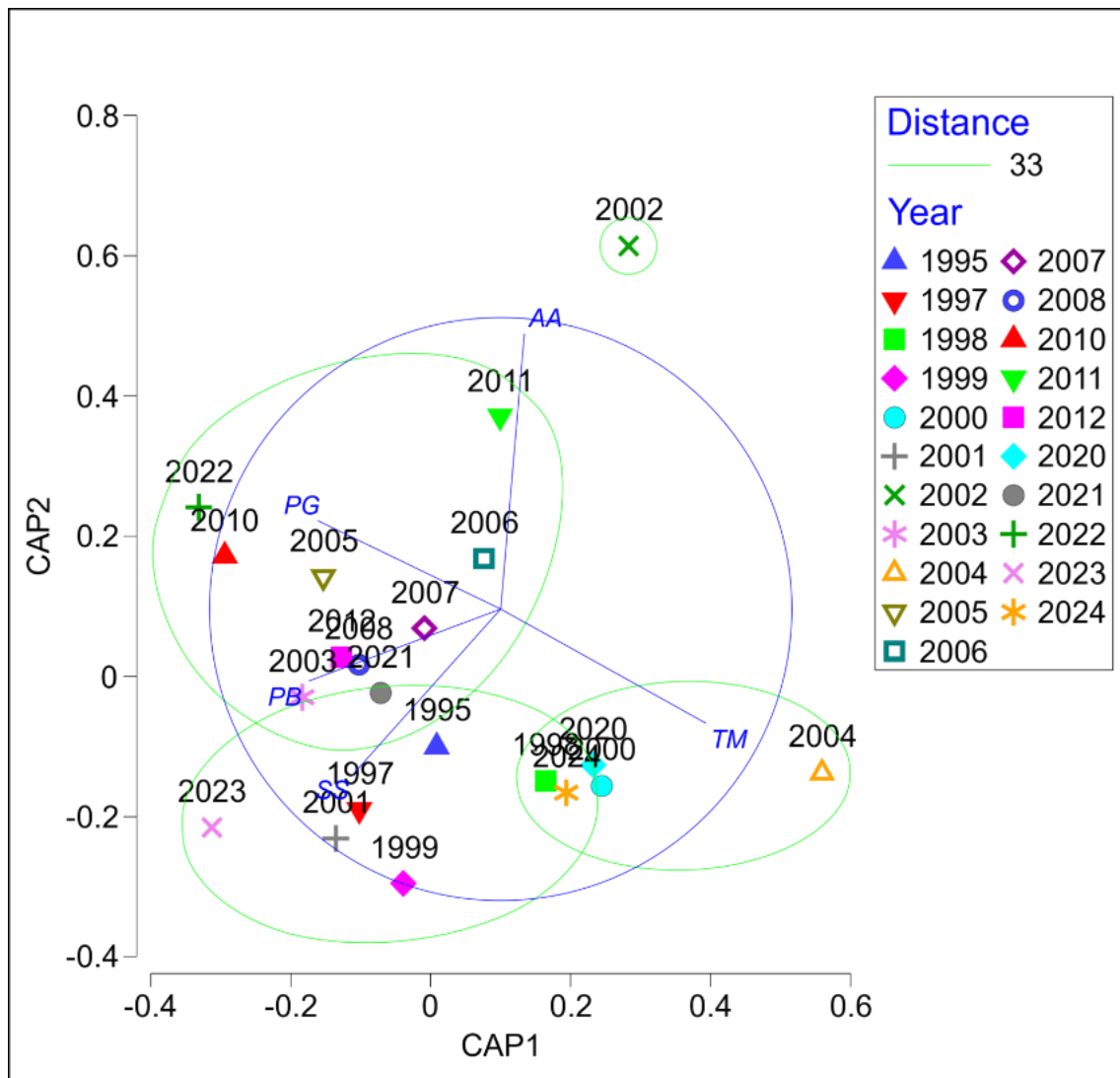


Figure 5.9. Canonical Analysis of Principal Coordinates (CAP) ordination plot of fish community structure in *Ecological Zone 5* based on the centroid resemblance matrix. Each point represents the general location of community structure in that year. Blue vectors indicate the main taxa contributing to differences among years, as identified by SIMPER analysis, while the green circles indicate similar groupings of community structure.

Table 5.7. Average relative abundances by cluster of the main taxa identified by SIMPER analysis as contributors to temporal differences in community composition within Ecological Zone 5. Cluster 1 includes year: 2003, 2005, 2006, 2007, 2008, 2010, 2011, 2012, 2021, 2022. Cluster 2: 1995, 1997, 1999, 2001, 2023, 2024. Cluster 3: 1998, 2000, 2004, 2020.

	AA	SS	PG	TM	PB
C1	0.330	0.587	0.311	0.083	0.1856
C2	0.059	0.791	0.112	0.108	0.174
C3	0.107	0.523	0.129	0.343	0.104
2002	0.733	0.396	0.071	0	0

The RELATE test resulted significantly (Spearman’s $Rho = 0.133$, $P < 0.05$), between the environmental variables and fish community while the distLM sequential test showed that temperature and evapotranspiration were the two statistically significant environmental parameters (SI Ch.5, Table S5). Temperature had a strong, negative association with the first and second axes. Evapotranspiration was highly correlated with the first axis and negatively correlated with the second axis. The first axis alone explained 11.92% of the total variance out of 13.28% (SI Ch.5, Table S9), (see also Methods for explanation of differences between numerical results and dbRDA figures based on yearly averages).

In the dbRDA graphs (Fig. 5.10), the left panel shows the significant environmental variables identified in the sequential test (SI Ch.5, Table S5), while the right panel shows the species most correlated with community structure in relation to environmental parameters. Specifically, TM which appeared in both the dbRDA vector projections and the SIMPER (SI Ch.5, Table S13), was the taxa most strongly associated with temperature and evapotranspiration variations.

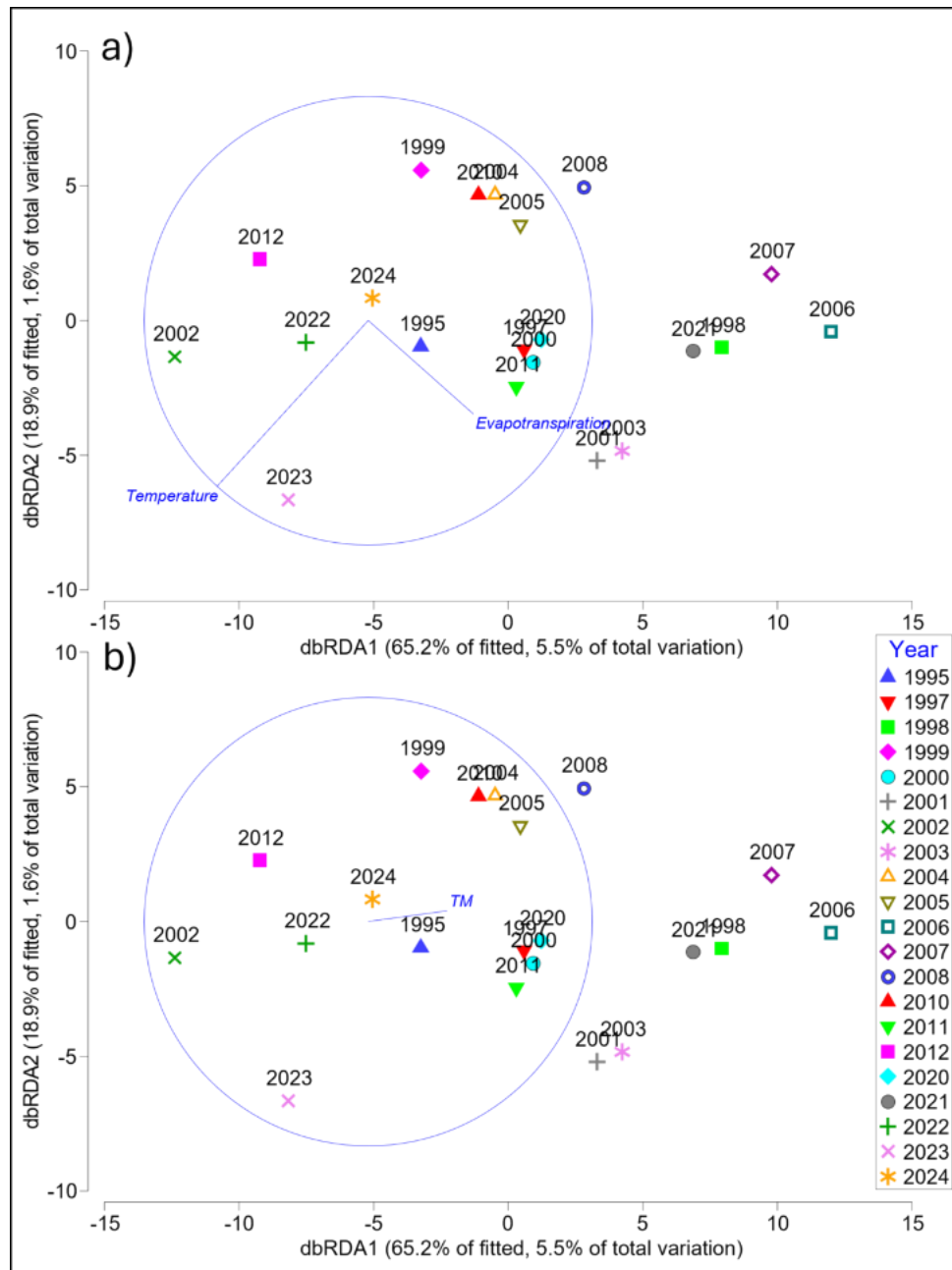


Figure 5.10. Distance-based redundancy analysis (dbRDA) of fish community structure in Ecological Zone 5. The ordination is based on Bray–Curtis resemblance of square-root transformed yearly centroid abundance data, matched to yearly average environmental variables. Panel a) shows the fitted vectors of environmental parameters, while panel b) displays the projection of the most influential species, identified both by a strong correlation (TM 35%) with environmental gradients and as a top contributor to community change in SIMPER.

