



UNIVERSITÀ DEGLI STUDI DI PALERMO

Dottorato in Scienze della Terra e del Mare
Dipartimento di Scienze della Terra e del Mare (DiSTeM)
Settore Scientifico Disciplinare BIO/07 - Ecologia

STRUCTURAL AND FUNCTIONAL RESPONSES OF FISH ASSEMBLAGES TO DIFFERENT DRIVERS OF CHANGE

IL DOTTORE
Gabriele Turco

IL COORDINATORE
Prof. Alessandro Aiuppa

IL TUTOR
Prof. Marco Milazzo

CO TUTOR
Dott. Michele Gristina

CICLO XXXI
ANNO CONSEGUIMENTO TITOLO 2019

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SUMMARY

Biotic and abiotic factors synergistically act on natural systems. As a result ecological communities adjust their structural and functional responses to persist in a given area. Worldwide, marine ecosystems have been changing through time with increasing anthropogenic pressure in the last centuries leading to only 13.2% of the total marine environments in a 'pristine' state. The consequences that direct and indirect anthropogenic pressures may have on marine organisms can largely differ, with both negative and positive effects of drivers of change of human origin. In the span of three years for my PhD thesis, I performed different experiments with the main goal of examining how direct or indirect drivers of change may affect fish assemblages. For this purpose, I focussed on positive effects of protection activities within Marine Protected Areas (MPAs) in the Mediterranean Sea and potentially detrimental impacts of increasing CO₂ concentrations in Italy, Japan and Papua New Guinea.

The effects of these drivers can be disparate. The same driver can jeopardize the very existence of certain organisms (e.g. the effect of rising levels of CO₂ on calcifying organisms), while at the same time enhancing processes crucial to others (e.g. The role of CO₂ on increasing the photosynthesis rate in many algae), overturning the seascape in fundamental ways. As noted by Boyd and Hutchins (2012) and Boyd et al. (2018), the term driver is effect-neutral, indicating changes aren't necessarily positive or negative, and for this reason it will be used in contrast with the, always negative, connotation of the term stressor. The term driver has been associated with a multitude of "human and natural-induced factor that directly or indirectly causes a change in an ecosystem". Directly when it "unequivocally influences ecosystem process", indirectly when it "operates more diffusely, by altering one or more direct drivers" (Nelson 2005).

Indeed, one of the greatest concern relative to management of marine ecosystems is the depletion of large predatory fish by intense exploitation activities (e.g. fisheries) and the parallel need to sustain local communities' welfare. To date, Marine Protected Areas (MPAs) and both national and international fishery regulations represent the most effective (and low-cost) tools to prevent fish depletion, food web simplification, and to sustain the use of marine coastal resources worldwide. Properly enforced MPAs may be effective at re-establishing large food webs through top predator release, increasing the ecological community resilience and benefitting the socio-economical context within their boundaries. In the **Chapter 2** of this thesis, I aimed at evaluating fish assemblages characteristics (relative abundance, species richness and biomass) and predation

pressure inside and outside 11 Mediterranean MPAs where different levels of human exploitation are in place. To achieve this goal, I exploited fishing pressure gradients from no-take zones, buffer zones (e.g., where only local small-scale fishery is allowed), to heavily fished areas. These results allowed me to infer both the ecological effectiveness of the 11 pilot MPAs, exhibiting different enforcement levels, protection schemes, and hence being or not being able to be effective in reaching their intended conservation goals. Moreover, here I validate for the first time the large-scale use of a user-friendly sampling tool for MPA managers, providing support for specific monitoring, policy and management choices, as well as setting up more effective fishery management models related to local fishers' communities.

As the pilot MPAs under consideration differed in management and protection schemes, I tested their ecological effectiveness using established standards, analysing specific predictive variables expressed in units per time: Max N, a measure of relative abundance per hour, S, species richness per hour, and biomass (gr) per hour. Although with mixed results, the investigated MPAs proved indeed capable of improving their trophic state. Whilst fish species richness was not necessarily higher inside than outside the MPA, relative abundance and biomass increased significantly across the borders, although not for all trophic groups. In some instances, the response varied between MPAs, and such responses have been discussed.

Chapter 3 focused on assessing the mesopredator release in the 11 pilot MPAs investigated, indeed a controversial and debated hypothesis in the literature. According to this theory, the number mesopredators should rise significantly in external zones due fishing efforts being focused on larger apex predators.

Through a simple assay, over 11 MPAs, predation pressure has been tested by means of an experimental design that provides apex and mesopredator comparisons between protected (no-take and buffer areas where all or almost all activities are forbidden) and unprotected (external areas) zones, where SSFs plays a crucial role in balancing trophic interactions. The results highlighted the role of MPAs on guaranteeing higher apex predators and mesopredators rates in protected than unprotected areas overall, but the results rejected the mesopredator release hypothesis that was previously verified in terrestrial environments. Overall predation rates were unaffected, despite a few MPAs showing higher rates of it inside than outside their boundaries.

Probably due to the nature of the liquid medium and the greater mobility of marine species, this controversial theory has not been confirmed in this context.

(Chapter 4) Therefore one of the goals of my PhD was to assess both direct and indirect OA effects on fish communities, via habitat change, and how this may lead to reduced top-down control (i.e. decreasing fish predation intensity). To have a general view of such hypotheses I worked along different CO₂ gradients located in three climatic zones (Temperate, Subtropical and Tropical regions). Baited Underwater Videos (BUV) were used to assess the responses of fish assemblages exposed to nowadays and expected $p\text{CO}_2$ levels along three CO₂ seeps where community shifts were already documented. In addition, a standard tethering experiment was employed by Squidpops to quantify predation pressure and potentially emphasize the generality of top-down processes strength under future ocean scenarios. Results showed that community shifts differed in the three CO₂ seeps as a result of CO₂ acting as a 'nutrient', and thus boosting macroalgae and low profile turfs in the temperate and subtropical zones, and as a stressor on habitat forming scleractinians in subtropical and tropical regions. Overall, the complexity of the shifting communities decreased with this process cascading on fish assemblages composition and structure. Differences in predation intensity were not statistically significant between high $p\text{CO}_2$ and controls. To reduce sampling subjectivity which is common when standard visual census techniques are employed, in all cases I used innovative techniques like BUVs (Baited Underwater Video systems) and even modified existing ones to better suit specific goals. Hence, an additional achievement of my study was the design of the new BUV structure, with better performance in surveying shallow water habitats.

Chapter 1

GENERAL INTRODUCTION

The role of Marine Protected Areas

The standard definition of an MPA has been given by the International Union for Conservation of Nature (IUCN) as follows: “Marine protected areas are 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.” (WCPA, 2008). Depending on the required reference target and desired purposes, protected areas can be further characterized so that additional specific criteria are met. Over time, these areas have become a valuable tool for managing and preserving marine resources as a whole (i.g. Higgins *et al.*, 2008; Lester and Halpern, 2008; Claudet *et al.*, 2008; Harmelin-Vivien *et al.*, 2008; Claudet and Guidetti, 2010a, 2010b).

In vulnerable environments such as MPAs, proper management plays a key role in maintaining ecosystem stability, and coexistence between all stakeholders (Francour *et al.*, 2001). The effects of human activity can lead to deleterious effects on the marine ecosystem over time, in particular: resource exploitation (i.e. fishing (55%), tourism *etc.*), habitat degradation (37%), pollution, global climate change, pollution and the introduction of alien species (8%); over time, these elements have caused direct and indirect consequences on biotic and abiotic features, compromising biodiversity (Worm *et al.*, 2008, Lubchenco *et al.*, 2003).

One of the main consequences of fishing is the reduction in abundance of target species (Pauly *et al.*, 2002). In particular, most apical predators and commercially-valuable species showed a significant decrease in number (Murawsky, 2000; Sadovy, 2005) because of it. These species are characterized by slow growing rates, large sizes, a long life cycle and low recruitment (Pauly *et al.*, 2002; Sadovy, 2005). The fishing action can also have an effect on fish stock, depleting the number of larger individuals within the population (Law, 2000). Thanks to the high resilience of fish populations, this trend can be reverted, but recovery times are long (Halpern *et al.*, 2002;

Hutchings *et al.*, 2004, Sadovy, 2005). Moreover, the fishing effort not only affects commercially valuable species, but also drastically reduces population at many different trophic levels, due to high rates of non-targeted species catch (Murawsky, 2000).

In this respect, an MPA can provide an increase to total abundance, biomass, and fish size within its boundaries, in particular relatively to species specifically targeted by fisheries, while combating impoverishment in adjacent areas as well (Mosquera *et al.*, 2000; Micheli *et al.*, 2004; Guidetti and Sala 2007). Being able to protect multiple species at different trophic levels can mitigate the effects of target-resource exploitation by fisheries. (Zhou *et al.*, 2010)

Nowadays, market demand favours larger top predators; such preference leads to a cascade of effects first described by Pauly *et al.* (1998), that has been defined as “*Fishing Down Marine Food Web*”. FDMFW has downstream consequences on the exploitation of target resources at progressively decreasing trophic levels.

In the early 2000s, the concept of Ecosystem-Based Fisheries Management (EBFM) was introduced to MPAs: this set of rules would take stock interactions, stock-prey relationships and stock-habitat requirements as the fundamental reasoning behind the management of an MPA (Pikitch *et al.*, 2004; Christie *et al.*, 2007). Even though this model can mitigate the impact of fishing on the entire ecosystem (Zhou *et al.*, 2010), it has proven to be insufficient, since it does not take the “human dimension of fishing” in adequate consideration, nor the relationship between economic requirements and ecosystem health (Garcia *et al.*, 2003).

One of the main threats looming over MPAs is represented by small-scale fisheries (SSF) whose pressure on the area is, differently from others stakeholders, characterized by higher frequency and intensity. An SSF is identified as any small capital investment fishery, using small boats and employing fishing techniques and gear that can be manned by just one or two professional fishers (Colloca *et al.*, 2004; Halkos *et al.*, 2018). Due to the specific cultural context changing along the Mediterranean, SSFs focus on different commercial species and use different tactics (Pelletier and Ferraris 2000) or fishing strategies (He *et al.* 1997; Halkos *et al.*, 2018) depending on their country of origin.

While observing MPAs, only SSFs will be taken under consideration because, contrary to industrial fisheries, their activity focuses on areas close to the coast and, consequently, protected areas (Colloca *et al.* 2004).

In this regard, regulation of SSFs could constitute an essential vector to maintain species health descriptors at high levels (i.e. abundances and biomass) through co-management between the managing body and the stakeholders. Furthermore, it should be noted that fisheries represent a major source of high-quality food, employment and economic benefit for the coastal areas (Llorent and Font, 2013; Halkos *et al.*, 2018), reasons that should make SSFs a focal point in the maintenance of the marine ecosystem.

Lost predatory interactions and community changes

The effects of fishing on target species – specifically on apex predators – have been extensively assessed (e.g., Edgar *et al.*, 2017; Valdivia *et al.*, 2017). Intensive exploitation of apex predators generates a drastic reduction in top-down control, promoting an exponential increase of lower level trophic groups. This phenomenon was first described in terrestrial environments, and was given the name of *mesopredator release*, which occurs when “the absence or negative change in the density or distribution of an apex predator results in an expansion in density or distribution, or behaviour of a middle-rank predator in a trophic web” (Crowell 1961; Prugh *et al.*, 2009; Brashares *et al.*, 2010). Empirical evidence suggests that higher mesopredator presence has been observed in systems characterised by low productivity levels, while highly productive systems are generally ruled by apex predators.

Ocean Acidification (OA)

Since the industrial revolution, the close and essential relationship between mankind and the environment has drastically changed. A rapport that had historically been a one-way dependency of the former on the latter, evolved into a diametrically opposite situation where mankind would change the environment to suit its industrial expansion needs. This has materialized through land exploitation, industrial production and an exponential increase in fossil fuel extraction and combustion that, over time, leading to the introduction of huge amounts of anthropogenic carbon

dioxide (CO₂) in the atmosphere (Houghton *et al.*, 2002). From the 19th to the 21st century, the amount of atmospheric CO₂ has raised from 280 parts per million (ppm) to more than 400 ppm (current) (https://climate.nasa.gov/climate_resources/24/graphic-the-relentless-rise-of-carbon-dioxide/).

A fundamental aspect that needs to be thoroughly examined as a consequence of such an increase, is the strong relationship between atmospheric CO₂ concentration and temperature.

The connection between the two has been proven by comparing time series data of temperature changes to the estimation of driver trends during glacial cycles, on a time scale of hundreds of thousands of years. Reconstructions of Paleoclimatic data, done through ice layering and oxygen isotope examinations, highlighted how increases or decreases in CO₂ levels during glacial cycles correspond to proportional increases or decreases in temperature. One of the most remarkable aspects of this phenomenon is represented by CO₂ solubility and its concentration in relation to temperature (<https://www.ncdc.noaa.gov/global-warming/temperature-change>).

Nowadays, it's well known that on a small temporal scale (from 19th to 21st century), fluctuations of these abiotic features, acting synergistically with other greenhouse gases, are driving a series of considerable and serious environmental changes (Stocker *et al.*, 2013).

The most glaring consequence of these interactions is the so-called "Global Warming" effect, described as: "a slow change in stressors driven by the atmospheric CO₂ concentration and other greenhouse gases" (IPCC, 2007; Godbold *et al.*, 2013).

As reported by the Fifth Assessment Report by IPCC, the ocean's average temperature has risen dramatically from 1971 to 2010, by approximately 0.1°C per decade, in the first sixty meters of the water column. Furthermore, as reported by Collins *et al.*, (2013), the global atmospheric temperature is projected to increase by up to 4°C by the end of this century.

Increases in temperature generate different alterations both in the water's chemical properties (*e.g.* by increasing the kinetics of CaCO₃ precipitation or the water density) and the species living in this medium (*e.g.* Physiological alterations) (Pörtner, 2008).