Discussion

This study investigated, for the first time, how the composition of fish communities in the eastern river basins of Emilia-Romagna changed over the last 30 years in relation to environmental changes, using three main variables identified as possible drivers of these dynamics: temperature, evapotranspiration, and precipitation. Temporal, spatial, and environmental drivers of community variation were disentangled across multiple ecological zones. Overall, the analyses revealed significant temporal dynamics, heterogeneous responses across ecological zones, and varying degrees of vulnerability of certain species to environmental and climatic changes that have important implications for freshwater biodiversity conservation.

As confirmed by the initial PERMANOVA analysis, which considered the years with the greatest overlap among zones, and by subsequent PERMANOVA analyses of individual zones, the factor Year significantly influenced fish community composition across most ecological zones. However, the intensity and direction of these changes varied: while some zones showed pronounced shifts in species structure over time, others remained comparatively stable, highlighting heterogeneous patterns of vulnerability within zones.

The basin-level PERMANOVA revealed no significant differences in community structure among the three basins tested. These results should be viewed with caution, as the test was limited to three basins and earlier years, but it nonetheless represents the most robust comparison available and indicates that ecological zones, rather than basins, are the dominant spatial unit driving community variation.

In Zone 1, the PERMANOVA analysis indicated that the Year factor explained a very high proportion of variance. However, this value, as well as the entire analysis of this Zone, should be treated with caution, as the low number of samples and the absence of replicates over several years may affect the solidity of these results. Subsequent analyses suggested that *Salmo trutta* complex (SX) remained the dominant species throughout the monitoring period; however, in recent years, an increase in the number of *Telestes muticellus*TM became apparent, indicating a gradual reorganisation of the community. *Padogobius 112onelli* (PB) was also present in the early years, as well as in warmer years, indicating a possible signal of a progressive expansion of tolerant species to higher temperatures and hydrological variability. In contrast, *Cottus gobio* (CG) appeared to be more strongly associated with colder periods, following a trend opposite to that of TM and PB. From an ecological perspective, these patterns highlight the role of temperature in shaping the community: SX and CG thrive in colder conditions (Fortini, 2018; Hari et al., 2006), while TM and PB increase with warming, corroborating broader evidence that temperature warming promotes the expansion of warm-tolerant species (Carosi et al., 2019; Reid et al., 2019). This suggests the onset of an altitudinal shift, with cold-water species increasingly forced to seek refuge in higher altitude zones, while more tolerant taxa gradually extend their range into zones previously dominated by salmonids.

Moving on Zone 2, temporal variation was still evident, although it occurred to a lesser extent compared with the other zones. Even in this zone *Salmo trutta* complex (SX) remained the most abundant species in the early years but its dominance gradually declined, with *Squalius squalus* (SS) and *Telestes muticellus*TM becoming more abundant in recent years. Even *Barbus plebejus* (BP) and *Protochondrostoma genei* (PG) became more abundant in recent years and contributed more to wetter years, consistent with their rheophilic ecology (Fortini, 2018). *Padanogobius lizonelli* (PB) contributed steadily across the time series without marked peaks, suggesting a microhabitat-driven, relatively stable presence rather than a strong climatic signal. Among environmental drivers, both temperature and precipitation were significant: precipitation was positively associated with rheophilic species (notably BP and PG), whereas higher temperatures corresponded to reduced dominance of salmonids (SX) and a relative increase in generalist species such as SS and TM. Overall, SS exhibited an intermediate behaviour along environmental gradients, confirming its generalist nature and its ability to adapt to both warming and hydrological variability (Pompei et al., 2011).

Zone 3 showed significant interannual variation, with the Year factor accounting for a large part of these changes in the communities. *Salmo trutta* complex (SX) was strongly associated with the earliest years, confirming its role as a dominant cold-water species (Hari et al., 2006; Fortini, 2018). In contrast, *Squalius squalus* (SS) and *Telestes muticellus* (TM) increased their abundances in more recent years, marking a community shift toward more tolerant cyprinids. *Barbus plebejus* (BP) and *Protochondrostoma genei* (PG) contributed at lower to intermediate levels throughout the time series, without clearly defining temporal clusters. The *Padogobius bonelli* (PB) also increased in more recent years. Regarding environmental variables, all three factors: temperature, precipitation, and evapotranspiration were significantly correlated with species distribution. This helps us understand how this zone is highly heterogeneous, not only because of its ecologically diverse assemblage, but also due to its transitional riverine characteristics, shifting from those typical of mountainous sectors to those of lowland plains. Such heterogeneity likely explains why this is the only zone where all three environmental variables were significant, as its transitional nature makes it more sensitive to multiple drivers of change. While Hari et al. (2006) provided evidence that trout populations decline under warmer conditions, the negative association with wetter years in our data may reflect indirect stressors linked to altered hydrology. Increased precipitation can lead to more frequent floods, substrate mobilization, turbidity, or habitat instability, which disproportionately affect cold-water salmonids (Gallagher et al., 2022), and this aligns with observations from other Mediterranean rivers, where high peak-flow events during egg incubation and fry emergence reduced the recruitment success of *Salmo trutta* (Sánchez-Hernández & Nunn, 2016).

SX was also positively aligned with increasing evapotranspiration, which could reflect indirect links between climatic water demand, reduced flows, and habitat contraction. However, no specific studies have addressed this relationship, so this interpretation should be considered with caution. In contrast, PB was associated

with more recent years, indicating an increase in abundance under recent climatic conditions. Although ecological information on PB remains limited, some studies have explored aspects of its feeding ecology and the influence of hydrological variability on population traits. These findings suggest that fluctuating flows may restrict movement and habitat exploration, potentially affecting growth and prey availability (Bo et al., 2011). Similarly, research on the congeneric *Padogobius nigricans* in Central Italy showed limited dispersal ability and no significant upstream shift under warming. Therefore, there are likely other dynamics not assessed in our study that are linked to its increase in abundance and deserve attention in further studies. Much more defined is the altitudinal expansion of rheophilic cyprinids such as TM (Carosi et al., 2019). More broadly, these dynamics are consistent with global evidence that cold-water salmonids are being progressively constrained to upstream refugia, while taxa more tolerant to higher temperatures and hydrological variability expand in lowland habitats (Reid et al., 2019).

In the first three zones, the increase in average annual temperatures and the decrease in rainfall provide evidence that climate change has had a direct impact on the basins in our study and, consequently, on the fish communities that live there. In particular, the increase in temperature may be related to changes in the spatial distribution of the species considered in our study (Carosi et al., 2019; Lorenzoni et al., 2006; Franchi et al., 2014). This certainly opens new scenarios, as species moving upstream along these rivers could encounter more frequent droughts, given the reduced size of the rivers in mountainous zones and the decrease in average annual rainfall, with the consequent possibility of local extinctions (Carosi et al., 2019; Lorenzoni et al., 2014).

Zone 4 exhibited high interannual variability, with no significant correlation between environmental variables and community composition RELATE test. The assemblage was characterized by irregular fluctuations in species contributions; indeed, *Salmo trutta* complex (SX) was present in some early years, but it underwent a rapid decline that led to its near disappearance in the following years. In contrast, *Squalius squalus* (SS) emerged as the most representative taxon of this zone, maintaining high abundance across both earlier and more recent years. This continuity highlights its generalist nature and ability to persist under diverse local conditions (Pompei et al., 2011). *Barbus plebejus* (BP), *Protochondrostoma genei* (PG), and *Telestes muticellus* (TM) contributed at intermediate and fluctuating levels, without defining clear temporal trends. Overall, the fish community of Zone 4 appears to be heterogeneous and unstable, shaped more by local habitat characteristics and stochastic interannual variability than by broad climatic drivers, with SS acting as the dominant and persistent element of this zone. One possible explanation for these results is that additional factors, not included among our variables, may have influenced communities in this zone. The rivers in these stretches are close to or even cross anthropized areas that are often home to agriculture or factories, which could have a much greater impact on fish communities than the environmental variables considered here.

Zone 5 displayed the weakest temporal signal among all zones, with only ~13% of community variation explained by the Year factor, confirming the relative stability of this assemblage over the three decades of monitoring. The most abundant species was *Squalius squalus* (SS), which consistently represented the main contributor across the entire time series. Its persistent abundance highlights its ecological generalism and ability to thrive under a wide range of local conditions. Other species contributed at lower levels: *Alburnus alburnella* (AA) displayed a remarkable peak in 2002, which appears to be an isolated event rather than a long-term trend; *Telestes muticellus* TM and *Protochondrostoma genei* (PG) showed modest and variable contributions, while *Padogobius 115onelli* (PB) remained consistently minor. Overall, this assemblage was characterized by stability and the dominance of the generalist taxon SS (Pompei et al., 2011), with secondary species fluctuating without establishing clear temporal trajectories. From the environmental perspective, Zone 5 showed only a weak association between species distribution and climatic variables. In the dbRDA, only TM was projected, indicating that it was the only species driving community change and displaying a detectable relationship with the environmental gradients. However, the overall explanatory power of temperature and evapotranspiration was low, suggesting that climatic drivers have limited influence on the structure of this assemblage. This weak signal is likely related to the downstream position of Zone 5, likely also at play in Zone 4, where stronger anthropogenic pressures (e.g. water pollution, hydromorphological alteration, flow regulation) and cumulative impacts from upstream catchments may overshadow climate-related trends. In addition, several studies have shown that changes in fish communities are related to abiotic and biotic alterations in ecosystems, such as habitat degradation, introduction of exotic species, and anthropogenic modifications of watercourses or flow regimes (Jackson et al., 2001; Bernardo et al., 2003; Gido et al., 2010). As a result, community composition in Zone 5 appears to be shaped more by local stressors than by regional environmental gradients.

The multivariate analyses conducted in this study described and confirmed the influence of climate change on fish communities within the basins of Emilia-Romagna. Among the environmental variables considered, temperature emerged as the parameter with the greatest influence, especially in Zones 1–3 at medium and high altitude. In these zones, dbRDA analyses showed a negative correlation between temperature increase and the presence of cold-water taxa (e.g., *Salmo trutta* complex, *Cottus gobio*), while tolerant cyprinids such as *Telestes muticellus* and *Squalius squalus* increased in abundance during warmer years. In our dataset, the progressive decline of the *Salmo trutta* complex in lowland and warmer reaches confirmed the sensitivity of salmonids to increasing temperatures. Similar results have been documented in central Spain, where recruitment success of young-of-the-year trout was negatively affected by rising temperatures and by peak-flow events during the fry emergence period (Sánchez-Hernández & Nunn, 2016). More broadly, salmonids are recognized as one of the most vulnerable groups under global warming scenarios, with expected contractions of their distribution ranges in Mediterranean rivers (Filipe et al., 2013). Precipitation and evapotranspiration also contributed to community

structuring, but their effects were more variable and often secondary to temperature. In Zone 2, precipitation appeared to interact with rheophilic cyprinids (e.g., *Barbus plebejus*, *Protochondrostoma genei*), which tended to increase in wet years, consistent with their preference for flowing habitats with higher discharge (Sánchez-Hernández & Nunn, 2016). Conversely, evapotranspiration played a significant role in Zone 3, highlighting how hydrological stress can compound the effects of warming. Our findings are corroborated by evidence of upstream range shifts of *Telestes muticellus* and *Squalius lucumonis* in the Tiber River basin, where both species expanded their distribution by 30–40 m in elevation over the last three decades to track their thermal optimum (Carosi et al., 2019). This trend is consistent with predictions that climate warming will progressively favour thermophilic cyprinids over cold-water taxa in Mediterranean catchments (Filipe et al., 2013). In contrast, Zone 4 exhibited weak and inconsistent associations with climatic drivers. Here, high inter-annual turnover suggested the prevalence of local or stochastic factors, possibly related to habitat alteration, flow regulation, or land-use pressures. Finally, Zone 5 showed almost no detectable response to climatic gradients. Instead, species distributions appeared largely decoupled from environmental parameters, with *Squalius squalus* dominating consistently and only *Telestes muticellus* showing some association with temperature. This suggests that anthropogenic pressures and water quality degradation in downstream sectors may override or mask the effects of climate, limiting the ability to detect clear ecological signals (Carosi et al., 2019).

Overall, our results fit well within the Mediterranean framework: cold-water salmonids are declining, while rheophilic cyprinids are expanding under warmer and more variable hydrological regimes, and an altitudinal shift is emerging as a common response. However, the persistence of these patterns is strongly modulated by local anthropogenic disturbances, which may mask climatic signals and exacerbate community vulnerability.

These findings reinforce the importance of maintaining standardised long-term surveys of freshwater fish communities to detect ecological shifts in response to changing environmental conditions. Other Italian and European studies (Abbà et al., 2024; Marchi et al., 2023; Friedrichs-Manthey et al., 2024) demonstrate how sustained observation efforts can reveal large-scale ecological trends and climate-related changes in community composition. Maintaining coherent, long-term monitoring networks is therefore essential to support species conservation and preserve the ecological integrity of freshwater systems in Italy and across Europe.

Future research should aim to strengthen the link between climatic and environmental drivers and ecological responses by integrating additional hydrological data with existing long-term biological datasets. It would also be desirable to extend similar approaches to other Italian river basins and native fish species in order to gain a broader understanding of freshwater ecosystem responses across spatial and temporal scales, and to identify potential indicators of climate-driven ecological change that can support adaptive conservation strategies under future scenarios.

Supplementary Information for Chapter 5

Table S1. Number of transects per year within each ecological zone.

Year	Ecological Zone 1	Ecological Zone 2	Ecological Zone 3	Ecological Zone 4	Ecological Zone 5
1995	0	1	1	1	3
1996	4	0	0	0	0
1997	3	1	2	7	1
1998	5	18	15	19	7
1999	2	3	8	13	5
2000	2	4	7	19	5
2001	1	6	5	7	4
2002	0	6	2	14	2
2003	3	9	11	18	13
2004	3	2	12	2	3
2005	0	7	10	9	5
2006	2	8	11	6	4
2007	2	6	10	4	3
2008	2	7	8	3	3
2009	0	0	4	0	0
2010	1	1	7	1	3
2011	0	0	2	2	8
2012	0	1	3	3	4
2019	1	2	2	4	0
2020	0	0	2	2	2
2021	2	0	3	3	3
2022	2	1	2	3	2
2023	1	2	2	5	1
2024	1	0	5	8	2

Section 1. DistLM sequential tests

The following tables report the results of DistLM sequential tests for each Ecological Zone (1–5). Variables were added sequentially (forward selection), with each variable explaining additional variation after accounting for those already included. Significant predictors ($p < 0.05$) are highlighted in bold. DistLM analyses were performed on the complete sample dataset, whereas dbRDA figures in the main Results were based on yearly averaged distance matrices of environmental parameters and community centroids.

Table S2. Results of DistLM Sequential Tests for *Ecological Zone 1*. The analysis was based on Bray–Curtis resemblance of square-root transformed abundance data. Significant predictors ($p < 0.05$) are reported in bold.

Sequential test							
Variable	R ²	SS (trace)	Pseudo-F	P	Prop.	Cumul.	Res.df
Temperature	0.15862	7505.3	6.5982	0.004	0.15862	0.15862	35
Evapotranspiration	0.15796	-30.91	-0.026377	0.997	-0.000065325	0.15796	34
Precipitation	0.17812	953.73	0.8093	0.401	0.020156	0.17812	33

Table S3. Results of DistLM Sequential Tests for *Ecological Zone 2*. The analysis was based on Bray–Curtis resemblance of square-root transformed abundance data. Significant predictors ($p < 0.05$) are reported in bold.

Sequential test							
Variable	R ²	SS (trace)	Pseudo-F	P	Prop.	Cumul.	Res.df
Temperature	0.035968	6842.1	3.0967	0.0386	0.035968	0.035968	83
Evapotranspiration	0.058157	4220.8	1.9318	0.1375	0.022188	0.058157	82
Precipitation	0.096572	7307.5	3.4442	0.0248	0.038415	0.096572	81

Table S4. Results of DistLM Sequential Tests for *Ecological Zone 3*. The analysis was based on Bray–Curtis resemblance of square-root transformed abundance data. Significant predictors ($p < 0.05$) are reported in bold.

Sequential test							
Variable	R ²	SS (trace)	Pseudo-F	P	Prop.	Cumul.	Res.df
Temperature	0.074034	21009	10.554	0.0001	0.074034	0.074034	132
Evapotranspiration	0.12802	15320	8.1101	0.0004	0.053984	0.12802	131
Precipitation	0.16281	9873	5.4024	0.0022	0.034791	0.16281	130

Table S5. Results of DistLM Sequential Tests for *Ecological Zone 5*. The analysis was based on Bray–Curtis resemblance of square-root transformed abundance data. Significant predictors ($p < 0.05$) are reported in bold.

Sequential test							
Variable	R ²	SS(trace)	Pseudo-F	P	Prop.	Cumul.	Res.df
Temperature	0.064434	7826.4	5.5098	0.0006	0.064434	0.064434	80
Evapotranspiration	0.12004	6754.1	4.9921	0.001	0.055606	0.12004	79
Precipitation	0.13281	1551.4	1.1488	0.3306	0.012772	0.13281	78

Section 2. dbRDA results

This section reports the results of the distance-based redundancy analyses (dbRDA) for ecological zones 1-3 and 5. Ecological zone 4 is not included as the Relate test resulted insignificantly. Environmental parameters significantly correlated with community structure variance are shown in bold. The tables summarize the associations between environmental variables and the principal axes, including the percentage of explained variance and the direction of correlation.

Table S6. Results of the distance-based redundancy analysis (dbRDA) for *Ecological Zone 1*. The upper section reports the correlations between environmental variables and the dbRDA axes (multiple partial correlations), while the lower section shows the percentage of variance explained by each axis, both relative to the fitted model and to the total variation.

Relationships between dbRDA coordinate axes and orthonormal X variables (multiple partial correlations)ei					
Variable	dbRDA1		dbRDA2		dbRDA3
Temperature	0.961		0.260		0.092
Evapotranspiration	0.070		0.093		-0.993
Precipitation	-0.266		0.961		0.071
Percentage of variation explained by individual axes					
	% explained variation out of fitted model		% explained variation out of total variation		
Axis	Individual	Cumulative	Individual	Cumulative	
Axis 1	91.56	91.56	16.31	16.31	
Axis 2	10.51	102.07	1.87	18.18	
Axis 3	-2.07	100	-0.37	17.81	

Table S7. Results of the distance-based redundancy analysis (dbRDA) for *Ecological Zone 2*. The upper section reports the correlations between environmental variables and the dbRDA axes (multiple partial correlations), while the lower section shows the percentage of variance explained by each axis, both relative to the fitted model and to the total variation.

Relationships between dbRDA coordinate axes and orthonormal X variables (multiple partial correlations)				
Variable	dbRDA1		dbRDA2	
Temperature	-0.680		-0.730	
Evapotranspiration	0.394		-0.279	
Precipitation	-0.619		0.624	
Percentage of variation explained by individual axes				
	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
Axis 1	91.91	95.91	9.26	9.26
Axis 2	5.31	101.21	0.51	9.77
Axis 3	-1.21	100	-0.12	9.66

Table S8. Results of the distance-based redundancy analysis (dbRDA) for *Ecological Zone 3*. The upper section reports the correlations between environmental variables and the dbRDA axes (multiple partial correlations),

while the lower section shows the percentage of variance explained by each axis, both relative to the fitted model and to the total variation.