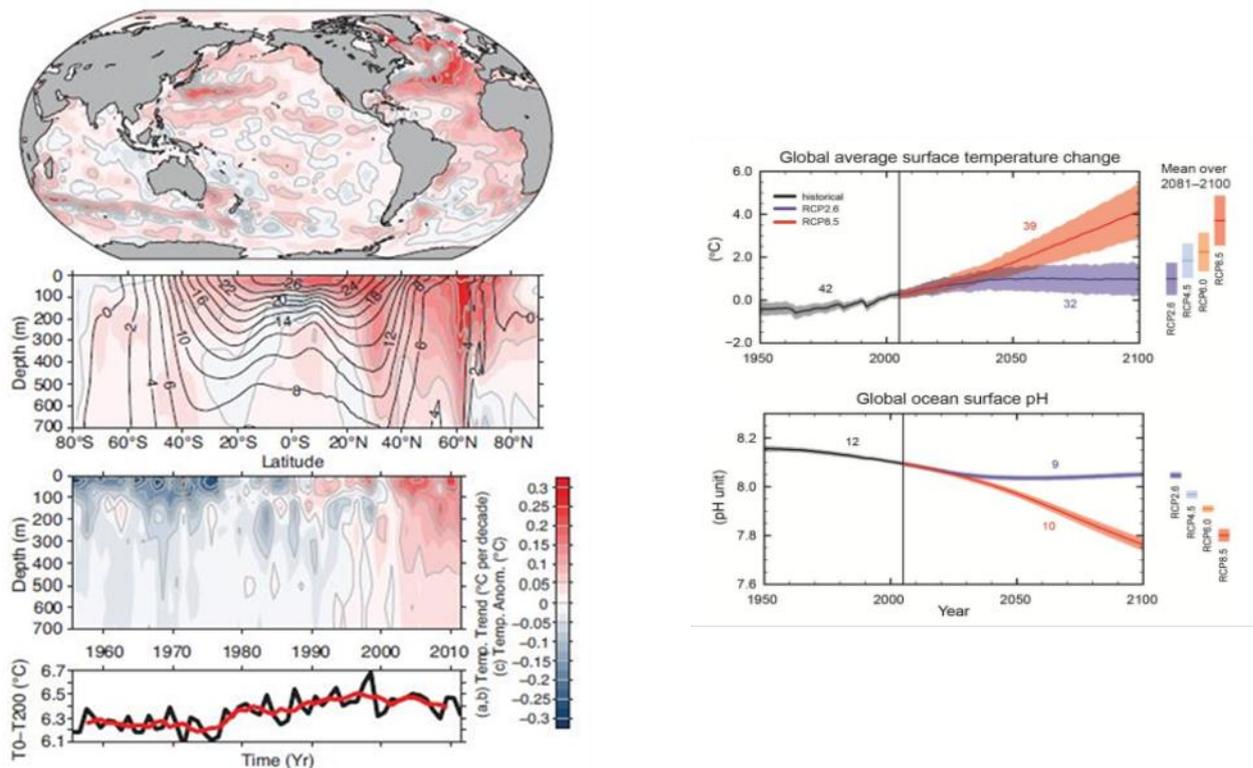
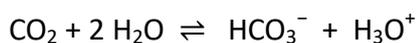
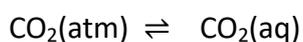


Figure 1.1. Global temperature distribution and trend per decade (left). Global average surface temperature and pH related to the different RCP scenarios (right).

Since the second industrial revolution, while the atmosphere has absorbed the bulk of CO₂ emissions, Prentice *et al.*, (2001) estimate that fewer than half of anthropic CO₂ emissions remain in the atmosphere. The remaining quantity is absorbed by the oceans and terrestrial biosphere. Without this oceanic uptake, the current atmospheric CO₂ concentration would be 55 ppm higher than currently observed.

The basic issue that gas brings to oceanic waters lies in the fact that when it transfers from the atmosphere to seawater, it binds to the water molecule, changing its chemical properties:



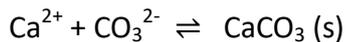
This process is known as Ocean Acidification (OA) (Caldeira & Wickett, 2003).

Usually, this gas is in equilibrium in the liquid medium, but exceeding the stoichiometric balance leads to a chemical reaction occurs that generates an increase of H⁺ ions. The oceans are the main reservoirs for CO₂ deriving from anthropic action (Rejmánek 2005). An increase of about 26% of H⁺ ion concentration has been estimated since the early years of the 20th century (decrease in

oceanic pH from 8.2 to 8.1), leading to a possible increase of more than 0.5 pH units by 2100 (Caldeira & Wickett, 2003).

As that pH it is expressed on a logarithmic scale, small variations on the scale could have large impacts, promoting a cascade of effects on both biotic components and the water column as a whole, that would be a precarious balance that is fragile in itself.

An immediate example would be calcificant species, for which calcium carbonate, in the form of calcite or aragonite, plays a primary role:



As a consequence of acidification, H^+ ions increase drastically, and carbonate ions are not able to bind to them.

This has an effect on both terrestrial and marine sectors, capable of causing, in some cases, total habitat loss.

From the late 20th century, climate change awareness by the international community has increased considerably. The Intergovernmental Panel of Climate Change (IPCC) hypothesized, in their fifth assessment report (AR5) (https://www.ipcc.ch/pdf/assessment-report/ar5/syr/SYR_AR5_FINAL_full_wcover.pdf), four different possible trajectories named Representative Concentration Pathways (RCP), leading to the theorization of climatic models based on different emission scenarios in the years to come (Fig. 1.2):

- RCP 2.6: assumes that global emission of Greenhouse gases (GHG) will be diminished between 2010-2020 (peak).
- RCP 4.5: assumes that global emission of Greenhouse gases (GHG) will be diminished after 2040 (peak)
- RCP 6: assumes that global emission of Greenhouse gases (GHG) will be diminished after the 2080 (peak)
- RCP 8.5: assumes emissions would continue to rise even after the 21st century.

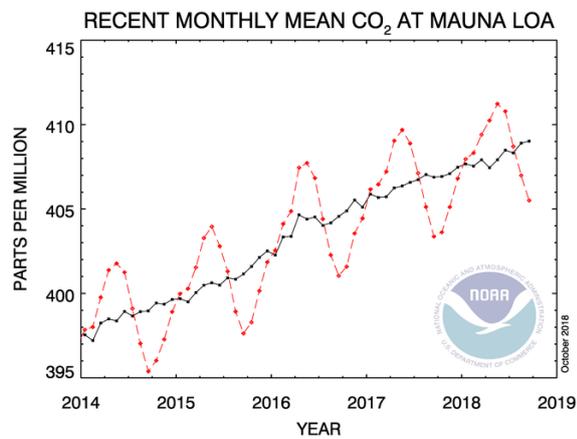
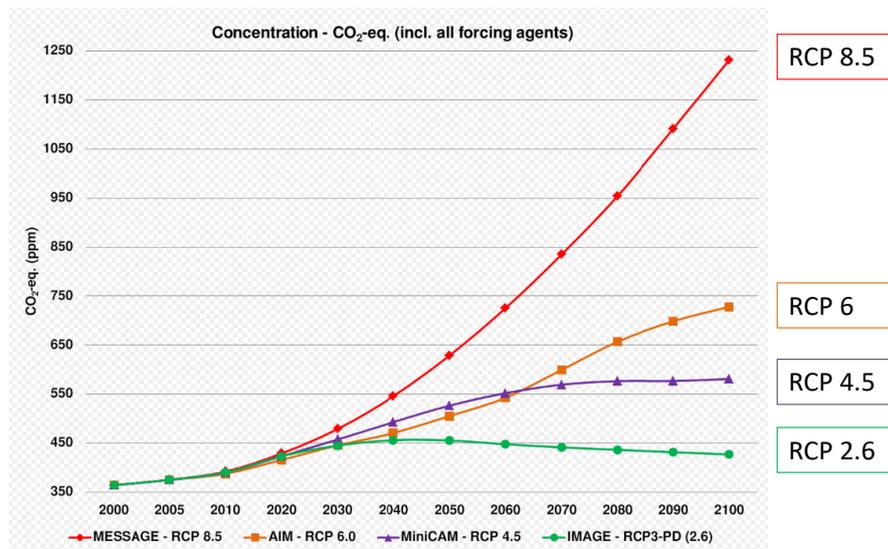


Figure 1.2. Representative Concentration Pathways of CO₂ emissions from the beginning to the end of the century. CO₂ values in the x-axis are reported as parts per million (ppm) (above). Monthly MAUNA LOA Trend reported during the month of October 2018 (<https://www.esrl.noaa.gov/gmd/ccgg/trends/index.html>) (below).

The RCP 8.5 scenario, with its emission pathways and GHG concentrations (“business-as-usual” case scenario), is one of the most used in scientific and research fields to hypothesize the possible changes to biotic factors associated with extremely high CO₂ concentrations. In particular, according to the scenario envisioned by the IPCC, not limiting emissions, without attempting to mitigate climate change, would generate a most disastrous environmental condition in the future (Fisher et al., 2007; IPCC 2008; Riahi et al., 2011).

To estimate and quantify the effects of ocean acidification on a future time-space scale, multiple authors have suggested the use of natural environments (as estuarine, upwelling and

hydrothermal vents zones) to emulate different scenarios, in place of the manipulative laboratory experiments that have been exclusively performed until some time ago (Barry *et al.*, 2010).

Recent studies have been done in such realistic conditions in order to analyse processes such as animal and vegetable species distribution, growth, survival, metabolic performance, physiological responses, calcification and dissolution rates, behaviour and species interaction, related to different $p\text{CO}_2$ levels (*e.g.* Fabry *et al.*, 2008; Munday *et al.*, 2008; Cigliano *et al.*, 2010; Fabricius *et al.*, 2011, 2017; Kroeker *et al.*, 2013; Nagelkerken *et al.*, 2015, 2017, 2018).

In a laboratory, it is possible to closely modulate the frequency and intensity of some abiotic variables (*e.g.* temperature, salinity, pH), examining the response of single species (Russell *et al.*, 2009). Conversely, in natural systems, it is possible to study OA's consequences on entire communities (Cigliano *et al.*, 2010; Fabricius *et al.*, 2011) and observe how these interact within physical-chemical factors (Sunday *et al.*, 2014, 2016). Moreover, focusing on areas with high CO_2 levels, it is possible to verify functional models of the whole ecosystem, estimating the possible ecological winners in future scenarios of global climatic changes, investigating mechanisms of adaptation and plasticity at different $p\text{CO}_2$ concentrations.

Effects of OA on ecosystems

The inertia of these vectors could amplify the system's enthalpy at a global scale, progressively producing changes to abiotic factors that would, over time, slowly accumulate into massive ones.

Aside from how CO_2 and its related stressors (*e.g.* temperature variations) might act on a global scale, its effects are evident on a local scale (Godbold *et al.*, 2013).

One of the primary objectives in the scientific field starting from the year 2000, has been to evaluate the possible effects induced by different drivers of change (*e.g.* pH, temperature, run-off, alien species) on an ecosystem's functioning.

Typically, an ecosystem is a set of interconnected elements generating from interactions between biotic and abiotic components.

The predictions of large increases in CO₂ levels before the end of the century made by the IPCC in the absence of drastic containment measures, have prompted the scientific community to envisage possible ecological winners and losers in a constantly exacerbating and eroding CO₂ scenarios.

Particularly, one of the main challenges in environmental sciences is to evaluate the direct and indirect effects of CO₂ on the environment and the organisms living in it (e.g. Nagelkerken *et al.*, 2015, 2017; Sunday *et al.*, 2016). The biogenic consequences that this driver produces on habitats, also act indirectly on biodiversity (Fig. 1.3). Some authors have highlighted the close relationship between these changes in structural habitat complexity and species richness (e.g. Sunday *et al.*, 2016). As reported by the abovementioned authors, the direct effect of pCO₂ on habitat-forming organisms is strictly linked to their physiology and life history (Fig. 1.4).

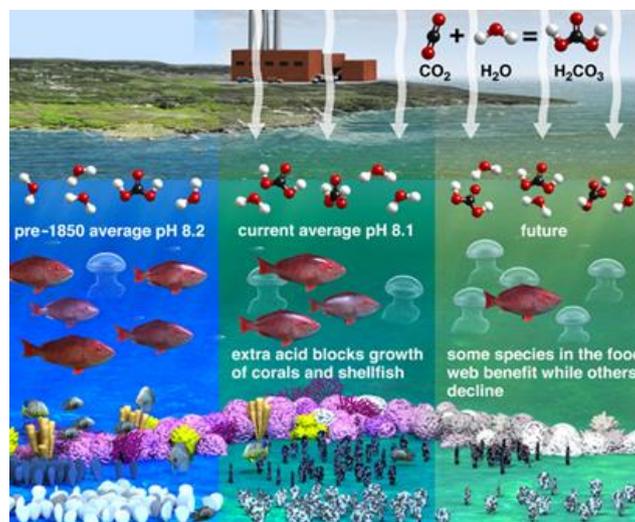


Figure 1.3. Past, present and future scenarios of Ocean Acidification on biogenic habitats and species.

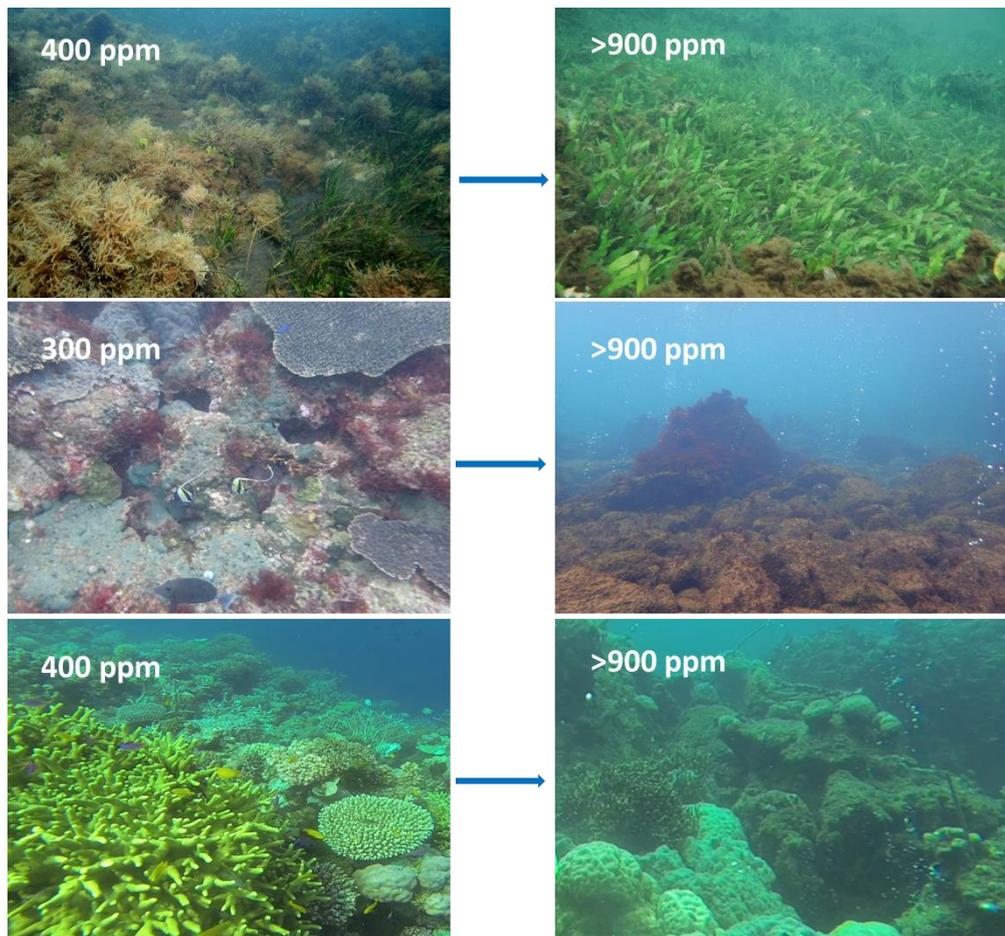


Figure 1.4. Habitat shifts at increased CO_2 levels at different climatic zones (temperate, sub-tropical and tropical)

While, for calcifying species, increases in pCO_2 generate deleterious effects on growth and calcification (Fabricius *et al.*, 2011; Kroeker *et al.*, 2013), autotrophic organisms such as macroalgae and seaweeds, enjoy an increase on photosynthetic rates (Falkenberg *et al.*, 2013). These habitat modifications generate habitat complexity changes that are not able to support the same species richness from before the disturbance. In order to assess the relationship between habitat complexity and species richness, Sunday *et al.*, (2016) evaluated, at a global scale (from temperate to tropical environment), the response of the principal habitat formers, and species richness, to pCO_2 changes.