Relationships between dbRDA coordinate axes and orthonormal X variables (multiple partial correlations)				
Variable	dbRDA1		dbRDA2	
Temperature	-0.647		-0.261	
Evapotranspiration	0.705		-0.561	
Precipitation	-0.289		-0.786	
Percentage of variation explained by individual axes				
	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
Axis 1	84.59	84.59	13.77	13.77
Axis 2	13.5	98.08	2.20	15.97
Axis 3	1.92	100	0.31	16.28

Table S9. Results of the distance-based redundancy analysis (dbRDA) for *Ecological Zone 5*. The upper section reports the correlations between environmental variables and the dbRDA axes (multiple partial correlations), while the lower section shows the percentage of variance explained by each axis, both relative to the fitted model and to the total variation.

Relationships between dbRDA coordinate axes and orthonormal X variables (multiple partial correlations)				
Variable	dbRDA1		dbRDA2	
Temperature	-0.629		-0.775	
Evapotranspiration	0.763		-0.600	
Precipitation	-0.150		0.199	
Percentage of variation explained by individual axes				
	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
Axis 1	89.78	89.78	11.92	11.92
Axis 2	8.71	98.49	1.16	13.08
Axis 3	1.51	100	0.20	13.28

Section 3. SIMPER detailed tables

The following tables report the results of SIMPER (Similarity Percentage) analyses performed within each Ecological Zone (1–5). SIMPER was applied to Bray–Curtis resemblance matrices calculated on square-root transformed species abundances, with groups defined by year. For each zone, taxa are ranked according to their average percentage contribution to between-year dissimilarities, with values indicating the relative influence of each species on temporal community variation.

Detailed tables include all taxa identified by SIMPER, including those with lower contributions, in order to provide a complete overview of species-level influences on temporal patterns of community structure.

Table S10. SIMPER results for Ecological Zone 1. Taxa are ordered by their average percentage contribution to between-year dissimilarities (Bray–Curtis resemblance, square-root transformed abundances).

Species	Average Contribution %
CG	64.1
PB	38.7
SX	34.5
BP	19.6
SS	16.5

Table S11. SIMPER results for Ecological Zone 2. Taxa are ordered by their average percentage contribution to between-year dissimilarities (Bray–Curtis resemblance, square-root transformed abundances).

Species	Average Contribution %
SX	24.8
TM	22.7
SS	21.5
PG	20.0
BP	16.5
PB	14.1

Table S12. SIMPER results for Ecological Zone 3. Taxa are ordered by their average percentage contribution to between-year dissimilarities (Bray–Curtis resemblance, square-root transformed abundances).

Species	Average Contribution %
SX	23.2
TM	25.1
SS	21.7
PG	19.2
BP	17.1

Table S13. SIMPER results for Ecological Zone 5. Taxa are ordered by their average percentage contribution to between-year dissimilarities (Bray–Curtis resemblance, square-root transformed abundances).

Species	Average Contribution %
AA	21.8
SS	17.8
PG	19.2
TM	16.2
PB	14.9

Chapter 6

Eight years of functional trait community monitoring at the SS Thistlegorm wreck (Red Sea)

Abstract

Coral reef ecosystems, including artificial reefs like the SS Thistlegorm in the Red Sea, play a critical role in supporting biodiversity and ecological functions. This study examined the functional trait community variance over eight years using data collected through the Scuba Tourism for the Environment recreational citizen science program, comparing these findings to previously studied taxonomic community variance. Using multivariate analysis of selected traits, the results reveal oscillatory patterns in functional trait variance, highlighting a relative stability of this artificial reef ecosystem over time. Despite changes in environmental circumstances, the functional trait community demonstrated a capacity for quicker recovery, underscoring its resilience. The traits that emerged as the main drivers of community variance through time were the morphological form massive-erect, colonial growth, large-group gregariousness, sexual reproduction and the potential for asexual reproduction, the size class 2, poisonous defense mechanisms, filter feeding diet, and pelagic mobility. By leveraging citizen science data, this research provides insights into the long-term functional dynamics of artificial reef ecosystems, contributing to broader efforts in reef conservation and management.

Introduction

Coral reefs are among the most biologically diverse systems on the planet. While only occupying 1% of the ocean they host approximately 25% of its species, providing critical habitat, nursery grounds, and feeding areas (McAllister et al. 1994). Beyond the inherent ecological significance, they also provide an array of ecosystem services to human communities including coastal protection, fisheries, and tourism valued to an estimated \$375 trillion per year (Constanza et al. 1997). However, these ecosystems are increasingly under threat due to climate change, overfishing, and pollution, which have resulted in widespread coral bleaching, declines in biodiversity, and reduced reef resilience (Hughes et al. 2017). To understand the health, dynamics, and potential threats to these ecosystems, long-term monitoring is critical. Coral reef monitoring programs have provided key insights into the impacts of human-induced environmental stressors such as climate change, ocean acidification, overfishing, and coastal development. Such programs, like the Global Coral Reef Monitoring Network (GCRMN), have not only proven invaluable in tracking changes in detecting trends and potential threats through biodiversity and species tracking (Wilkinson 2004), but they also provide a key tool for comparing the success of various management strategies such as MPAs or restoration efforts by providing clear insights as to how reef ecosystems respond to such interventions through time (Dunham et al. 2017). As coral reefs face increasingly significant and severe disturbances, robust and ongoing monitoring becomes crucial to ensure their resilience and survival.

The Red Sea offers a unique region for coral reef research, with its reefs being among the most resilient in the world (Fine et al. 2013; Kleinhaus et al. 2020). These reefs are characterized by high levels of endemism and the ability to thrive under stressful environmental conditions, such as elevated salinity and sea surface temperatures (Fine et al. 2013), making the Red Sea a critical natural laboratory for studying coral reef dynamics and the responses of reef ecosystems to environmental stressors. Despite its importance, however, the region remains relatively understudied compared to more well-known systems like the Great Barrier Reef and the Caribbean (Berumen et al. 2019). In this context, artificial reefs, such as the SS Thistlegorm, provide valuable insights into reef ecology by contributing to biodiversity through increased habitat complexity, fish abundance, and overall habitat availability, while also serving sociocultural goals (Higgins et al. 2022; Lee et al. 2023). As a historic World War II shipwreck located in the northern Red Sea, the Thistlegorm has not only become a vibrant artificial reef but it is also a significant cultural and economic asset due to its global popularity as a recreational dive site (Brown et al. 2020). The taxonomic community structure of the SS Thistlegorm and its variation throughout eight years of monitoring, indicate a thriving and well-established community on this artificial reef (Lee et al. 2023; Chapter 1).

While taxonomic analyses have provided important insights into “who” inhabits these ecosystems and how they drive community variance through time (Lee et al. 2023), they offer a limited understanding of “what” functions these organisms perform and how these functions interact or change over time. Functional trait ecology

addresses this gap by using trait-based approaches to establish critical links between biological communities and the abiotic components shaping ecosystem dynamics (McGill et al. 2006). Emerging as a scientific discipline in the latter half of the 20th century, functional ecology gained prominence in the early 2000s, as researchers recognized its potential to uncover novel insights into ecosystem functioning and resilience (McGill et al. 2006; Petchey & Gaston, 2006).

On coral reefs, functional traits of key groups such as reef fish and corals play a pivotal role in determining ecosystem structure and function. For instance, coral traits such as colony growth forms, skeletal density, and reproductive modes can influence habitat complexity as well as reef recovery potential (Darling et al. 2012; Graham and Nash 2013; Darling et al. 2017). Similarly, fish traits like feeding guilds, body size, and nutrient cycling can dictate, algal control, and energy transfer across trophic levels (Brandl et al. 2019). Applied to artificial reefs like the SS Thistlegorm, this approach could help uncover how functional roles are distributed within the community and how these roles vary through time. The Thistlegorm harbors a complex assemblage of corals and reef-associated fish (Lee et al. 2023), and understanding the drivers of functional change, whether linked to species additions, losses, or shifts in dominant traits is essential in predicting the long-term viability.

Building on previous work, this study takes a functional trait-based approach to examine long-term changes in the functional community structure of the SS Thistlegorm. Rather than directly linking functional traits to specific ecosystem processes, this research focuses on analyzing how the functional composition of the artificial reef community has varied over time. By identifying which functional traits are driving these temporal shifts, this study aims to uncover patterns of change in the functional roles of the resident species. Such an approach provides valuable insights into the stability, adaptability, and potential vulnerabilities of artificial reef communities in response to temporal and environmental pressures. Furthermore, this work contributes to a broader understanding of how artificial reefs, like the Thistlegorm, can support functional diversity and ecosystem resilience under changing marine conditions.

Materials and Methods

Data collection and isolation

The Scuba Tourism for the Environment—Red Sea Biodiversity Monitoring Program (www.steproject.org) involved the collection of extensive spatial-temporal biodiversity data in the Red Sea from 2007 to 2015 using a recreational citizen science approach where the behavior of underwater tourists remained unchanged during surveys (Branchini et al. 2015). Volunteer participants filled out questionnaires providing general demographic information (name, country of residence, address, diving certification, etc.), dive site and environment type (sandy bottom, wreck, open water, or coral reef), six diving parameters (year (PY), maximum depth (MD), depth most

of their time was spent at (AD), temperature (T), dive time (RTD), and time of day (FD)), and sightings of 72 target taxa which were recorded on the questionnaires immediately following the dive (Branchini et al. 2015). For each taxon sighted, divers also recorded an estimated abundance using three classes: 1, 2, and 3 (rare, frequent, and very frequent, respectively). These classes were weighted according to the taxon's individual expected occurrence. The 72 faunal taxa were selected because they 1) represent the main ecosystem trophic levels in the Red Sea, 2) are common/abundant, and 3) are easily identifiable by recreational divers (Branchini et al. 2015). Data collected by volunteers from the Thistlegorm wreck during the project were isolated and then aggregated by year.