The study was conducted in naturally acidified environments, along varying pH gradients. In such environments, it was possible to estimate the effects that acidity has on habitat complexity and, as a consequence, on species richness, by comparing present pH value to future trends. In a pristine environment like Papua New Guinea, on a coral reef, habitat complexity decreases along a pH gradient. As a consequence, Sunday *et al.*, (2016) measured a negative impact on the associated

invertebrates. There was no evidence of changes in fish composition and structure responses, probably attributable to the small extension of the studied area, or to the high mobility of fish.

Furthermore, they examined the consequences of decreasing pH on mussel beds and associated species (such as encrusting sponge, ephemeral and filamentous algae, and barnacles).

Their results showed a trend similar to the coral reef, with a decrease of species richness connected to the habitat.

An opposite trend was found in seagrass shoot density, because increases in CO₂ correspond to increases in photosynthetic rates. Conversely, none of the associated species were gaining any benefits (like incrusting epiphytes). A peculiar effect was observable in relation to the macroalgae population in two natural CO₂ seeps (Vulcano and Ischia Island, Italy): even though these species also benefited from an increase in photosynthetic rates caused by CO₂, they appeared to be replaced by low profile algae, specifically turf algae, which were quicker to colonize the bottom substrate, not allowing the establishment of larger erect algae (Fig. 1.4).

Effects of OA on species

The effects of OA on animal behaviour (Cattano *et al.*, 2018), as well as biological interactions, ecosystemic structure and functioning (Clements and Hunt, 2015), have also been subject to analysis. OA can cause behavioural disturbances in many species of fish, from impaired learning, to auditive and olfactive ability (Simpson *et al.* 2005; Vail & McCormick 2011), along with indirect effects on fish assemblages via habitat modifications, as reported above.

Increasing *p*CO₂ levels act as a driver of change to the neurosensorial capacity of fish, altering their innate ability to distinguish different environmental stimuli, and their behaviour as a consequence. Specifically, these authors have highlighted olfactive discrimination to different stimuli (Munday *et al.*, 2009, 2014), visual perception of predators (Ferrari *et al.*, 2012) and retinic function (Chung *et al.*, 2014), an impact on cerebral functions and fish survival (Ferrari *et al.*, 2012), lateralization (the tendency for some neural functions or cognitive processes to be selective to one side of the brain or the other, verified by different decision-making tasks involving left rather than right hemisphere

responses to environmental stimuli) (Nilsson, 2012; Domenici 2011, 2014), feeding (Chivers, 2014) and homing ability (Devine, 2012a).

Some evidence suggests that altered behavioural responses, under elevated levels of CO_2 , could be promoted due to the action of the neurotransmitter gamma-aminobutyric receptor (GABA-A), which is involved with inhibitory cognitive functions in the nervous system of vertebrates (Nilsson et al. 2012, Hamilton et al. 2014, Chivers et al. 2014, Lai et al. 2015, Nasuchon *et al.*, 2016). Specifically, in normal conditions, the ion gradient allows the inflow of Cl^- and HCO_3^- ions, leading to the hyperpolarization and inhibition of the neuron, by the opening of the GABA-A receptor. This process allows a reduction in neuronal activity to maintain a resting state, as normal neuronal Cl^- gradients are relatively close to electrochemical balance (Nilsson *et al.*, 2012)(Fig. 1.5). In conditions of ocean acidification, fish accumulate intracellular HCO_3^- ions in order to avoid acidosis and, as a consequence, Cl^- ions are pumped outside the cellular membrane, generating a depolarization of the neuron, and causing an excitation of the neural pathway not dissimilar to conditions of overactivity observed in epilepsy, resulting in altered behaviour.

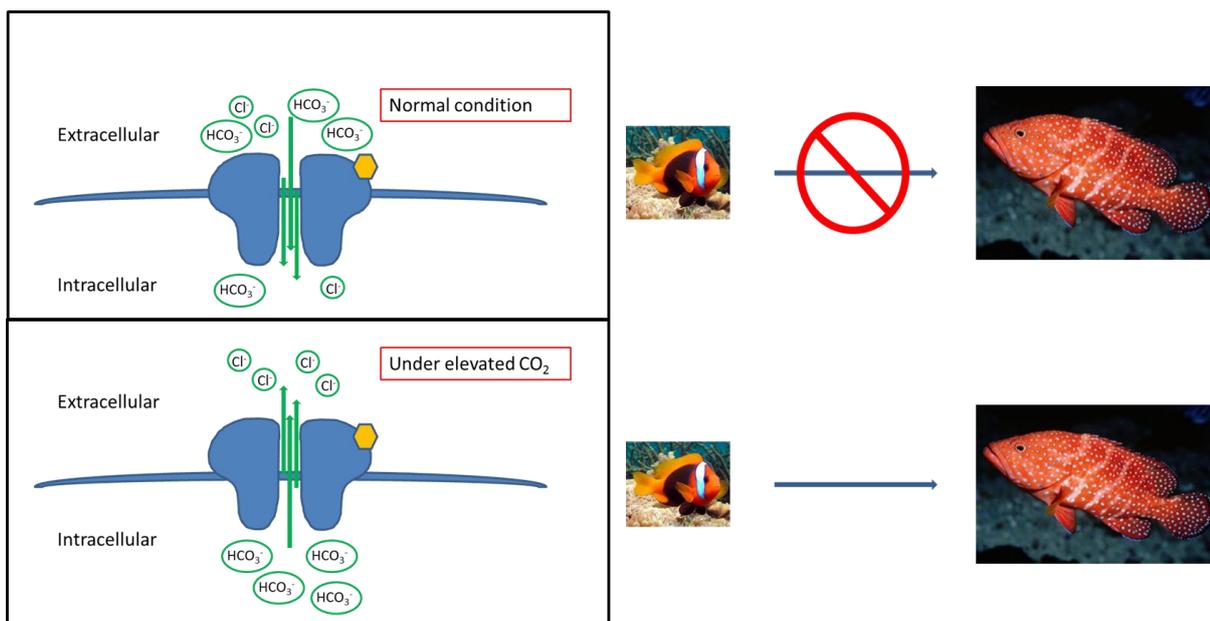


Figure 1.5. Different chemical environment within GABA-A receptors in normal and elevated CO_2 conditions (left) and example of the consequent change in behaviour (right)

It should be noted that not all species respond in the same way. As reported by Allan *et al.* (2014), the acclimation process can limit these effects in at least some. Through a transgenerational acclimation experiment (acclimating to 880 ppm), he noticed an attenuation of the effects of high

CO₂ in *Amphiprion melanopus* (prey) in its escape response to predator cues (described as predation rates according to success/number of attacks). However, he suggested that this acclimation process does not completely compensate the effects of high CO₂ on escape responses. Aside from transgenerational acclimation, some organisms can overcome these adverse conditions by carrying out different adaptations, such as evolutionary adaptation and epigenetic flexibility.

Aims of thesis

The broader purpose of my thesis was to investigate the structural and functional response of fish assemblages to different drivers of change. Particularly, with the use of novel video techniques and tethering experiments on the field, I assessed the effects that small-scale fisheries (SSF) have on the structure and functioning of fish assemblages along a gradient of protection in the context of Mediterranean MPAs.

Furthermore, using the same methodologies, I evaluated the effects of ocean acidification on temperate, tropical and sub-tropical coastal fish assemblages, by observing how CO₂-mediated changes in habitat complexity along a natural pCO₂ gradient may affect the composition and structure of the associated fish communities.

The specific chapters of my dissertation are briefly presented as follows:

Initially, I describe how, using a Baited Underwater Video system, I examined the main fish community descriptors in 11 Mediterranean MPAs along a gradient of exploitation (from No-take to Buffer to External areas) by SSFs, in order to estimate the health status and functioning of Western-Mediterranean MPAs. Through a meta-analytical approach, I evaluate and quantify the response of multiple-use MPAs, and how fishing activities could influence the effectiveness of protected zones compared to unprotected ones (Chapter 2). The subsequent chapter deals with a well-studied terrestrial theory: mesopredator release. With a tethering experiment, I evaluated predation rates in protected and unprotected zones in the context of MPAs, in order to quantify the effectiveness of top-down control compared to apex and mesopredator species relationship, both quantitatively and qualitatively (Chapter 3). In Chapter 4, I tackled the effects of ocean acidification on the ecosystem. Ecological surveys on both benthic habitats and fish assemblages were carried out in three volcanic seeps belonging to diverse climatic zones in the Mediterranean

and the Pacific ocean, with the main aim of assessing similar response trends. Particularly, through various techniques, I assessed both direct and indirect effects of Ocean Acidification (OA) on fish communities due to habitat changes and benthic community shifts, and whether such processes might lead to reduced top-down control (i.e. decreased fish predation intensity). Concluding remarks, key results and potential for future work are reported in Chapter 5.

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Chapter 2

Testing the ecological role of small scale fishery in Mediterranean marine protected areas

INTRODUCTION

In marine ecosystems, one essential factor involved in the efficiency of ecological communities is the relative strength of bottom-up and top-down control, as vectors for biomass distribution (Borer *et al.*, 2006): the combination of richer amounts of nutrients, leading to an increase in primary producers, on one side (bottom-up), and the effects of predation on the other (top-down), can actively control biomass at all trophic levels (Fretwell, 1977; Oksanen *et al.*, 1981).

In the literature, multiple studies have described the ability of an MPA to re-establish the carrying capacity of an ecosystem. In particular, its benefits consist of a multitude of positive ecological effects: increases in size, density and biomass of commercially exploited species, composition and structure of whole fish assemblages as well as a rise in trophic interactions (e.g. Guidetti and Sala, 2007; Lester and Halpern, 2008; Giakoumi *et al.*, 2017). The driving force behind the beneficial effects of MPAs is the so-called “reserve effect”: this concept is closely tied to the capability of unfished areas to re-establish biomass and target species abundance in entirely protected areas compared to fished ones (Garcia-Charton *et al.*, 2004). This phenomenon can ensure an increment in outgoing rate (e.g. from the no-take zone to adjacent areas) of fish biomass and the number of reproducers and juveniles: the spillover effect (Halpern *et al.*, 2010; Di Franco *et al.*, 2012; Di Lorenzo *et al.*, 2016), which benefits both the food web and small-scale, coastal fishing (Claudet *et al.*, 2008; Guidetti and Claudet, 2010). Meta-analysis studies though, show that this process is not consistent between different studies (Claudet *et al.*, 2008; Lester *et al.*, 2008; Sala *et al.*, 2012; Sciberras *et al.*, 2015; Giakoumi *et al.*, 2017), one of the issues consisting in the large age and size differences between the different areas under examination (Claudet *et al.*, 2008).

The same meta-analysis also demonstrated how increases in MPA performance are not necessarily related to buffer zone sizes but, more noticeably, are directly linked to lifetime and extension of entirely no-take areas (Claudet *et al.*, 2008). According to the above-mentioned authors, the buffer

zone represents a “double-edged sword”: as fishing activity is not strictly forbidden, the buffer can be exploited to the point of becoming a threat to target species in the whole area. Furthermore, the reciprocal conflict between authorities that manage MPAs and stakeholders (extractive users), makes the effectiveness of an MPA, at times, uncertain. As reported by Schroeder and Love (2002), “MPA performance” is driven by the range of activities permitted, as recreational fishing and recreational angling represent the primary source of fish mortality. “The only practical way to address the spatial distribution of community structure and its relationship with environmental and human factors is by measuring community structure across gradients of these factors” (Sala *et al.*, 2012).

Hence the idea, as suggested by Guidetti and Claudet (2010), to institute a co-management plan involving all parts that come into play in this context. Particularly, they emphasize the key-role of fishermen themselves as those designated to regulate fishing activities besides no-take and buffer zones. Their study revealed that, after two years, biomass and abundance related to catch per unit effort (CPU) were higher compared to the initial period.

European context

Due to the high amount of coexisting endemisms, and biotic and abiotic features that uniquely characterize it, the Mediterranean basin is considered a unique terrestrial and marine biodiversity hotspot (Coll *et al.*, 2010), on which the above-mentioned global trend of increasing coastal and marine human activities (tourism, pollutions, aquaculture, maritime transport, fishing) make it an area of particular ecological interest (Coll *et al.*, 2010; Rodríguez-Rodríguez *et al.*, 2015).

In this basin, MPAs are coordinated by different management bodies that act according to their own government (Gabrié *et al.*, 2012): most of them follow national policies, others are directly managed at the local or regional level, and only a small fraction are handled by non-governmental organizations (Gabrié *et al.*, 2012; Di Franco *et al.*, 2014). Furthermore, the wide range of different designations of MPAs (e.g. Marine Protected Area, Nature Reserve, National Park, Marine Reserve, Nature Park) (see Tab.XXX), can create additional issues.

Here Small Scale Fisheries (SSFs) - defined by the European Maritime and Fisheries Fund (EMFF) by the use of “fishing vessels of an overall length of fewer than 12m and not using towed fishing gear”- play a crucial role in maintaining the local population and have considerable socio-economic relevance (Quetglas *et al.*, 2016). In the Mediterranean Sea, their catch represent a large portion of

total fish caught (Natale *et al.*, 2015). These realities make use of smaller scale trammel nets and gillnets, trawl and set longlines (Natale *et al.*, 2015), each more or less species-selective. Obviously, the depletion of top predators (again characterized by slow-growing rates, large size, long life cycle and low recruitment) caused by fishing can bring a substantial benefit to the species these prey upon (Yodzis, 2001), but this phenomenon can lead to additional shifts in trophic interactions, altering their natural balance (Daskalov *et al.*, 2007). Theoretically, in an MPA, given the presence of no-take zones (where all activities are forbidden), a higher presence of top-predators should be expected, when compared to external areas that are not subject to the same limitations on fishing. Furthermore, the abundance biomass and species richness of whole fish assemblages should be greater in unfished zones (highest protection levels) than fished ones.

This study focuses on the performance of 11 Mediterranean MPAs, in order to investigate the potential effect of local artisanal fisheries on fish communities. By comparing the protected (No-take and Buffer) within unprotected (External) zones I will evaluate the health status of some Mediterranean MPAs by evaluating fish communities predictable variables.

METHODS

MPAs, sampling sites and spatial replication



Figure 2.1. Map of the 6 countries and 11 MPAs involved in the study

Fish assemblages were surveyed from June to September 2017 (Fig. 2.1, Table 2.1) at 11 Mediterranean Protected Areas, located in six different countries (France, Spain, Greece, Italy, Croatia and Slovenia), with varying management/protection schemes. Each Marine Protected Area (MPA) is governed by a specific national policy, resulting in differing zonation schemes. As an example, Italian and Spanish MPAs (Isole Egadi, Torre Guaceto, Portofino, Es Freus and Cabo de Palos) share a similar three-level protection scheme: Zone A (i.e. no-take, no-access), Zone B (buffer zone with local artisanal fishery permitted) and Zone C (general/buffer zone usually with only local artisanal fishery permitted). By contrast, the Greek MPA I worked on (Zakynthos) has a 6-month, non-permanent protection scheme, Slovenian and Croatian MPAs (Strunjan and Telašćica) have a two-level protection scheme (Take vs No-Take Zone), whilst French ones are quite mixed. This variability considered, a common, experimental design with 3 levels of protection was employed: No-Take, Buffer and External zones, with two random sites within each zone.

Table 2.1. Scheme of the selected MPAs with considered descriptors.

DESIGNATION	LOCATION	COUNTRY	IUCN CATEGORIES	ECOREGION <i>(Notarbartolo di Sciarra and Agardi (UNEP-MAP-RAC/SPA, 2010))</i>	VESSELS ALLOWED
Marine reserve	Es Freus	Spain	Not Reported	Algerian Provencal Basin	18
Marine Reserve	Cabo de Palos	Spain	VI	Algerian Provencal Bacin	19
Fisheries reserve	Cap Roux	France	V	Algerian Provencal Bacin	6
Marine Park	Côte Bleue	France	Not Assigned	Algerian Provencal Bacin	27
Natural Reserve	Bonifacio	France	IV	Tyrrhenian Sea	38
MPA	Torre Guaceto	Italy	IV	Ionian Sea	5
MPA	Egadi islands	Italy	IV	Tyrrhenian Sea	40
MPA	Portofino	Italy	V	Tyrrhenian Sea	22
Natural park	Telascica	Croatia	V	Adriatic Sea	7
Landscape park	Strunjan	Slovenia	V	Adriatic Sea	10
Marine national park	Zakynthos	Greece	II	Ionian Sea	35

Samplings were performed at every level of protection present in each MPA (no-take, partially protected and unprotected). In two of them (Cote Bleue and Cap Roux), only the no-take zone is present, with no partial protection (buffer) zones between the no-take and the unprotected (external) areas around. In these two MPAs, external sites at a different distance from MPA borders have been sampled (2 close to it, and 2 sites at a higher distance) (Fig. 2.2): this has allowed us to draw parallel samplings between any sort of MPA structure.

According to the IUCN (International Union for Conservation of Nature), each MPA (understood as every protection's form in a spatially explicit environment) has a primary protection objective. These are subdivided into 6 categories each of them with a mainly managing features:

- Category Ia- Strict Nature Reserve
- Category Ib- Wilderness Area
- Category II- National Park
- Category III- Natural Monument of Feature
- Category IV- Habitat/Species Management Area
- Category V- Protected Landscape/Seascape
- Category VI- Protected Area with sustainable use of natural resources

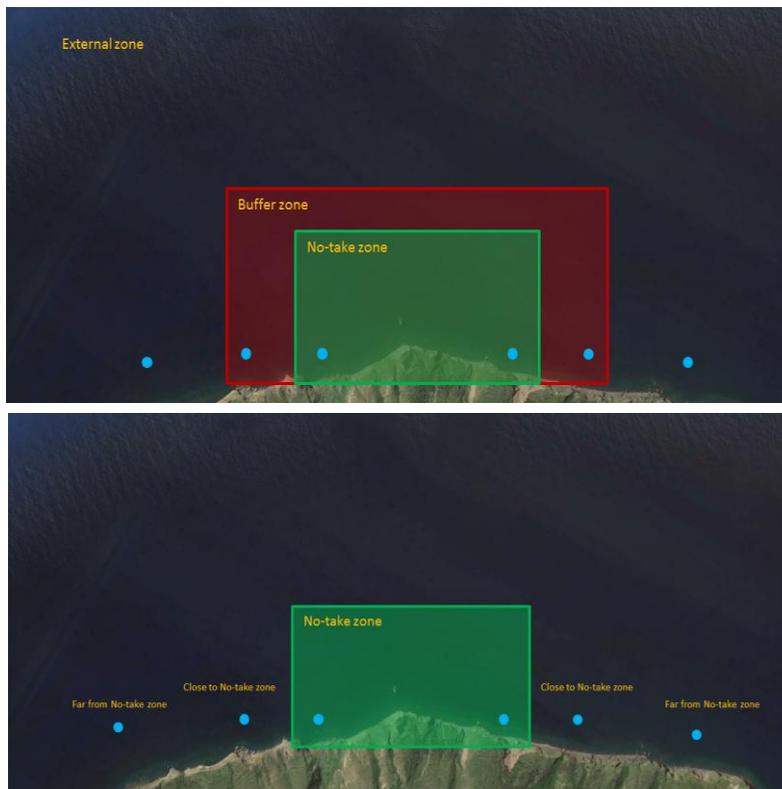


Figure 2.2. Scheme of MPA zoning for a) multi-use “standard” MPA; b) no-take only MPA (also called marine reserve); blue dots represent BUUV sampling positions.

To achieve the desired outcome, two sampling techniques have been used, which are suitable for cross-sectional studies. Instead of more common visual census techniques, two novel techniques were employed to study fish assemblages composition and their processes: *Baited Underwater Video* (BUV) and *Squidpop* surveys. These methods avoid the possible influence caused by the presence of the diver, that could otherwise lead to biased estimations relatively to fish behaviour.

To assess the spatial variability of shallow water fish assemblages in the MPAs, the BUV systems were deployed in three different zones (no-take, buffer and external zones) at two random sites each. Thirty-six replicate samplings were collected for each MPA for a total of 378 deployments.

Rocky reefs represent the most vital bottom type to be protected, as it commonly hosts most of the species targeted by fishing (Boero *et al.*, 2005; Guidetti *et al.* 2008). For this reason, samplings were carried out on rocky bottoms at depths between 5 and 15 meters at the same daylight hours

of 8:00 A.M. and 3:00 P.M. In addition, GPS coordinates were marked down for each replicate sampling.

In order to assess fish composition, abundance, and biomass of different trophic guilds, (including pelagic species that tend to be shyer and harder to detect by scuba divers), fish data was collected using the Stereo-Baited Underwater Video system technique (BUVs) (e.g. Stobart *et al.*, 2007; Whitmarsh *et al.*, 2016).

To assess and obtain a quantifiable and standardized estimate of predation pressure, and to characterize predatory fish metrics (species composition and abundance) along the examined gradient of protection, and the extent of exploitation by small-scale fisheries, a total of 144 Squidpops have been deployed within the eleven MPAs. For each protection level (no-take, buffer and external), replicates were randomly located within 2 sites (with about fourteen replicates within each MPA). Like the BUVs, each Squidpop's replicate sampling was deployed during daylight hours and positioned 500m in rocky bottoms at depths between 5 and 15 meters, at a safe distance from the BUV systems, in order to avoid interference between the two bait types.

Stereo Baited Underwater Video system

The Baited Underwater Video system is a novel technique that consists of a stainless steel frame (120x80 cm, respectively the primary and secondary axis) (Fig. 2.3) equipped with two GoPro Hero 3 high definition cameras (720p@60 fps) with a wide-angle lens, continuously recording for one hour. In front of the cameras, at 1.20 m distance, a mesh bag with 400 g of crushed *Sardina pilchardus* was placed to attract fish inside the field of view. Due to their high oil dispersion, this bait type ensured the arrival of different species belonging to different trophic levels. As reported in multiple studies, sardines represents the most effective type of bait at attracting fish thanks to Odour-plume dispersion (Westerberg *et al.*, 2010; Wraith *et al.*, 2013). To ensure proper stabilization during descent, a lead has been fixed at each extremity of the secondary axis of the frame.

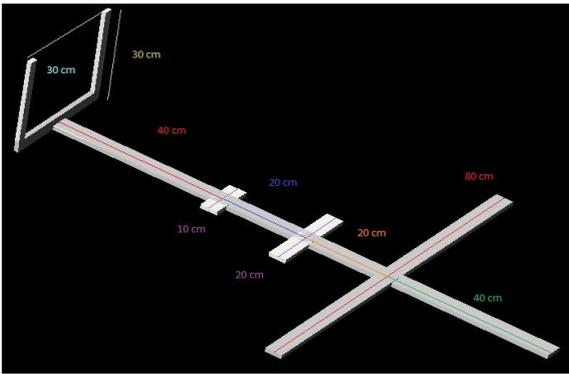


Figure 2.3. Baited Underwater Video system scheme

BUVs were lowered from the boat to the sea floor and recovered using a rope attached to a surface buoy marking the location. Each replicate was left recorded for 60 min around, and the start and end time were checked. To avoid the pseudo-replication, each BUV system were deployed 150 meters apart in the same location.

Up to 12 BUV replicates were dropped in a single day with freshly crushed sardines filled for each deployment. A total of 378 were deployed during the survey campaign. Only in Strunjan's MPA were made four replicates for each random site due to the small extension of MPA. Due to the low visibility or the instrument sideways dropped, some replicates were deleted from the total count. Particularly 370 was used in total for the analysis.

BUVs Analyses

For each 60min video, species richness (S) and MaxN (a conservative measure of abundance calculated as the maximum number of fish of the same species identified over the observation period) were successively analyzed and chronicled. To evaluate species richness, every fish sighted in the field of view was considered, regardless of proximity, whilst $MaxN$ was calculated taking only species present within 2 meters around the bait in consideration (Fig. 2.4).

All length measurements were made by using Myrtus (M.I.T. GmbH company) after prior calibration. Footage from each camera (left and right) was synchronized and the length of fishes was considered as total length (TL) and measured at time of MaxN (Watson *et al.*, 2010; Barley *et al.*, 2017).

For each individual was used the allometric relationship between weight (W) and length (L).

$$W=aL^b$$

where “a” and “b” are species-specific constant available on FishBase (<https://www.fishbase.de>).

The total biomass of a species per sample was calculated by multiplying the maximum number of individuals with the maximum average size of the same multiplied by the conversion coefficients.



Figure 2.4. Different species attracted to a Baited Underwater Video system in different MPAs.

Analysis

Meta-analytical approach on fish assemblages

In order to assess and summarize an overall effect of the principal variables analysed, a meta-analysis summarizing the general trend of fish to different levels of protection was used. A meta-analytical approach has been used to summarize data collected from eleven MPAs, providing results of independent experiments. Results are reported as Effect-size analysed in the metric of

the natural logarithm of the ratio among the values of the response variable (i.e. species richness, MaxN) between no-take vs external zones and buffer vs external zones, in order to examine the potential role of fisheries in areas where fisheries are strictly forbidden or local artisanal fisheries is allowed as response ratio (Micheli et al., 2004; Guidetti et al., 2008). A positive response ratio means an individual abundance or species richness greater within experimental groups rather than in controls (i.e. higher values in no-take rather than external zones). A negative response means the opposite trend (i.e. the abundance and/or species richness is higher in fished zones than unfished). Null effect means that there are no differences between experimental and control groups.

The Effect size estimate was made by determining the log response ratio (Ln(RR)) calculated as:

$$\text{Ln}(\bar{Y}_E/\bar{Y}_C) \rightarrow (\bar{Y}_{\text{no-take}}/\bar{Y}_{\text{external}}) \text{ and } (\bar{Y}_{\text{buffer}}/\bar{Y}_{\text{external}})$$

where \bar{Y}_E and \bar{Y}_C were the mean values of a variable (species richness or MaxN) in experimental ($\bar{Y}_{\text{no-take}}$ or \bar{Y}_{buffer}) and control areas ($\bar{Y}_{\text{external}}$) (protected and unprotected zones respectively). To test how far samplings vary from the average value, the variance associated with the estimate means of different groups were analysed.

$$S_{\text{LRR}} = \frac{S^2_E}{n_{(E)} \bar{Y}^2_E} - \frac{S^2_C}{n_{(C)} \bar{Y}^2_C}$$

These was provided by the ratio between the mean square for the no-take zones and the sample size for each group. The equation represents the variance of Ln(RR), where S^2_E and $n_{(E)}$ constitute the variance and number of replies in experimental groups and S^2_C and $n_{(C)}$ are the variance and number of replies in control groups. The methods “continuous fixed-effect inverse variance” was utilized. This method performs a fixed-effect meta-analysis with inverse variance weighting. It is important to note that a significant effect occurs when the 95% confidence limit around the average response ratio value does not fit into zero (Guidetti and Sala 2007).
Confidence Interval:

$$\text{CI} = \text{Ln}(\text{RR}) \pm (t_{\alpha/2}(n-1)) * S_{\text{LRR}}$$

where t is a critical value determined from the t ($n-1$) distribution.

Furthermore, the responses to protection have been also measured as effect size on commercial value level (Claudet *et al.*, 2008) and functional level (i.e. trophic groups) based on Guidetti (2014).

RESULTS

A total of 370 BUV deployments and 154 Squidpop were carried out in 11 MPAs from June to September 2017.

BUVs Results

A total of 69 fish taxa belonging to 26 Families were recorded from BUV surveys. Overall, the main Families represented were Labridae (15 species) and Sparidae (15 species). Approximately 40% of fish taxa were present in 6, or more, out of 11 MPAs. Most of the species recorded could be considered frequent, while some species were exclusive to certain MPAs (i.e. the herbivores *Sparisoma cretense*, *Siganus luridus* and *S. rivulatus* in Zakynthos Marine National Park or *Diplodus cervinus*, *Gymnothorax unicolour*, *Parapristopoma octolineatum*, *Myliobatis aquila* in Cabo de Palos Marine Reserve) (Tab. 2).

It was possible to identify exact species from all families except for Clupeidae, Mugilidae and Gobiidae, whose identification was not possible in many cases.