Functional Trait Assessment

Similarly to other studies that have applied functional groupings in both sessile benthic organisms (Teixido et al. 2018) and reef fishes (Stuart-Smith et al. 2013; Denis et al. 2019), the selected taxa were assigned functional traits based on 8 different functional groups: size, diet, gregariousness, period of activity (foraging), position in the water column, defenses, potential of asexual reproduction, and morphological form (SI Ch.6, Table S1). Size was assigned based on 4 different size classes (class 1 from 1 to 9 cm; class 2 from 10 to 50 cm; class 3 from 51 to 100 cm; and class 4 from 101 to 300 cm). Taxa were assigned diets based on being either filter feeders, mixotrophic (mainly symbiotic coral), carnivores, herbivores/grazers/omnivores, invertivores, or piscivores. Colonial, solitary (sessile invertebrate), solitary (mobile), small groups, and large groups were assigned to taxa based on gregariousness. Taxa were either strictly nocturnal, strictly diurnal, or diurnal-nocturnal in their periods of activity. Sessile, benthic, benthopelagic, or pelagic were assigned for a taxa's position in the water column. Taxa defenses were classified as being without, calcareous spicules and sclerites, venomous, external carbonate, external shell, poisonous, or electric. As for reproduction, taxa were labeled as either having the ability to reproduce asexually (including those that also produce sexually), or not. The morphology of taxa could be branched, foliose-erect thallus (sheets/blades), tree-like, massive-erect, worm-like, slug-like, bivalve, fusiform, articulated, star-like, spherical, filiform, compressiform, or depressiform.

All functional traits were assigned based on extensive research through Fish Base (www.fishbase.se), Sea Life Base (www.sealifebase.se), and literature. Trait assignments were primarily based on Fish and Sea Life Base, then, if information was not available a literature review.

Preliminary treatment and analyses

To assess the quality of volunteer-collected data, validation trials were performed in the previous analysis on dives within the Thistlegorm dataset (details on the parameters tested and results are available in Lee et al. 2023, and the standard methodology is described in Meschini et al. 2021).

Much of the preliminary work conducted on the yearly data of the SS Thistlegorm was completed in the previous study where the annual taxa community structure was analyzed (Lee et al. 2023). In the current study, we retained this filtering method to ensure consistency. Using Primer-e software (Clarke and Gorley 2015) the original taxa community matrices were converted into functional trait matrices by assigning indicator values to each taxon. Trait counts were then summed for each sample, based on the presence and abundance of each taxon in the corresponding questionnaire (sample). Both abundance and presence/absence matrices were analyzed, with yearly non-metric multidimensional scaling (MDS; Clarke and Gorley 2015) plots used to visualize the trait community. BVSTEP BEST tests (Clarke and Gorley 2015) were conducted for each year to identify the traits most influential in explaining community variance. For simplicity, traits that were not identified as "Best" results for community variance in any year were excluded from the temporal analysis.

Temporal Analysis

The yearly trait community matrices were merged for the temporal analysis, and two datasets were elaborated: one based on abundance and the other on presence/absence of traits throughout the study. Both matrices were standardized by samples to account for relative differences in the composition of functional traits across years. This approach addresses the potential for some traits to be highly repetitive among taxa (e.g., potential for asexual reproduction) or, conversely, highly specific to certain taxa (e.g., an electrical defense mechanism). By standardizing across samples rather than variables, the analysis emphasizes the relative contribution of traits within each year rather than giving all traits equal weight (Clarke and Gorley 2015). This provides a more nuanced view of the functional trait community structure over time.

A Euclidean distance matrix (Euclidean distance; Clarke and Gorley 2015) was then created from the normalized diving parameters for testing whether community structure was primarily driven by temporal changes rather than diving parameters. To test this, two Relate tests (RELATE; Clarke and Gorley 2015) were performed between the diving parameter matrix and the two trait matrices. The first was an overall RELATE test, while the second examined the relationship between the datasets within the levels of the factor "Year".

To test for significant differences among the years, two Permutational Multivariate Analyses of Variance (PERMANOVA; Clarke and Gorley 2015) were conducted for each trait matrix, with "Year" as the factor (8 levels: 2007-2014), applied to the corresponding Bray-Curtis similarity matrices (Bray-Curtis similarity; Clarke and Gorley 2015). Both the main test and post-hoc pairwise comparisons were performed. To visualize yearly variance in community structure, MDS plots were generated using centroid distances for the "Year" factor. Significantly similar year groupings were identified through hierarchical clustering using SIMPROF, and the traits most influential to community variance were determined with the BIOENV BEST procedure (Clarke and Gorley 2015). These traits and yearly clusters were then overlaid onto the MDS plots to illustrate the inter-year community trait dynamics of the SS Thistlegorm.

Results

Data collection and isolation

The results for this section of the methods are consistent with the previous analysis. The year 2015 was also excluded due to not meeting the minimum quota of 10 questionnaires per year, deemed necessary for a sufficient sample size based on validation trials conducted across the entire STE project and at the SS Thistlegorm wreck site (Meschini et al. 2021; Lee et al. 2023). The table showing the number of questionnaires per year and the significant decline over time can be found in Supplementary Information Chapter 6 (Table S2).

Functional trait assessment

Of the 72 target taxa included in the STE project (Branchini et al. 2015), only 61 were incorporated into the analyses of this study. Taxa listed as "Other..." (e.g., Other Fish or Other Sea Urchins) were excluded due to the difficulty of assigning specific traits to such broad taxonomic groups. For groups larger than the species level, size was assigned based on the largest species native to the Red Sea within the group, while other functional characteristics were generally consistent throughout. The complete list of taxa and their assigned traits can be found in the supplementary materials (SI Ch. 6, Table S1). Fifty-four unique functional entities were identified from the 61 taxa groups considered in the analysis.

Temporal Analysis

There was no significant interaction between the environmental parameters and overall trait community variance throughout the years. The Relate tests within certain years for both the trait abundance data as well as the trait presence/absence data did show significant results (Table 6.1; $P < 0.01$) however, given that the Rho values were both below 0.12, the relationship of the diving parameters to the trait community variance within years was minimal (Table 6.1).

Table 6.1. Relate test results of environmental parameters to the trait community variance including the overall relate test and the within levels of factor (Years) relate test. *Indicates a significant test statistic.

	Overall		Within Levels of Factor	
	Rho	P	Rho	P
Trait Abundance	0.039	0.072	0.109	0.001*
Trait Presence/Absence	0.03	0.117	0.113	0.001*

According to the results of the Main PERMANOVA tests, both abundance and presence/absence functional communities were significantly different among years (Main tests: $F = 4.905$, $df = 7$, $P = 0.0001$ and $F = 5.077$, $df = 7$, $P = 0.0001$ respectively). From the resemblance matrices, the average between-group similarity was 29% for the taxonomic community and 59% for the functional community. For context, we also calculated the average between-group similarity for the previously published Thistlegorm dataset (Lee et al., 2023), which

had not been reported or calculated in that study. Overall, 61% of the pairwise comparisons were significantly different for both the presence–absence and abundance datasets (see SI Ch.6, Table S3 for full results). Figure 6.1 illustrates the temporal changes in functional community traits using an MDS plot based on yearly centroidal distances from the functional abundance dataset. The BEST analysis identified the explanatory traits driving these community changes (projected onto Fig. 6.1 in blue) as the morphological form massive-erect, colonial growth, large-group gregariousness, the potential for asexual reproduction, and the size class 2. Three significant clusters of trait communities were identified (Figure 6.1): 2012 grouped with 2009, 2007 with 2010 and 2011, and 2008 with 2014, and 2013 being an anomalous year. While a sequential shift over time is not apparent, an oscillatory pattern emerges, marked by fluctuations in the prevalence of asexual reproduction and colonial traits. Specifically, these traits show an increase from 2007 to 2008, a decline in 2009, subsequent increases in 2010 and 2011, and another dip in 2012 before stabilizing by 2013, with a final spike in 2014. Alongside these oscillations, a general decline is observed in the abundance of massive-erect forms and large groups, while the prevalence of size class 2 gradually increases over time. Notably, 2013 displays abnormal variance compared to other years, with a sharp increase in size class 2 and significant decreases in massive-erect and large-group traits. By 2014, the community variance appears to return to a more typical pattern.