Table 2.2. List of fish taxa recorded through BUVs deployments ($n=370$) in eleven MPAs (number of BUV deployments for each MPA in brackets). Commercial value, trophic group, MaxN of each taxon and Max % presence of all species within each MPA is reported.

Species	Commercial value	Trophic group	Max % presence	Bon	CdP	CR	CB	EF	Ega	Por	Stru	Tel	TGA	Zan
				(36)	(27)	(36)	(36)	(36)	(34)	(35)	(25)	(36)	(36)	(33)
<i>Anthias anthias</i>	NC	PL	9							3				
<i>Apogon imberbis</i>	NC	PL	55	1		2	2		2	1		2		
<i>Atherina boyeri</i>	LC	PL	27					16					3	49
<i>Balistes carolinensis</i>	LC	CA	18					1				1		
<i>Blennidae</i>	NC	CA	9									2		
<i>Boops boops</i>	C	PL	91	9	14	3	20	12	9		22	81	2	9
<i>Chromis chromis</i>	NC	PL	100	87	120	56	52	69	80	150	41	119	30	74
<i>Clupeidae</i>	C	PL	27			20		2						1
<i>Conger conger</i>	C	AP	36				1			1	1	1		
<i>Coris julis</i>	NC	CA	100	19	16	9	19	10	15	20	3	5	9	4
<i>Ctenalabrus rupestris</i>	LC	CA	18				1					1		
<i>Dactylopterus volitans</i>	NA	CA	9					2						
<i>Dasyatis pastinaca</i>	LC	CA	18					2						1
<i>Dentex dentex</i>	C	AP	82	2	3	1	1		1	3		1	2	1

<i>Dicentrarchus labrax</i>	C	AP	18							1			1	
<i>Diplodus annularis</i>	C	CA	100	9	4	11	2	26	2	15	2	17	2	9
<i>Diplodus cervinus</i>	C	CA	9		2									
<i>Diplodus puntazzo</i>	C	CA	82	2	3	1	1	2	1	1		14		1
<i>Diplodus sargus</i>	C	CA	100	7	8	10	13	4	5	24	2	4	2	11
<i>Diplodus vulgaris</i>	C	CA	100	16	9	6	4	13	102	20	2	8	3	6
<i>Epinephelus costae</i>	C	AP	18		7			1						
<i>Epinephelus marginatus</i>	C	AP	45		1			1		2		1		1
<i>Gobiidae</i>	NC	CA	55	1		1			1		3		1	1
<i>Gymnothorax unicolor</i>	NC	AP	9		2									
<i>Labrus bergylta</i>	C	CA	27	1				1		1				
<i>Labrus merula</i>	C	CA	45	1		1	1			1	2			
<i>Labrus viridis</i>	C	CA	55	1		1	1	1	1	1				
<i>Lithognathus mormyrus</i>	C	CA	18			4		4						
<i>Mugilidae</i>	C	DE	55			2	2	1		30	2	10		
<i>Mullus barbatus</i>	C	CA	9								5			
<i>Mullus surmuletus</i>	C	CA	91	2	3	1	1	4		9	5	3	1	1
<i>Muraena helena</i>	C	AP	82	1	2	2	1	2	2	2		3		3
<i>Myxeroperca rubra</i>	C	AP	18		2					1				
<i>Myliobatis aquila</i>	NC	AP	9		1									
<i>Oblada melanura</i>	C	PL	100	8	7	23	5	6	1	64	14	36	1	11
<i>Pagellus erythrinus</i>	C	CA	9								10			
<i>Pagellus sp.</i>	C	CA	9										4	
<i>Pagrus pagrus</i>	C	CA	36				1			1	1		2	
<i>Parablennius gattoruggine</i>	NC	CA	9							1				
<i>Parablennius pilicornis</i>	NC	CA	36		3		1				2	2		
<i>Parablennius rouxi</i>	NC	CA	27				1					2	1	
<i>Parapristopoma octolineatum</i>	LC	CA	9		8									
<i>Pseudocaranx dentex</i>	LC	CA	9									3		
<i>Raja sp.</i>	C	CA	9					1						
<i>Sardinella aurita</i>	C	PL	9					1						
<i>Sarpa salpa</i>	LC	HE	82	22	180	23	17	21		70	12	1	1	
<i>Sciaena umbra</i>	C	CA	36		7		5	3		12				
<i>Scorpaena maderensis</i>	C	CA	9									1		
<i>Seriola dumerili</i>	C	AP	45	2	11	1						11		8
<i>Serranus cabrilla</i>	C	CA	91	3	2	2	5	1	2	7		2	1	2
<i>Serranus hepatus</i>	NC	CA	18								7		1	
<i>Serranus scriba</i>	LC	CA	100	3	3	4	3	3	20	5	2	5	2	3
<i>Siganus luridus</i>	LC	HE	9											10
<i>Siganus rivulatus</i>	NC	HE	9											1
<i>Sparisoma cretense</i>	LC	HE	9											8
<i>Sparus aurata</i>	C	CA	73	1	1	1	1	1		12	1	3		
<i>Sphyaena sp.</i>	C	AP	45		26	2		1	4	4				
<i>Spicara maena</i>	LC	PL	27	6		1						10		
<i>Spicara smaris</i>	LC	PL	55	22			3	2	3		8			5
<i>Spondyliosoma cantharus</i>	C	CA	100	17	2	3	2	1	1	10	1	12	4	1
<i>Symphodus bailloni</i>	NC	CA	18		1				1					
<i>Symphodus cinereus</i>	NC	CA	36			1			1		3		1	
<i>Symphodus doderleini</i>	NC	CA	18			1			1					
<i>Symphodus mediterraneus</i>	NC	CA	91	3	1	2	2	5	2	3		1	2	2
<i>Symphodus melanocercus</i>	NC	CA	82	1	1	1	3	1	2	2		3		1
<i>Symphodus ocellatus</i>	NC	CA	91	12	4	7	3	5	6	2		1	1	1
<i>Symphodus roissali</i>	NC	CA	64	2	1	1	2	1	1	2				
<i>Symphodus rostratus</i>	NC	CA	82	2	1	6	1	8	2	1		1		1
<i>Symphodus tinca</i>	LC	CA	91	8	3	5	17	8	6	3		2	1	1
<i>Thalassoma pavo</i>	NC	CA	82	2	45	3	1	27	23	1		6		14
<i>Trachurus sp.</i>	C	CA	18						1		5			
Tot.				31	35	35	34	38	29	36	24	35	24	30

When considering all 11 MPAs together, no-take zones exhibit the highest variability of species (66 fish taxa) whereas Buffer and External zones showed lower values (62 and 59 respectively)(Fig. 2.6a). The lowest mean value (\pm SD) of species richness was found in the no-take area of Strunjan, Slovenia (2.8 ± 1.5), the the highest in the no-take zone of Cabo de Palos (14.8 ± 2.6).

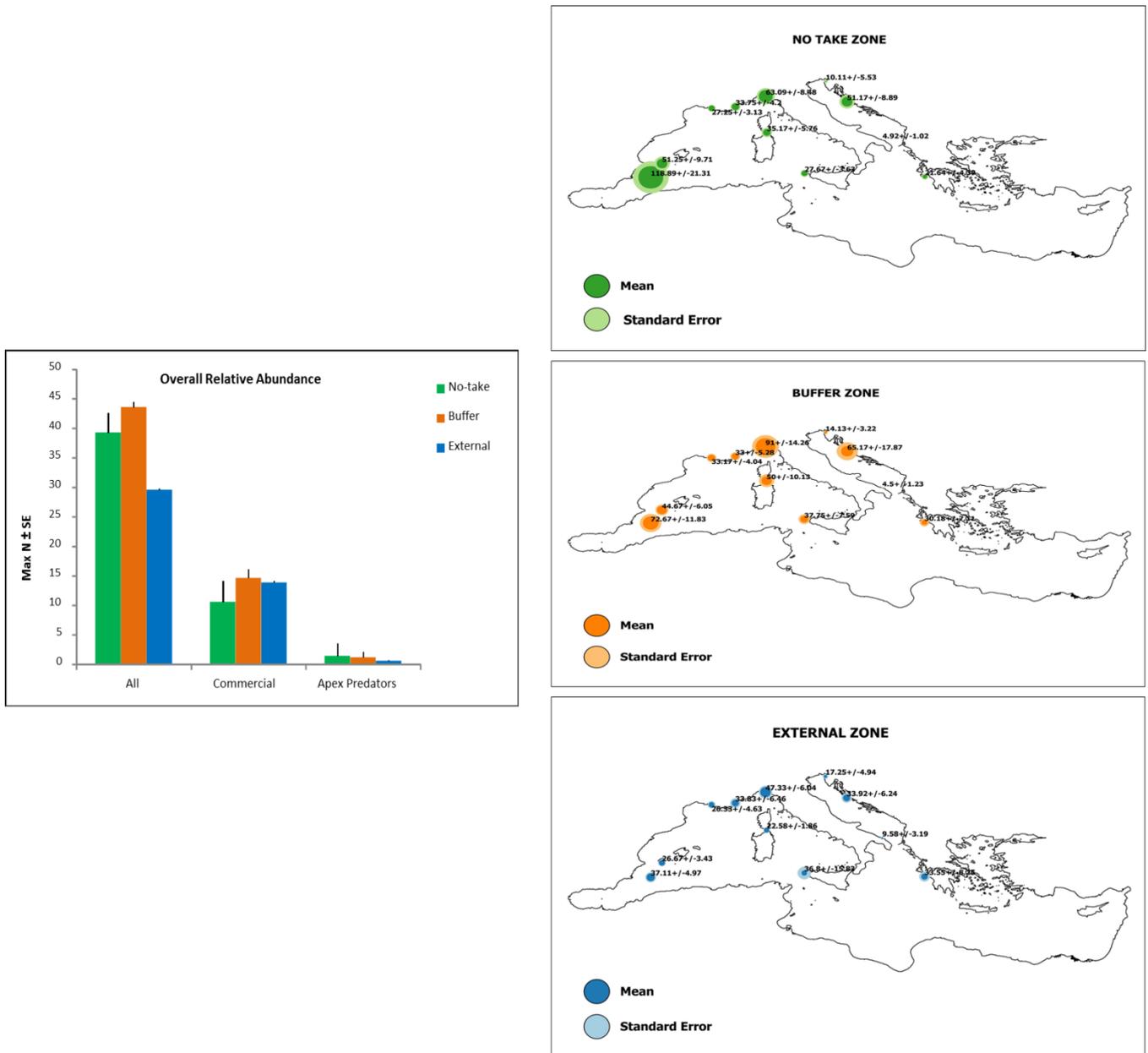


Figure 2.5. Relative abundance (MaxN) (mean \pm SE) of all 11 MPAs reported for each level of protection (No-take, Buffer and External zone).

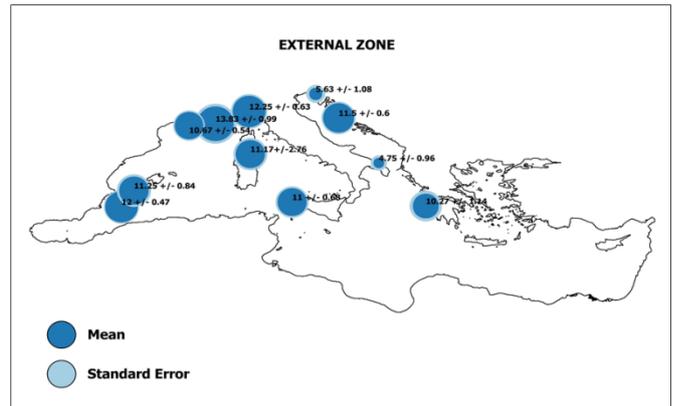
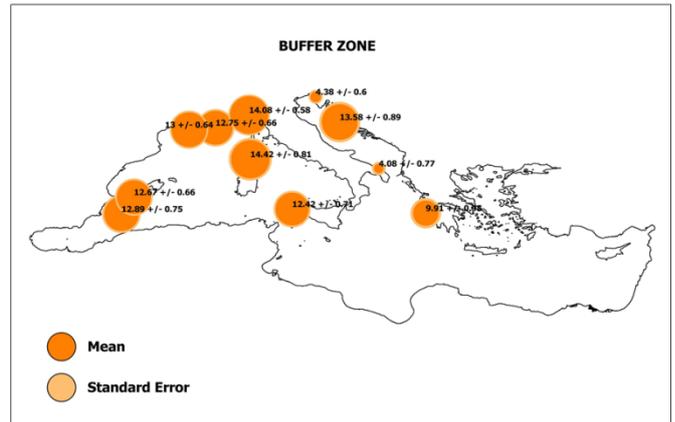
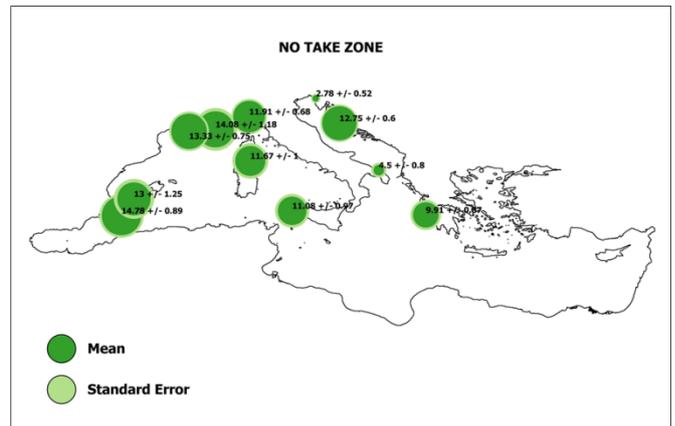
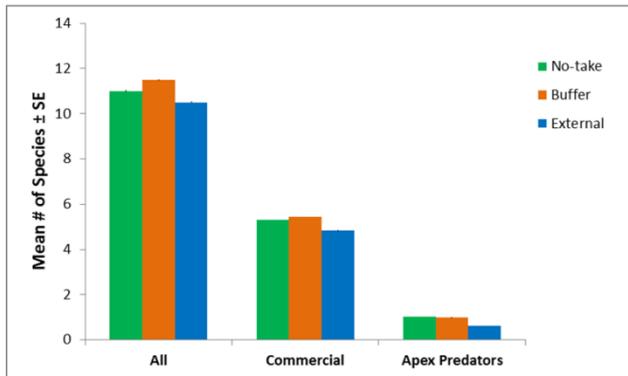


Figure 2.6. Species Richness (mean ± SE) of all 11 MPAs reported for each level of protection (No-take, Buffer and External zone).

In order to highlight potential effect that protected areas have on species that should primarily benefit from protection, they were categorized according to which are mainly targeted by commercial and recreational fishing.