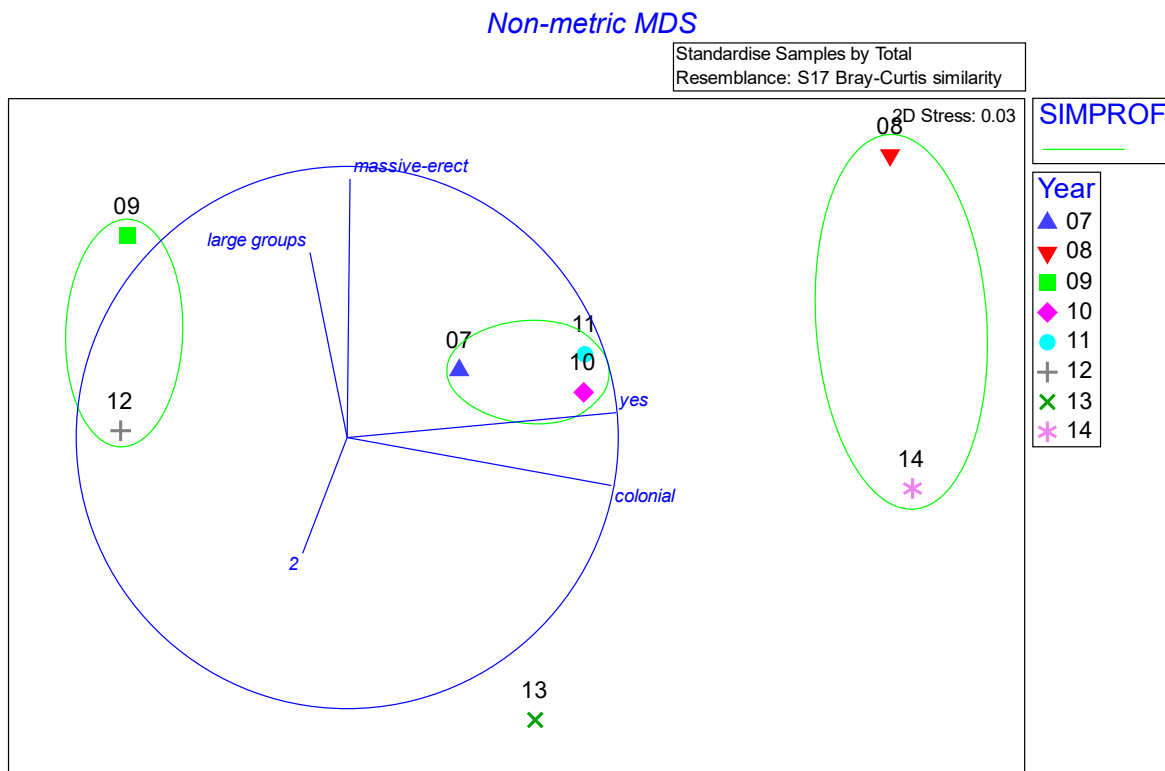


Figure 6.1. MDS of the functional trait abundance community structure from 2007-2014. Each point represents the central location of the community within that year. Green circles indicate similar groupings of years while the blue lines indicate the directional change in abundance of the projected BEST functional traits.

Similarly, Figure 6.2 depicts the temporal dynamics of functional traits based on an MDS plot of yearly centroidal distances derived from functional presence/absence data. The BEST analysis identified the traits most strongly influencing these patterns: pelagic mobility, poisonous defense mechanisms, only sexual reproduction, filter-feeding habits, and size class 2. In the presence–absence analysis, significant clusters included 2009 with 2012 and 2007 with 2010 and 2011, whereas 2008, 2013, and 2014 appeared as anomalous years (Figure 6.2). Pelagic and poisonous traits cluster distinctly, almost directly contrasting with the filter feeder trait. An oscillatory pattern can be observed between the clusters (similar to the functional abundance data) and is markedly driven by only sexual reproduction. Different from the abundance data, a vertical oscillatory trend can also be observed, where pelagic and poisonous traits exhibit synchronous variations, while filter-feeding traits show an opposing pattern. Similar to the abundance data, this dataset also highlights a notable phenomenon in 2013, characterized by an increase in filter-feeding traits, followed by 2014, which, while still deviating from the community's typical variance, shows a trend toward returning to normal variability (Fig. 6.2). Here however, 2008 is also a standalone year characterized by markedly low number of no asexual reproduction. This dataset underscores the persistence of key traits across years, revealing a complementary perspective to the abundance-driven dynamics observed in Figure 6.1.

Non-metric MDS

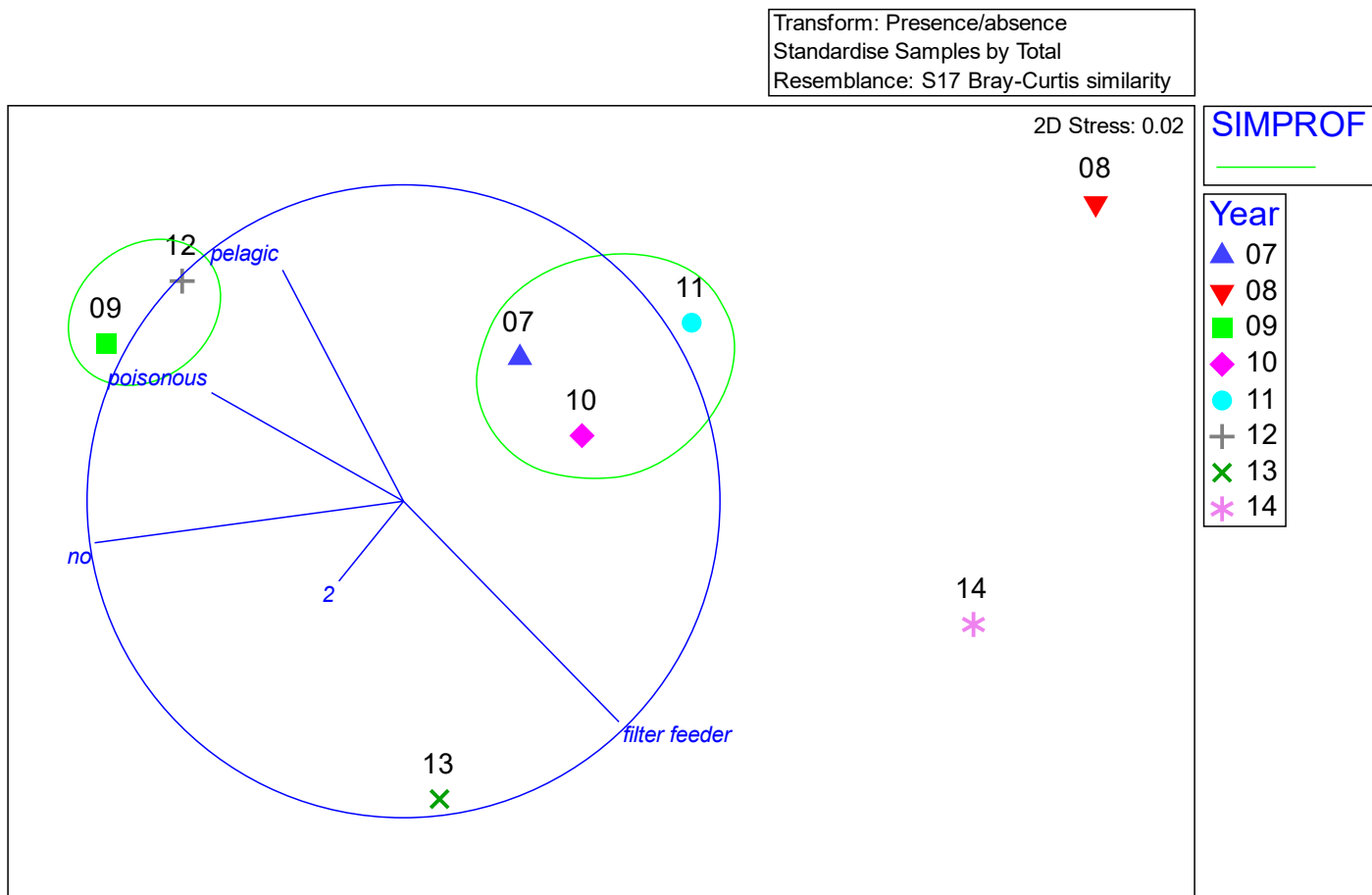


Figure 6.2. MDS of the functional trait presence/absence community structure from 2007-2014. Each point represents the central location of the community within that year. Green circles indicate similar groupings of years while the blue lines indicate the directional change in the presence of the projected BEST functional traits.

Discussion

This study highlights the temporal dynamics and variability of functional trait community at the world-renowned SS Thistlegorm wreck dive site, offering unique insights into its stability and ecological redundancy. The findings indicate that functional trait-based community variance is characterized by oscillatory patterns and driven by a few select traits. Average group similarity was higher in the functional trait matrices compared to the taxonomic community matrices, indicating that functional composition remained more stable across years than taxonomic identity. This suggests that, although species turnover occurred, many taxa shared similar functional roles, leading to a more conservative pattern of change at the trait level. Such patterns highlight the complementarity of functional and taxonomic approaches: while taxonomic analyses capture changes in species identities, functional analyses emphasize the persistence of ecological roles. This difference suggests greater temporal stability within functional traits, potentially indicating higher redundancy within the functional community. However, this study's

reliance on citizen science data required the use of broader taxonomic categories, deviating from traditional functional trait analyses that are often restricted to a single clade such as reef fishes or parrotfishes (D'Agata et al. 2014; Adam et al. 2018; Denis et al. 2019). By encompassing the entire coral reef community, this approach provides a broader, though less taxonomically detailed, perspective. It is also worth noting that the taxonomic categories themselves represent broad clades (e.g., parrotfishes, squirrelfishes, sharks), which already reduces resolution in the taxonomic analyses. This likely leads to an underestimation of functional redundancy, meaning that the true redundancy within the community may be higher than reported here. Accordingly, the higher average similarity observed in functional trait matrices should be interpreted with caution, as it may partly reflect the coarseness of both taxonomic and functional groupings.

Interestingly, both functional and taxonomic analyses identified 2013 as an anomalous year, marked by significant deviations from typical community variance (Lee et al. 2023). The functional community, however, exhibited a faster return to baseline variance by 2014. This suggests that functional traits may respond more rapidly than species to environmental perturbations, potentially due to higher functional redundancy buffering ecological processes against disruptions. This aligns with findings from studies on reef resilience, where high functional redundancy has been shown to stabilize ecosystem functions under environmental stress (Nash et al. 2016; Nyström 2006). The reduced dimensionality of functional traits (46 traits) compared to taxonomic categories (61 taxa) in this study inherently lowers the resolution of variation but emphasizes the resilience of ecological functions across time. The disturbance in 2013 and subsequent community changes may be related to reduced tourism due to the political upheaval of the Arab Spring (Lee et al. 2023), as this year was reported as one of the worst years on record for the Egyptian tourism industry which began recovering by the following year (Ecchevarria and Garcia-Enriquez 2020; Groizard et al. 2021). This impact from the reduction in tourism may have indirectly influenced the observed changes in trait community variance.

The traits identified as drivers of functional community variance—pelagic mobility, poison as a defense mechanism, colonial growth, asexual reproduction, only sexual reproduction, and size class 2—underscore key ecological strategies and resilience mechanisms. For instance, asexual reproduction in coral reef organisms, such as corals and starfish, plays a crucial role in population maintenance and recovery, especially under conditions of environmental stress (Sammarco 1982; López-Márquez et al. 2021). The energy trade-offs involved in asexual vs sexual reproduction can lead to oscillations in reproductive cycles, as organisms invest varying amounts of energy in growth, reproduction, and survival depending on the environmental context (Leuzinger et al. 2012). The results of this study could potentially be displaying these changes in reproductive cycles for organisms that have the potential to asexually reproduce.

Turning to the behavior of pelagic organisms, the reduced tourism in the study region could have significant implications for habitat preferences, particularly for more mobile species. Pelagic organisms (primarily

fish in this study), are known to alter their behavior and movement patterns in response to human activity, such as tourism and recreational diving (Albuquerque et al. 2014; Gairen et al. 2024). In regions with reduced tourism, these organisms may experience less disturbance, leading to a shift in habitat use. For instance, reef areas that are typically subjected to heavy tourist traffic, like Sharm el-Sheikh, may become "open for business" for mobile organisms once that stressor is reduced. Therefore, the reduction in tourism could explain why there is a lower abundance of pelagic organisms in 2013 allowing them to exhibit different habitat preferences when compared to periods of heavier human activity.

As for the observed decline in massive-erect coral morphology throughout the study, this trend may reflect broader ecological shifts and the vulnerability of certain coral growth forms. Massive morphological coral species tend to dominate in low-stress environments, where their large and robust structures enable them to outcompete other species for space and resources (Darling et al., 2012). However, as stress levels increase due to factors such as ocean acidification, thermal stress, and human impacts, these massive corals are often the first to decline and are subsequently replaced by more stress-tolerant, weedy, and generalist taxa, which are often characterized by branching morphologies (Darling et al., 2012). Recent evidence suggests that such community shifts toward stress-tolerant traits have contributed to the "flattening" of Caribbean reefs, a process marked by the loss of structurally complex coral species (Alvarez-Filip et al., 2009). Although the Red Sea is home to an unusually high number of heat-tolerant coral species, which has resulted in significantly fewer bleaching events even during some of the highest recorded temperature anomalies (Fine et al. 2013), evidence still indicates a general decline in colony size and increasing species homogenization throughout the region (Riegl et al. 2012). The observed decrease over time in massive-erect colony morphologies, though small, supports these findings and may indicate that, while the Red Sea has shown resilience to high temperatures thus far, there could be a limit to the ability of these reefs to endure such extreme conditions, which are expected to intensify in the future.

Size Class-2 organisms, encompassing the 10–50 cm range, represent an intermediate size class that varies in ecological significance across clades in coral reef systems. This range, although broad, was determined to accommodate the entire taxonomic set in the study, including invertebrates, corals, and fish. Within this size class, the notable taxa included a few soft corals, the mushroom coral (*Fungia* spp.) as the sole hard coral, many mollusks, as well as the mid-sized reef fish. As these traits were assigned based on the average size recorded within that species or taxa group, there were no in-situ measurements of organismal size. Mollusks in this range likely benefit from reduced predation risks and efficient foraging capabilities, while mid-size reef fish often occupy key trophic roles, optimizing mobility and habitat adaptability (Bellwood et al., 2003). Interestingly, it has been shown that resource scarcity and higher competition tends to increase the diversity of size classes in fish (Quintana et al. 2015; Schoener 1974). As this intermediate-size class evidently increased during the year 2013, this could be further evidence of the reduced stress due to diminished tourism in that region. The diversity of

taxa within this size class also underscores its functional importance, with intermediate-sized organisms possibly acting as ecological stabilizers and contributors to overall reef resilience (Mouillot et al. 2014).

One notable and previously mentioned limitation of this study is the broader taxonomic scope necessitated by citizen science data. Unlike traditional analyses that may focus on detailed functional traits within specific clades (e.g., herbivory in reef fishes or functional roles of parrotfish), this study evaluated functional traits across a wide range of taxa, including invertebrates, corals, bony and cartilaginous fish, as well as dolphins and sea turtles. While this approach provides a comprehensive view of community function, it may reduce the resolution of functional trait analyses and obscure subtleties that could emerge in studies focusing on narrower taxonomic groups. However, this broader perspective is particularly valuable for understanding ecosystem-wide functional dynamics, as it captures the contributions of less-studied taxa to ecological resilience. The observed patterns of oscillation and recovery align with ecological theories of functional redundancy and stability. Communities with high functional redundancy are better equipped to maintain ecosystem processes despite taxonomic fluctuations. The faster recovery of functional variance compared to taxonomic variance underscores this buffering effect.

Future research should investigate the environmental drivers or ecological interactions that may contribute to anomalies like those observed in 2013. Coupling trait-based analyses with environmental monitoring data could provide a deeper understanding of how factors such as temperature anomalies, nutrient dynamics, or anthropogenic pressures influence functional and taxonomic community structures. Additionally, further exploring the implications of broad taxonomic scope on functional trait analyses could refine methodologies for integrating citizen science data into ecological research.

Supplementary Information for Chapter 6

Table S1. Complete taxonomic list and their corresponding functional trait assignments including morphological, ecological, and behavioral characteristics.

Taxon	Size	Diet	Gregariousness	Period of Activity	Mobility/ Position in water column	Physical Defenses	Potential Asexual Reproduction	of	Morphological Form
<i>Tube Sponge</i>	3	filter feeder	colonial	diurnal-nocturnal	sessile	without	yes		branched
<i>Fire Coral</i>	4	mixotrophic	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		foliose thallus, sheets/blades
<i>Leather Coral</i>	3	mixotrophic	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		tree-like
<i>Soft Tree Coral</i>	2	mixotrophic	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		tree-like
<i>Sea Fan</i>	2	mixotrophic	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		tree-like
<i>Red Sea Fan</i>	3	carnivores	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		tree-like
<i>Sea Whip</i>	3	carnivores	colonial	strictly nocturnal	sessile	calcareous spicules sclerites	and yes		tree-like
<i>Sea Carpet Host Anemones</i>	4	mixotrophic	solitary (sessile Invert.)	strictly nocturnal	sessile	venomous	yes		massive-erect
<i>Plating Acropora</i>	4	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		branched
<i>Porcupine Coral</i>	4	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		branched
<i>Bubble Coral</i>	3	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		massive-erect
<i>Mushroom Coral</i>	2	mixotrophic	solitary (sessile Invert.)	strictly nocturnal	benthic	external carbonate	yes		massive-erect
<i>Lettuce Coral</i>	3	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		foliose thallus, sheets/blades
<i>Pineapple Coral</i>	3	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		massive-erect
<i>Black Coral</i>	4	mixotrophic	colonial	strictly nocturnal	sessile	external carbonate	yes		tree-like
<i>Christmas Tree Worm</i>	1	filter feeder	solitary (sessile Invert.)	diurnal-nocturnal	sessile	external carbonate	yes		worm-like
<i>Cowrie</i>	1	herbivores/grazers /omnivores	solitary	strictly nocturnal	benthic	external shell	no		slug-like

Taxon	Size	Diet	Gregariousness	Period of Activity	Mobility/ Position in water column	Physical Defenses	Potential Asexual Reproduction	of	Morphological Form
<i>Spanish Dancer</i>	1	invertivores	solitary	strictly nocturnal	benthic	poisonous	no		slug-like
<i>Coriacea</i>	1	invertivores	small groups	diurnal-nocturnal	benthic	poisonous	no		slug-like
<i>Tridacnae</i>	2	mixotrophic	solitary (sessile Invert.)	diurnal-nocturnal	sessile	external shell	no		bivalve
<i>Wing Oyster</i>	2	filter feeder	solitary (sessile Invert.)	diurnal-nocturnal	sessile	external shell	no		bivalve
<i>Squid</i>	2	piscivores	solitary	strictly nocturnal	benthopelagic	without	no		fusiform
<i>Big Fin Reef Squid</i>	2	piscivores	small groups	strictly nocturnal	benthopelagic	without	no		fusiform
<i>Banded Boxer Shrimp</i>	1	invertivores	paired	strictly nocturnal	benthic	external carbonate	no		articulated
<i>Hermit Crab</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	external shell	no		articulated
<i>Sea Lillies</i>	2	filter feeder	solitary	diurnal-nocturnal	benthic	without	no		star-like
<i>Sea Cucumber</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	calcareous spicules and sclerites	no		worm-like
<i>Pearl Red Star</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	without	yes		star-like
<i>Spiny Starfish</i>	2	invertivores	solitary	strictly nocturnal	benthic	venomous	yes		star-like
<i>Fire Urchin</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	venomous	no		spherical
<i>Pencil Urchin</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	without	no		spherical
<i>Giant Moray</i>	4	piscivores	solitary	strictly nocturnal	benthic	without	no		filiform
<i>NeedleFish</i>	1	invertivores	solitary	diurnal-nocturnal	benthic	without	no		filiform
<i>Squirrelfish</i>	2	invertivores	small groups	strictly nocturnal	benthic	without	no		compressiform
<i>Groupers</i>	3	piscivores	solitary	strictly nocturnal	benthopelagic	without	no		compressiform
<i>Black Spotted Rubberlip</i>	2	invertivores	small groups	strictly nocturnal	benthopelagic	without	no		compressiform
<i>Humpback Batfish</i>	3	herbivores/grazers /omnivores	small groups	diurnal-nocturnal	benthopelagic	without	no		compressiform
<i>Red Bass</i>	3	piscivores	solitary	strictly nocturnal	benthopelagic	without	no		compressiform
<i>Glassfishes</i>	2	invertivores	small groups	strictly nocturnal	benthopelagic	without	no		compressiform
<i>Goatfishes</i>	2	invertivores	small groups	strictly nocturnal	benthopelagic	without	no		compressiform
<i>Map Angel</i>	2	Invertivores	solitary	diurnal-nocturnal	benthopelagic	without	no		compressiform
<i>Butterflyfishes</i>	2	invertivores	small groups	strictly nocturnal	benthopelagic	without	no		compressiform