A particular emphasis was put on commercially valuable species (based on Claudet et al., 2008) and their trophic group in general as apex predators (based on Guidetti et al., 2014). It should, however, be noted that not all species have the same commercial value in all of the examined countries, due to historical and cultural factors. (Tab. 2.2).

When considering all 11 MPAs together, External zones exhibit the highest presence of commercial species (35 fish species) followed by no-take areas (34 fish species), while species of lower commercial value were most common in Buffer zones (Fig. 2.6).

A higher variety of apex predators was found in no-take (9 species) and buffer (8 species) zones rather than external ones (7 species) (Fig. 2.6)

The relative mean abundance (\pm SD) of different species, per level of protection, ranged from 118.8 (\pm 63.9) in the no-take zone of Cabo de Palos Marine Reserve to 4.5 (\pm 4.2) in the partially protected site of Torre Guaceto MPA.

As shown in figure 2.5, mean relative abundance result was found to be higher inside MPAs (both in buffer and no-take zone) than outside. Furthermore, the mean Max N (\pm SD) of commercially valuable species, was similar between buffer and external zones (14.6 (\pm 16.6); 13.9 (\pm 10.6) respectively), and lower in totally protected zones (10.5 \pm 10.3) (Fig. 2.5).

Regarding the mean (\pm SD) relative abundances per hour of apex predators, along the same gradient of protection/exploitation, no-take and buffer zones exhibit a higher number of individuals (1.39 (\pm 2.7); 1.17 (\pm 3.16)) than outside, where abundance was considerably lower (0.6 (\pm 0.8)).

Overall Species Richness, relative abundance (MaxN) and fish biomass

The overall MaxN, species richness (S) and biomass of fish assemblages were significantly greater in protected areas than in unprotected areas nearby (RR MaxN_(No take/External) = 0.282, CI= 0.22; RR MaxN_(Buffer/External) = 0.388, CI= 0.212; RR S_(Buffer/External) = 0.09, CI= 0.089; RR Biomass_(No take/External) = 1.711, CI = 1.203; RR Biomass_(Buffer/External) = 1.279; CI= 0.609), excepting for no-take vs external species richness ratio (Fig. 2.7).

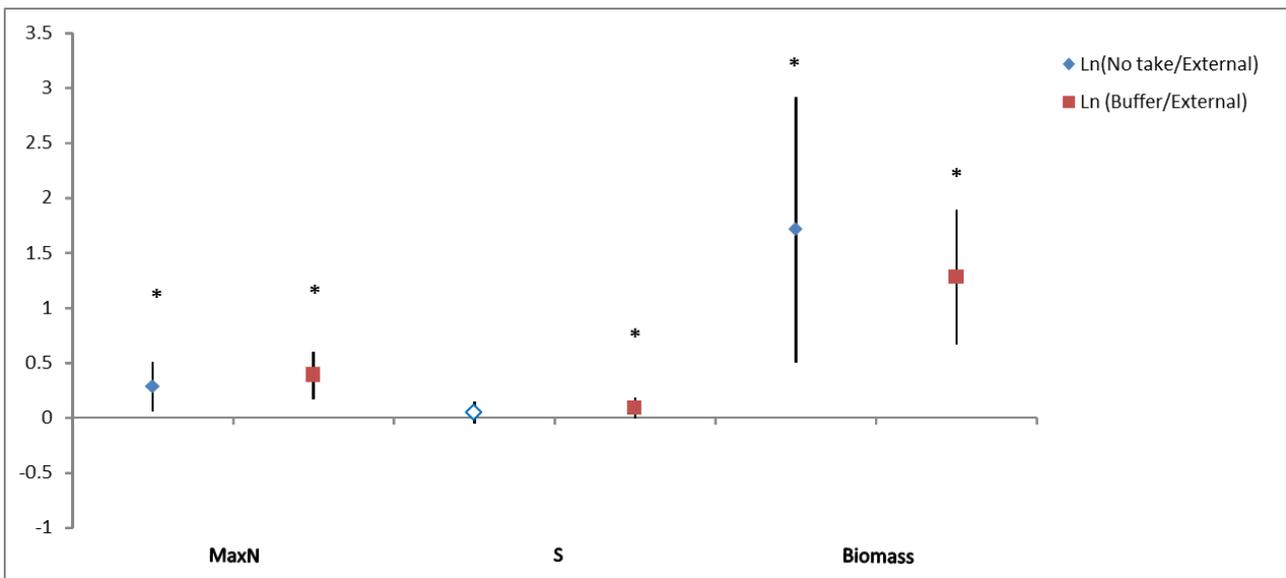


Figure 2.7. Ln(RR) of MaxN, species richness and biomass. response ratio between no-take and External areas(blue diamond), response ratio between Buffer and External areas(red square). Bars indicate 95% C.I.; the asterisks a significant effect on response ratio.

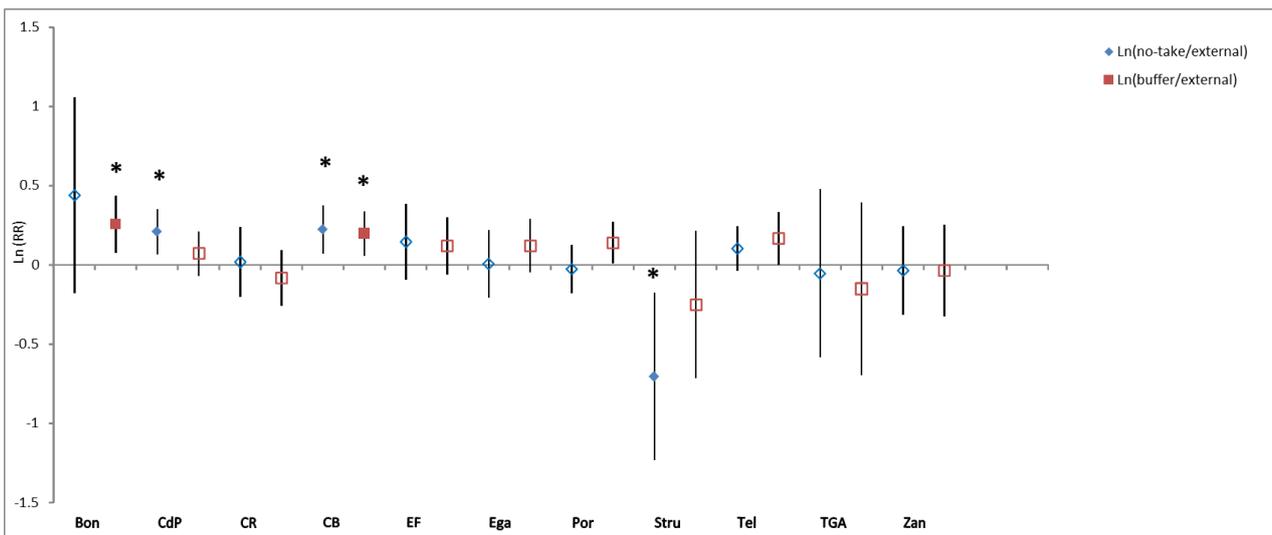


Figure 2.8. Ln(RR) of species richness. Blue diamonds represent the response ratio between no-take and External areas, Red squares the response ratio between Buffer and External areas. From left to right: Bonifacio (Bon), Cabo de Palos

(CdP), Cape Roux (CR), Cote Bleue (CB), Es Freus (EF), IE (Egadi Islands),Portofino (Por), Strunjan (Stru), Telasciça (Tel), Torre Guaceto (TG) and Zante (Zan). Bars indicate 95% C.I.; asterisks mark a significant effect on response ratio.

The response ratio recorded between protected and unprotected zones regarding total species richness was very diverse among the 11 MPAs (Fig. 2.8). Specifically, while *Cabo de Palos* and *Cote Bleue* showed a significant effect when comparing no-take and external zones (RR= 0.208, CI=0.141 and RR= 0.223, CI= 0.149 respectively), there was a negative effect in the ratio between the no-take and external zone in *Strunjan Landscape Park* (RR= -0.706, CI= 0.527). When comparing buffer zones, only *Bonifacio* and *Cote Bleue* showed a significant and positive effect (RR= 0.255, CI= 0.178 and RR= 0.198, CI= 0.139 respectively), while every other area did not.

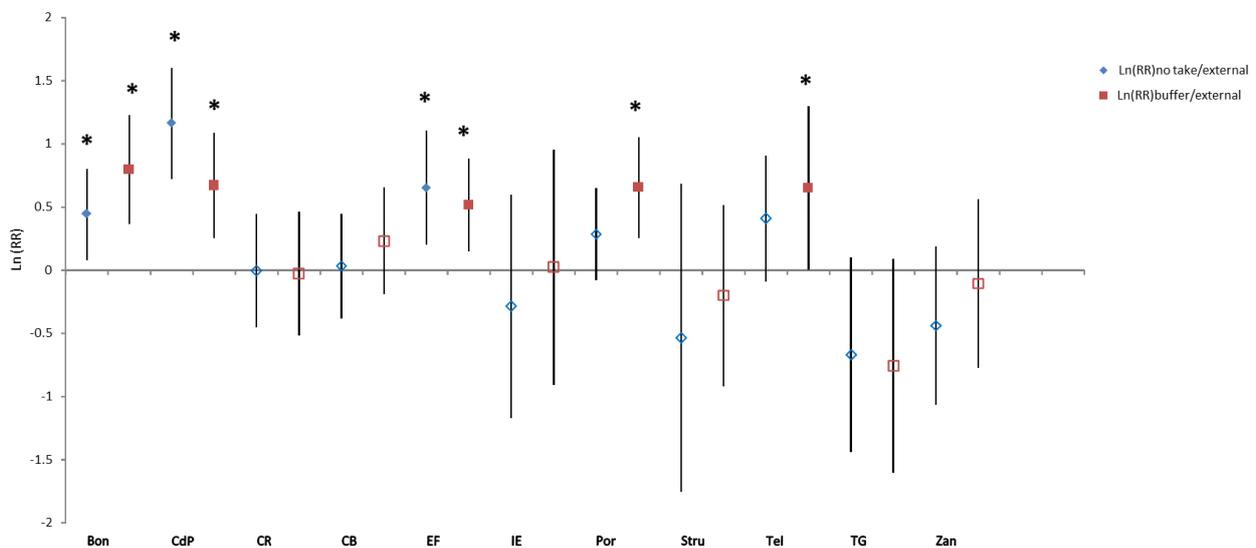


Figure 2.9. Ln(RR) of MaxN. Blue diamonds represent the response ratio between no-take and External areas, Red squares the response ratio between Buffer and External areas. From left to right: Bonifacio (Bon), Cabo de Palos (CdP), Cape Roux (CR), Cote Bleue (CB), Es Freus (EF), IE (Egadi Islands),Portofino (Por), Strunjan (Stru), Telasciça (Tel), Torre Guaceto (TG) and Zante (Zan). Bars indicate 95% C.I.; asterisks mark a significant effect in response ratio.

Analysing the mean relative abundance for each MPA (Fig. 2.9), their effect on protection was distinct. In some cases, such as *Bonifacio Natural Reserve* ($RR_{(no-take/external)}=0.443$, $CI_{(no-take/external)}=0.36$; $RR_{(buffer/external)}=0.795$, $CI_{(buffer/external)}=0.429$), *Cabo de Palos* ($RR_{(no-take/external)}=1.164$, $CI_{(no-take/external)}=0.438$; $RR_{(buffer/external)}=0.672$, $CI_{(buffer/external)}=0.413$) and *Es Freus Marine Reserve* ($RR_{(no-take/external)}=0.653$, $CI_{(no-take/external)}=0.448$; $RR_{(buffer/external)}=0.516$, $CI_{(buffer/external)}=0.366$), the Response Ratio between the analysed groups showed positive effects.

In *Portofino* and *Telasciça*, a positive effect was documented in the ratio between the relative abundance in buffer zones compared to external (RR=0.654, CI= 0.396; RR=0.653, CI= 0.647 respectively).

The remaining MPAs did not show significant differences in response ratios.

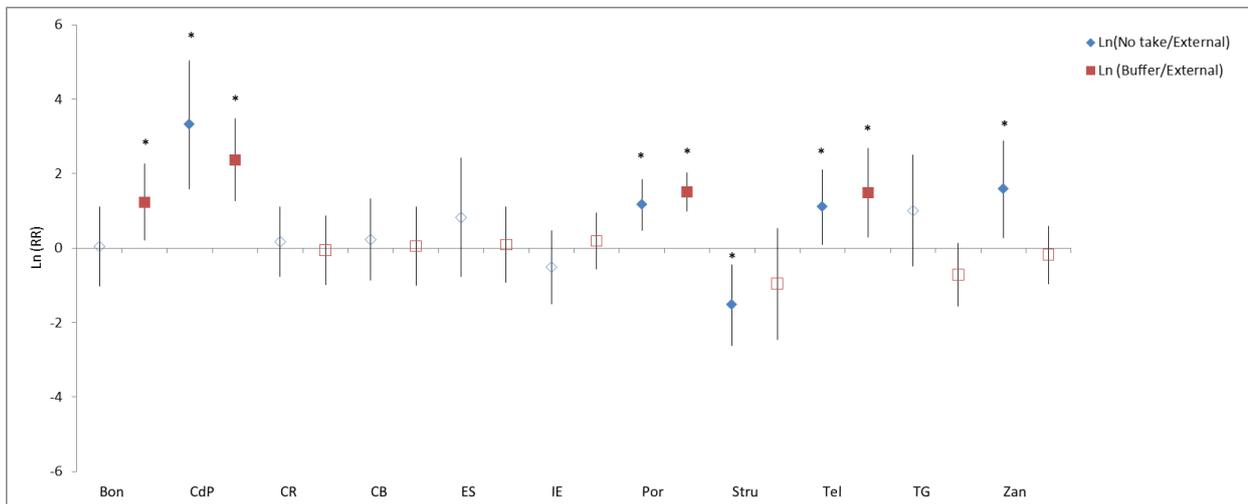


Figure 2.10. Ln(RR) of biomass. Blue diamonds indicate the response ratio between no-take and External areas, Red squares the response ratio between Buffer and External areas (red square). From left to right: Bonifacio (Bon), Cabo de Palos (CdP), Cape Roux (CR), Cote Bleue (CB), Es Freus (EF), IE (Egadi Islands), Portofino (Por), Strunjan (Stru), Telasciça (Tel), Torre Guaceto (TG) and Zante (Zan). Bars indicate 95% C.I.; asterisks mark a significant effects on response ratio.