Taxon	Size	Diet	Gregariousness	Period of Activity	Mobility/ Position in water column	Physical Defenses	Potential Asexual Reproduction	of	Morphological Form
<i>Longnose Hawkfish</i>	2	invertivores	paired	strictly nocturnal	benthic	without	no		compressiform
<i>Red Clownfish</i>	2	herbivores/grazers /omnivores	paired	diurnal-nocturnal	benthic	without	no		compressiform
<i>Napolean Wrasse</i>	4	piscivores	solitary	diurnal-nocturnal	pelagic	without	no		compressiform
<i>Parrotfish</i>	3	herbivores/grazers /omnivores	small groups	strictly diurnal	benthopelagic	without	no		compressiform
<i>Barracuda</i>	3	Piscivores	large groups	diurnal-nocturnal	pelagic	without	no		compressiform
<i>Sohal Surgeonfish</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthopelagic	without	no		compressiform
<i>Caranxes</i>	3	piscivores	large groups	diurnal-nocturnal	pelagic	without	no		compressiform
<i>Lionfish</i>	2	piscivores	solitary	strictly nocturnal	benthic	venomous	no		compressiform
<i>Spotted Flatheads</i>	3	piscivores	solitary	diurnal-nocturnal	benthic	venomous	no		compressiform
<i>Titan Triggerfish</i>	3	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	without	no		compressiform
<i>Box Fish</i>	2	invertivores	solitary	diurnal-nocturnal	benthic	poisonous	no		compressiform
<i>Blowfishes</i>	2	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthic	poisonous	no		compressiform
<i>Porcupine Fishes</i>	2	invertivores	solitary	diurnal-nocturnal	benthopelagic	poisonous	no		compressiform
<i>Sharks</i>	4	piscivores	solitary	strictly nocturnal	benthopelagic	without	no		depressiform
<i>Blue Spotted Stingray</i>	2	invertivores	small groups	diurnal-nocturnal	benthic	venomous	no		depressiform
<i>Manta Ray</i>	4	invertivores	solitary	diurnal-nocturnal	pelagic	without	no		depressiform
<i>Torpedo</i>	3	piscivores	solitary	strictly nocturnal	benthic	electric	no		depressiform
<i>Turtles</i>	3	herbivores/grazers /omnivores	solitary	diurnal-nocturnal	benthopelagic	without	no		depressiform
<i>Dolphins</i>	4	piscivores	small groups	strictly nocturnal	pelagic	without	no		fusiform

Table S2. Yearly counts of completed questionnaires, taken from Lee et al. 2023.

Year	2007	2008	2009	2010	2011	2012	2013	2014	Total
Number of Questionnaires	84	62	59	59	72	29	12	13	390

Table S3. Pairwise PERMANOVA results for relative abundance and sighting frequency by the factor “Year”, describing the strength of differences between pairs of years (T as the t-statistic). * Indicates significant p-value.

Groups	Relative Abundance		Sighting Frequency	
	T	P-value	T	P-value
2007, 2008	3.114	0.0001*	3.347	0.0001*
2007, 2009	2.27	0.001*	2.087	0.0027*
2007, 2010	1.391	0.086	1.351	0.101
2007, 2011	1.424	0.079	1.619	0.0367*
2007, 2012	1.797	0.013*	1.478	0.064
2007, 2013	1.187	0.201	1.208	0.194
2007, 2014	1.52	0.054	1.417	0.092
2008, 2009	4.521	0.0001*	4.716	0.0001*
2008, 2010	2.437	0.0006*	3.247	0.0001*
2008, 2011	2.307	0.001*	2.26	0.0026*
2008, 2012	3.498	0.0001*	3.639	0.0001*
2008, 2013	1.841	0.0102*	2.228	0.0014*
2008, 2014	1.388	0.0832	1.496	0.0545*
2009, 2010	2.789	0.0003*	2.455	0.0008*
2009, 2011	2.808	0.0001*	2.817	0.0003*
2009, 2012	1.429	0.0067*	1.162	0.221
2009, 2013	1.944	0.0049*	1.641	0.0272*
2009, 2014	2.654	0.0001*	2.437	0.0005*
2010, 2011	1.077	0.287	1.612	0.0462*
2010, 2012	2.125	0.0042*	1.698	0.035*
2010, 2013	1.131	0.242	1.272	0.153
2010, 2014	1.098	0.274	1.29	0.142
2011, 2012	2.115	0.0039*	2.023	0.009*
2011, 2013	1.167	0.215	1.355	0.114
2011, 2014	1.315	0.135*	1.201	0.191
2012, 2013	1.521	0.0621	1.324	0.134
2012, 2014	2.442	0.0007*	2.143	0.0054*
2013, 2014	1.383	0.117	1.441	0.096

Conclusion

This dissertation presents a multi-system investigation of how aquatic community composition varies across space and time, using a consistent multivariate analytical framework. Across six standalone chapters, it integrates marine and freshwater datasets, citizen science and expert surveys, and both taxonomic and functional approaches to detect patterns of ecological change. While the ecological contexts range from Red Sea and Caribbean coral reefs to Mediterranean benthic assemblages and riverine fish communities in Emilia-Romagna, all chapters share a common goal: to understand how communities respond to environmental pressures, anthropogenic impacts, and management strategies. By applying comparable statistical methods across systems, this body of work not only provides insight into individual ecosystems, but it also enables cross-system comparison and broader inference about community-level responses to change.

Across the marine chapters, a central theme emerges: the evolution of reef-associated communities through time, shaped by varying degrees of protection, human pressure, and ecosystem governance. While each study focused on a distinct spatial context, from an artificial reef in the Red Sea to natural reef systems in the Mediterranean and Caribbean, they all applied long-term datasets to uncover patterns of ecological reorganization. These chapters collectively highlight how temporal monitoring, when paired with spatial contrasts in management strategy, can reveal subtle but meaningful signals of community reorganization. From the ridge-to-reef gradient in the Red Sea, to the overlapping influence of human impact and management zones in the Mediterranean, to the stratified marine protection network in the Cayman Islands, the results emphasize that the structure of biological communities is not static but shifts in response to cumulative local and regional pressures. These findings reinforce the value of using community-level approaches to better understand the ecological consequences of different conservation frameworks across spatially diverse reef systems.

Extending this spatial-temporal framework beyond marine systems, the freshwater study applied the same community-level approach to long-term monitoring data across riverine ecosystems in Emilia-Romagna. Although differing in setting and taxonomic focus, the analysis echoed key patterns found in marine environments: significant changes in community structure over time, shaped by the interaction between broad environmental gradients and spatial context. By dividing rivers into ecological zones that reflect altitudinal, hydrological, and geomorphological differences, the study captured spatial heterogeneity in community vulnerability, demonstrating that zones at higher altitudes exhibited stronger correlations with temperature and precipitation, while downstream zones displayed greater variability and appeared more influenced by local anthropogenic context. This transition from primarily climatically driven to more disturbance modulated regimes reinforces the importance of spatial setting when interpreting long-term biological change. Together with the marine chapters, the freshwater analysis illustrates how multivariate tools can be consistently applied across ecosystems to reveal the layered drivers of community transformation.

The integration of functional trait-based analysis marked a methodological and conceptual progression in this thesis, moving beyond species identities to consider the ecological roles organisms play across time. This shift reflects a broader theme running through the work: that understanding community change requires not only identifying which species are present but interpreting how ecosystems are organized and persist under disturbance. By applying this lens to a site previously analyzed using taxonomic metrics, the thesis demonstrates how trait-based approaches can compliment species-based interpretations and reveal additional dimensions of community structure. While this perspective is not generalized across all systems examined here, it underscores the value of methodological flexibility and innovation in expanding ecological inference at focal sites.

Taken together, the chapters of this thesis demonstrate that community-level analysis, when applied consistently over space and time, can reveal nuanced patterns of ecological change that would be missed by single-metric or short-term studies. Across tropical and temperate systems, in both marine and freshwater environments, community structure emerged as a sensitive indicator of ecological processes shaped by climate, management context, and human activity. These findings reinforce the importance of long-term monitoring programs that incorporate species-specific and, where possible, functional information. They show that datasets with inherent limitations, such as uneven sampling, or citizen science origins, can yield meaningful ecological insights when analyzed within robust multivariate frameworks. This has direct relevance for regions where biodiversity is threatened, and ecosystems are undergoing rapid change, but long-term data remain scarce.

Across the diverse systems examined in this thesis, a unifying pattern emerges regarding the relative influence of environmental forcing, management, and local human context on community change. Where aquatic communities are buffered from sustained human pressure, through remoteness, effective protection, or lower intensity of surrounding development, broader environmental gradients and management strategies tend to exert a clearer and more sustained influence on long term community trajectories. In contrast, in systems situated closer to persistent disturbance, local pressures often dominate community organization, increasing variability and obscuring or dampening the signal of larger climatic or management signals. This context dependence suggests that ecosystem state is not governed by a single driver, but by the balance between background environmental change and the intensity of local human influence, a relationship that recurs across both marine and freshwater environments in this dissertation.

This thesis contributes to a growing body of research that embraces community composition, not simply biodiversity, as a lens for understanding ecological change. The results underscore that ecological communities are not static collections of species, but dynamic systems shaped by the interaction of environmental forcing, management, and disturbance. By integrating diverse ecosystems and data sources within a shared analytical framework, this work highlights how common patterns of community reorganization can be identified across

habitats and timescales, helping to bridge the gap between monitoring and action and informing conservation efforts with more context-sensitive and ecologically grounded understanding.

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