Looking at the biomass response ratio (Fig. 2.10), the trend between inside and outside behaviour was diverse. A particularly positive effect was documented in *Cabo de Palos* ($RR_{(no-take/external)}= 3.315$, CI= 1.726; $RR_{(Buffer/external)}= 2.273$, CI= 1.113), *Portofino* ($RR_{(no-take/external)}= 1.164$, CI = 0.688; $RR_{(Buffer/external)}= 1.514$, CI= 0.518) and *Telasciça* ($RR_{(no-take/external)}= 1.104$, CI= 1.003; $RR_{(Buffer/external)}= 1.489$, CI= 1.188), where the Buffer to External zone biomass ratio suggest a trend towards the so-called “reserve effect”. In *Bonifacio*, only the Buffer/External biomass ratio showed a positive effect ($RR_{(Buffer/external)}= 1.239$, CI= 1.027), while in *Zakinthos* only the No take/External ratio did ($RR_{(no-take/external)}=1.58$, CI= 1.305). There was no significant protection effect on biomass in *Cape Roux*, *Cote Bleue*, *Es Freus* and *Isole Egadi*, while in *Strunjan*, as was also the case for species richness, biomass resulted higher outside the MPA compared to the No take zone ($RR_{(no-take/external)}= -1.528$, CI= 1.085).

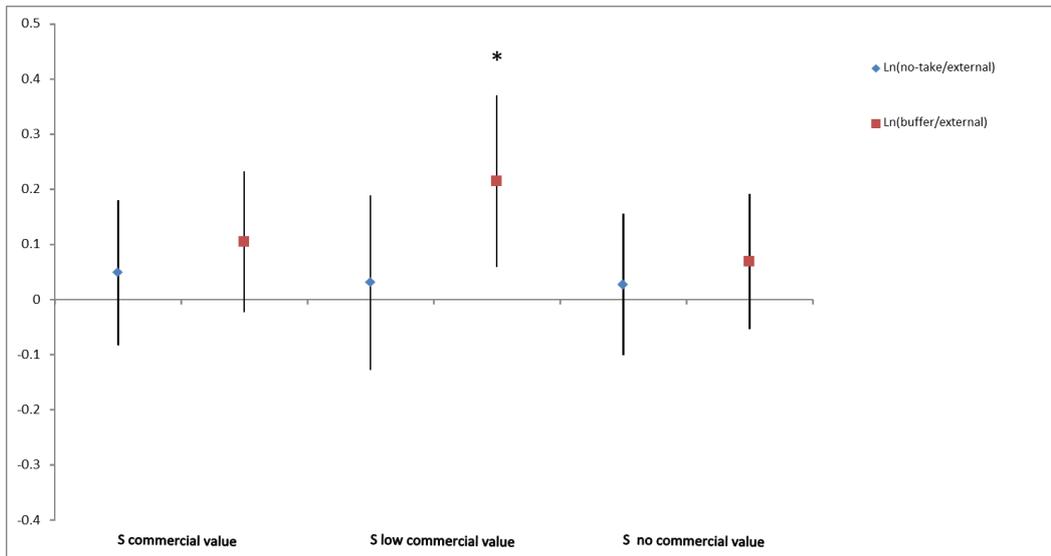


Figure 2.11. Ln(RR) of species richness related to commercial value species (high, low and no commercial value). Blue diamonds indicate the response ratio between no-take and External areas, Red squares the response ratio between Buffer and External areas (red square). Bars indicate 95% C.I.; asterisks mark a significant effect on response ratio.

Going deeper in detail, the response in species richness was analysed by dividing this variable into “sub-variables” to highlight the effect that protection has on species that are targeted by fishing compared to those that are not. With the exception of the ratio between buffer and external zones of species with low commercial value, which showed a positive effect ($RR_{(buffer/external)}=0.215$, $CI=0.155$), for all other species (S commercial and no commercial) the confidence interval overlapped the zero value, indicating that the differences were not statistically significant (Fig. 2.11). This can probably be explained by the relatively low number of species sampled.

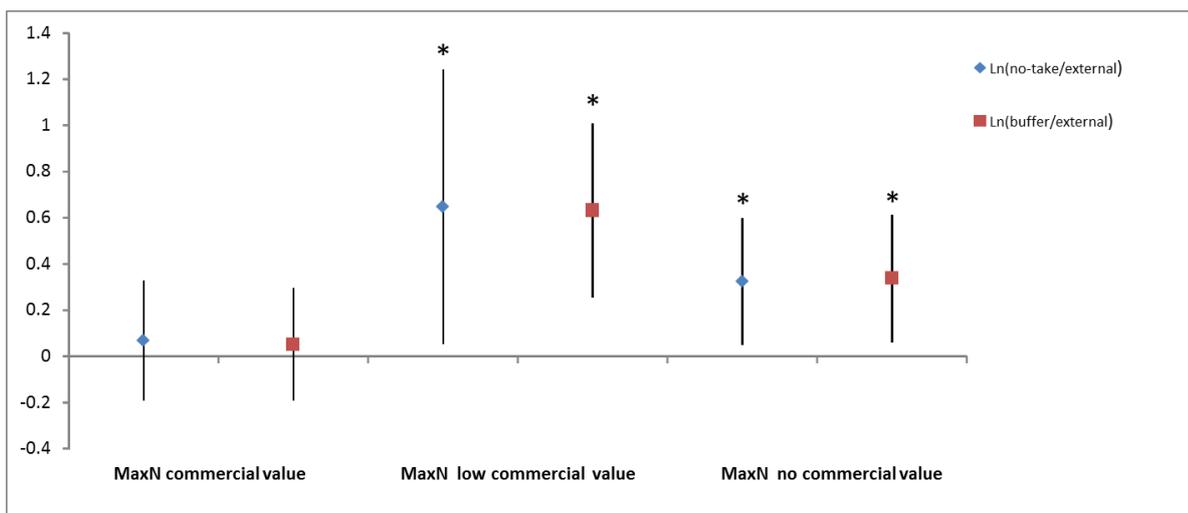


Figure 2.12. Ln(RR) of MaxN related to commercial value species (high, low and no commercial value). Blue diamonds indicate the response ratio between no-take and External areas, red squares the response ratio between Buffer and External areas. Bars indicate 95% C.I.; asterisks mark a significant effect on response ratio.

The overall relative abundance response to commercial species showed a null effect both in no-take and in buffer zones. In all other cases, for both MaxN of species with low commercial value and for species of no commercial value, between protected vs unprotected zones, showed significant positive effects (RR MaxN low commercial value $_{(no-take/external)} = 0.649$, $CI_{(no-take/external)} = 0.594$; $RR_{(buffer/external)} = 0.632$, $CI_{(buffer/external)} = 0.375$ while RR MaxN no commercial value $_{(no-take/external)} = 0.324$, $CI_{(no-take/external)} = 0.273$; $RR_{(buffer/external)} = 0.338$, $CI_{(buffer/external)} = 0.275$) (Fig. 2.12).

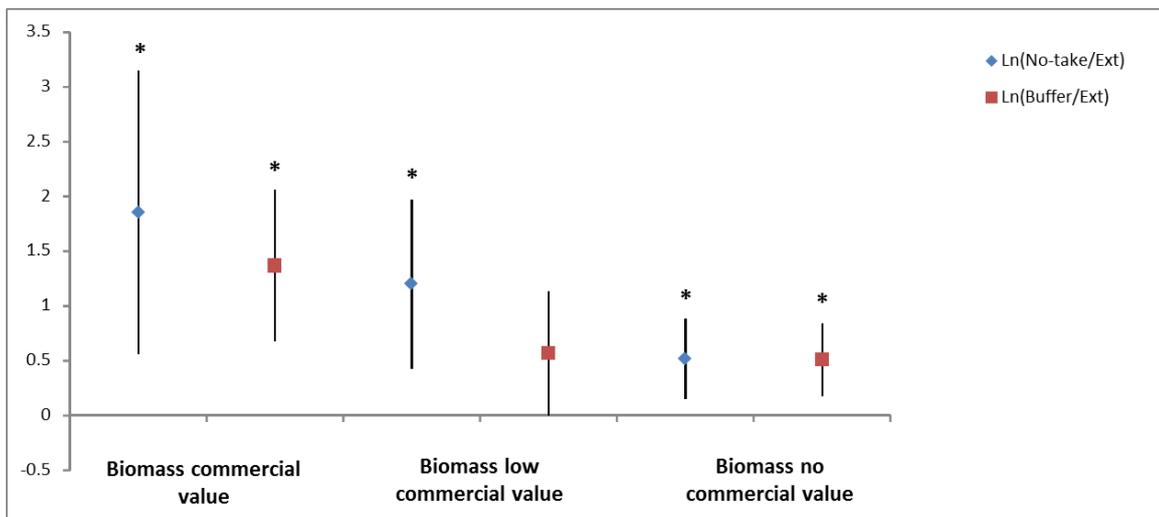


Figure 2.13. *Ln(RR) of Biomass related to commercial value species (high, low and no commercial value). Blue diamonds indicate the response ratio between no-take and External areas, red squares the response ratio between Buffer and External areas. Bars indicate 95% C.I.; asterisks mark a significant effect on response ratio.*

In order to highlight the effects that protection has on species biomass, I analyzed the Ln(RR) of high, low and no commercial species value between all MPAs (Fig. 2.13). Biomass displayed a positive effect both for the No take/External and Buffer/External ratios among high, low and no commercial species values (RR Biomass commercial value $_{(No-take/External)} = 1.851$, $CI = 1.291$; RR Biomass low commercial value $_{(No-take/External)} = 1.2$, $CI = 0.769$; RR Biomass no commercial value $_{(No-take/External)} = 0.514$, $CI = 0.365$; RR Biomass commercial value $_{(Buffer/External)} = 1.364$, $CI = 0.717$; RR Biomass low commercial value $_{(Buffer/External)} = 0.565$, $CI = 0.562$; RR Biomass no commercial value $_{(Buffer/External)} = 0.506$, $CI = 0.332$) with the exception of the Buffer/external ratio of low commercial species. Here, the confidence interval overlapped the zero value showing no effect on this group along the protection gradient.

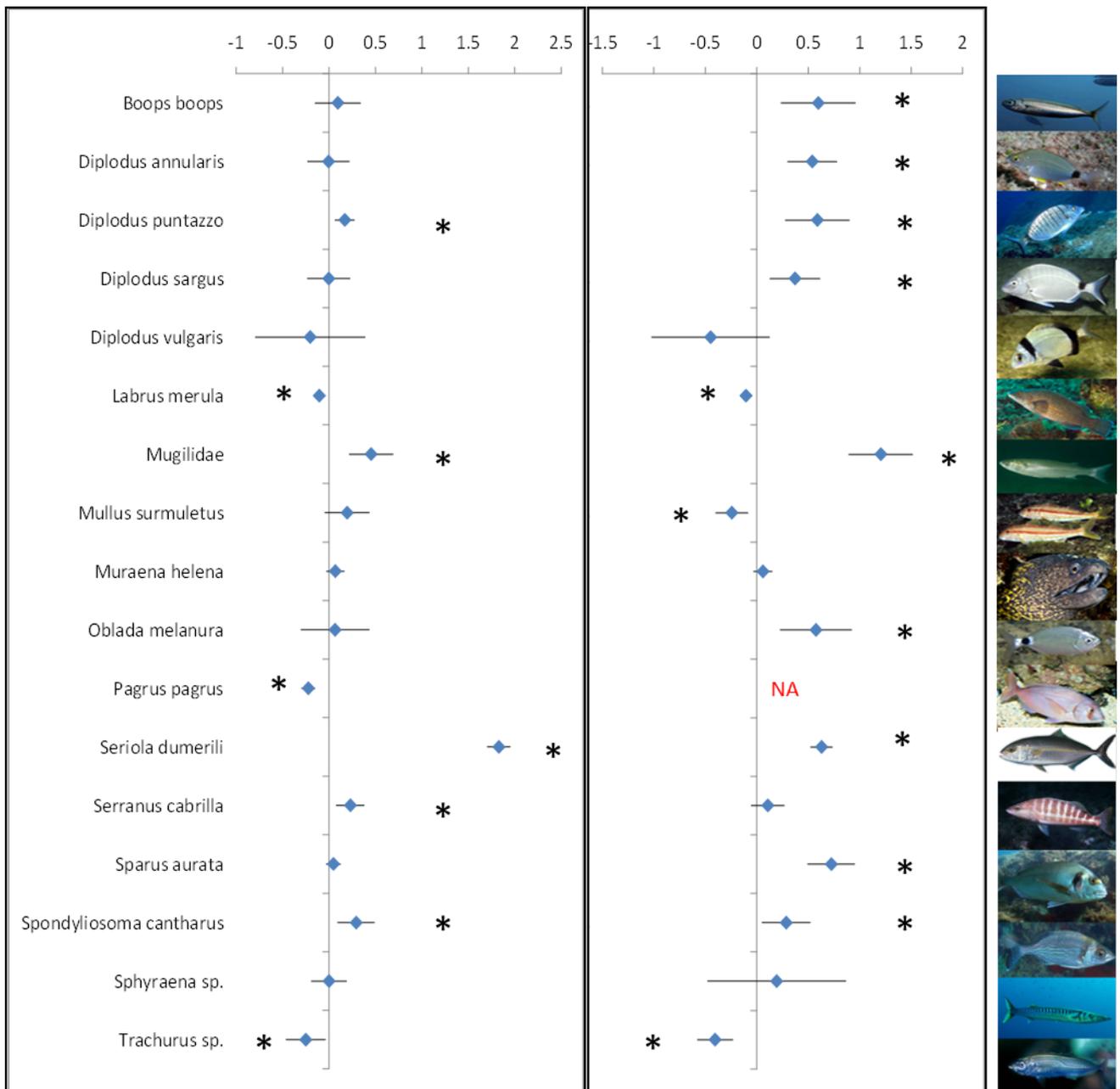


Figure 2.14. Response of MAXN of Commercial value species to protection, measured as the natural log response ratio ($\ln(\text{no-take}/\text{external})$ on left, $\ln(\text{Buffer}/\text{External})$ on right). Bars indicate 95% C.I.; asterisks mark a significant effects on response ratio.

The overall relative abundance per hour (MaxN) of species with high commercial value between no-take zones vs external ones (overall RR= 0.80, CI=0.29) and buffer vs external zones have been shown a positive effect (RR=0.1, CC=0.036). The response ratio of the same species between groups exhibit different trends. Only certain species like *Diplodus puntazzo* (RR=0.16, RR=0.58; CI=0.104, CI=0.31) and the Genus Mugilidae (RR_(no-take/external)= 0.454, CI_(no-take/external)= 0.235; RR_(buffer/external)= 1.204, CI_(buffer/external)= 0.31), *Seriola dumerili* (RR_(no-take/external)= 1.828, CI_{(no-}

take/external)= 0.122; $RR_{(buffer/external)} = 0.629$, $CI_{(buffer/external)} = 0.106$), *Spondyliosoma cantharus* ($RR_{(no-take/external)} = 0.292$, $CI_{(no-take/external)} = 0.198$; $RR_{(buffer/external)} = 0.285$, $CI_{(buffer/external)} = 0.234$) showed a positive effect in response ratio for both.

Besides, only two of the species analysed, *Trachurus sp.* ($RR_{(no-take/external)} = -0.251$, $CI_{(no-take/external)} = 0.211$; $RR_{(buffer/external)} = -0.405$, $CI_{(buffer/external)} = 0.172$), *Labrus merula* ($RR_{(no-take/external)} = -0.105$, $CI_{(no-take/external)} = 0.054$; $RR_{(buffer/external)} = -0.105$, $CI_{(buffer/external)} = 0.054$) exhibited a negative response ratio in both cases.

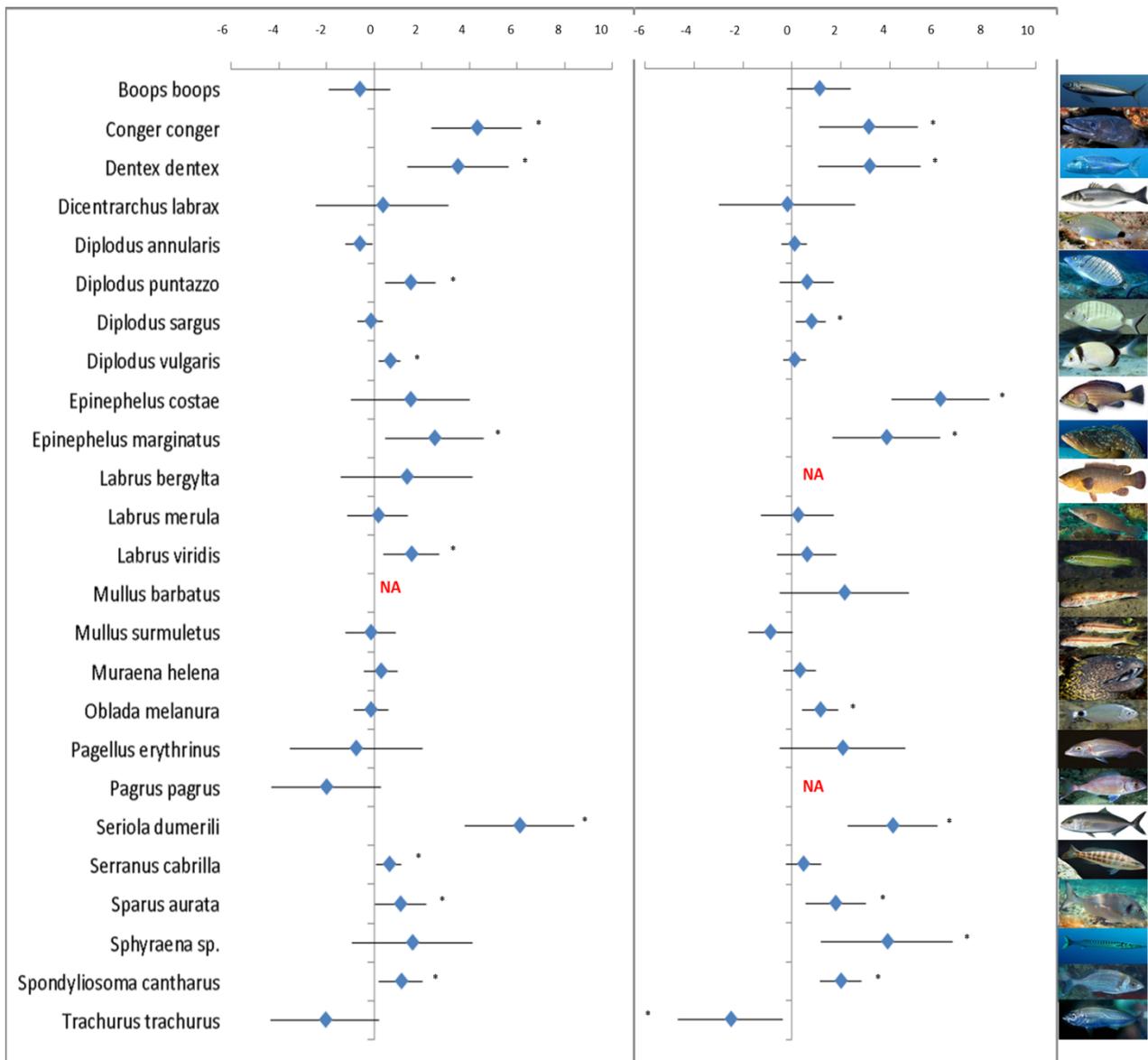


Figure 2.15. Response of Commercial value species biomass to protection, measured as the natural log response ratio ($\text{Ln}(\text{no-take}/\text{external})$ on left, $\text{Ln}(\text{Buffer}/\text{External})$ on right). Bars indicate 95% C.I.; asterisks mark a significant effects on response ratio.

Looking at the biomass of highly valuable commercial species, for some species it was significantly greater in fully protected (no take and buffer) than unprotected zones. This is the case of *Conger conger*, *Dentex dentex*, *Epinephelus marginatus*, *Seriola dumerili*, *Sparus aurata* and *Spondyliosoma cantharus*, for which protection appeared to have a stronger effect. In all other cases, higher biomass was only measured in one of the two comparisons, either between no take/external or buffer/external, not both. Certain species, such as *Diplodus puntazzo*, *Diplodus vulgaris*, *Labrus viridis*, *Serranus cabrilla* showed significant differences between in the No

take/External ratio, but not in Buffer/external, where no effect was found. Conversely, some other species were only found to have higher mean biomass when comparing the buffer zone to the outside, such as *Diplodus sargus*, *Epinephelus costae*, *Oblada melanura* and *Sphyraena sp.*

The remaining species did not show significant differences in biomass response ratio. Only the species *Trachurus trachurus* displayed a controversial trend with protection. Its biomass was higher in unprotected zones rather than protected.

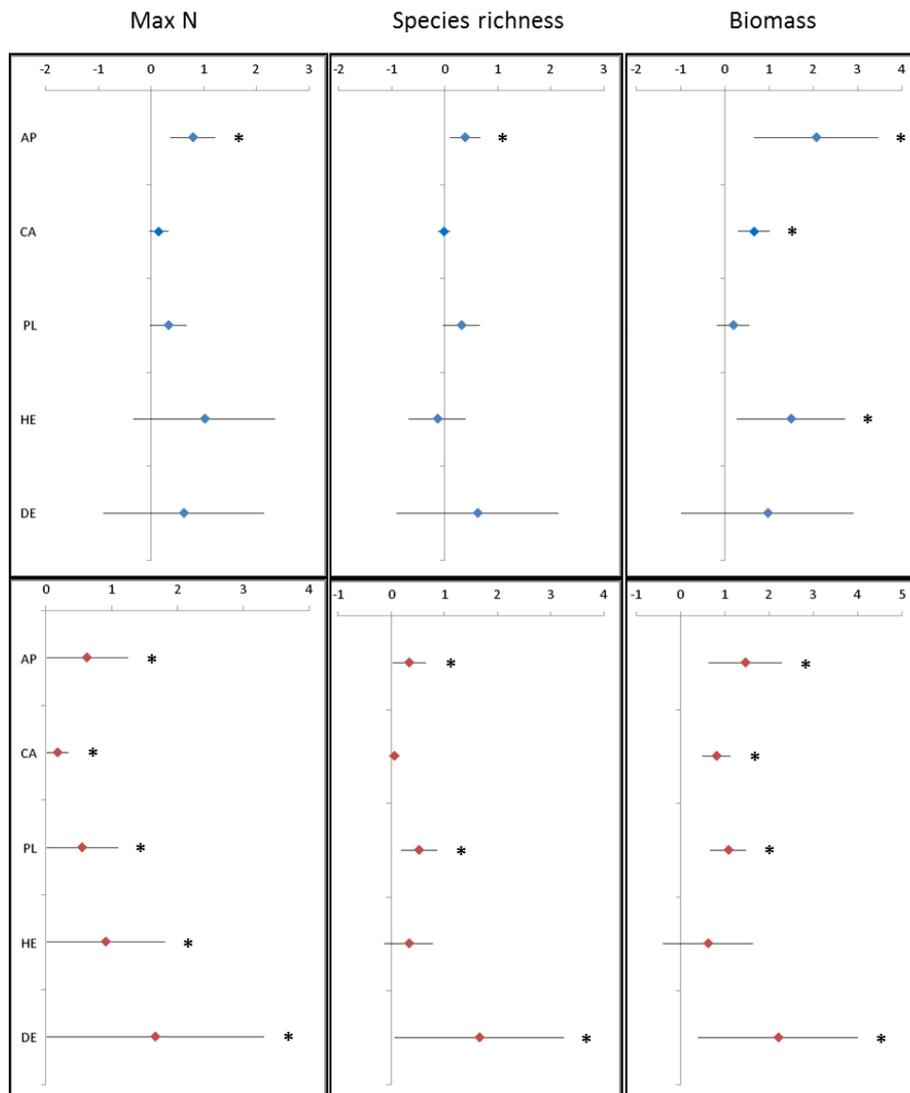


Figure 2.16. Response of trophic groups of species to protection, measured as the natural log response ratio of MaxN, species richness and biomass for each group (Ln(no-take/external Blue diamonds), Ln(Buffer/External Red Diamonds). Bars indicate 95% C.I.; the asterisks a significant effect on response ratio. Apex Predators (AP), CA (Carnivores), PL (Planktivores), HE (Herbivores) and DE (Detritivores).

Going into detail, response within the main variables was analysed by dividing the whole fish assemblage into separate trophic groups. Trophic groups displayed varying patterns in terms of effect sizes between No-take and External (Fig. 2.16.a) or Buffer and External zones (Fig. 2.16.b). Averaging all MPAs, apex predator species (AP) showed a significant difference between No take and External zones for every considered variable ($S \ln(RR_{AP(\text{no-take/external})}) = 0.384$, $CI = 0.288$ **MaxN** $\ln(RR_{AP(\text{no-take/external})}) = 0.793$, $CI = 0.422$; **Biomass** $\ln(RR_{AP(\text{no-take/external})}) = 2.064$, $CI = 1.406$) (Fig. 2.16 a). **MaxN** and species richness of carnivores (CA), planktivores (PL), herbivores (HE) and detritivores (DE) did not show statistically significant differences between No take and External zones ratios. The biomass of AP, CA and HE groups recorded instead of a significant increase attributable to protection ($\ln(RR_{CA(\text{no-take/external})}) = 0.663$, $CI = 0.366$; $\ln(RR_{HE(\text{no-take/external})}) = 1.486$, $CI = 1.225$), while PL and DE did not. The response pattern slightly changed when looking at the ratio among Buffer and External zones (Fig. 2.16 b). A positive effect was recorded for AP, PL and DE for each response variable (**MaxN** $RR_{AP(\text{buffer/external})} = 0.624$, $CI = 0.529$), planktivores ($RR_{PL(\text{buffer/external})} = 0.548$, $CI = 0.358$) and detritivores ($RR_{DE(\text{buffer/external})} = 1.658$, $CI = 1.596$), **Species Richness** ($RR_{AP(\text{buffer/external})} = 0.34$, $CI = 0.316$), planktivores ($RR_{PL(\text{buffer/external})} = 0.524$, $CI = 0.335$) and detritivores ($RR_{DE(\text{buffer/external})} = 1.658$, $CI = 1.596$) and **Biomass** ($RR_{AP(\text{buffer/external})} = 1.456$, $CI = 0.83$), planktivores ($RR_{PL(\text{buffer/external})} = 1.07$, $CI = 0.408$) and detritivores ($RR_{DE(\text{buffer/external})} = 2.199$, $CI = 1.813$)). **MaxN** and biomass of Carnivores displayed significant protection effects (**MaxN**: $RR_{CA(\text{buffer/external})} = 0.172$, $CI = 0.164$; **Biomass**: $RR_{CA(\text{buffer/external})} = 0.809$, $CI = 0.318$) whereas responses in species richness were null. A higher relative abundance of herbivores was observed between Buffer and external zones ($RR_{HE(\text{buffer/external})} = 0.904$, $CI = 0.869$) although no effect on biomass and S was recorded.

Detailed relative abundance and biomass for all species, categorized by trophic group, can be consulted from the Supplementary Data.

DISCUSSION

Relative abundance and biomass of fish assemblages were significantly higher in fully and partially protected zones than unprotected, while species richness was found to be higher in buffer zones and displayed a null difference in no take. While the significantly higher descriptors found within protected areas compared to the outside can be attributed to management measures that limit spill-over from protected to unprotected areas (Giakoumi *et al.*, 2017), they may also be attributable to artisanal, recreational, industrial and illegal fishing efforts that limit resource displacement in control areas.

Confirming what was reported by previous studies carried out in the Mediterranean Sea (Guidetti and Sala, 2007; Lester and Halpern, 2008; Giakoumi *et al.*, 2017), the biomass of commercially valuable species was higher in protected than control areas. Sedentary species, such as the dusky grouper, were more abundant and larger within protected areas compared to unprotected, strengthening findings from Giakoumi *et al.* (2017).

These findings also appear to verify this study's initial hypothesis that SSFs conduce a crucial role in the effectiveness of MPAs.

While fishing efforts certainly focus apex predators from higher trophic levels more intensely, these are certainly not the only affected ones. Fishing, in most cases, is hardly selective to a single target group species: in line with previous findings from Murawsky (2000), non-commercial species richness wasn't statistically higher compared to unprotected areas, although relative abundance was higher in reserves than outside.

Most of the fishers use a different kinds of traditional fishing gear, which is not species-specific, such as fixed nets, which are used by practically all the fishermen, and bottom longlines.

There is also gear, particular to certain MPAs, used exclusively in some areas of the Mediterranean Sea, that targets specific species: examples are the '*rossettara*', a small net used to target a tiny Gobid typically consumed near the Portofino MPA, and the '*bonitolera*', a net used to target medium-size scombridae (personal remarks).

As previously noted by Guidetti and Sala (2007), the findings presented here confirm that the structure of fish assemblages doesn't, on average, reveal a universal pattern of reserve effect in the whole region.

When looking at specific areas though, different MPAs displayed a widely different performance, indicating that different implementations of a marine reserve can provide largely different results.

While the differences observed between different MPAs can be partially attributed to varying features of the sub-basin. Differences in latitude, abiotic factors and ecoregion could be a possible vector to explain the presence (or absence) of certain species in the 11 MPAs analysed and those factors could represent some of the factors that could explain the high heterogeneity of abundance and richness of the observed species, but attempting to find data links supporting these factors alone did not indicate a clear pattern that could explain such extreme differences in the effectiveness of different MPAs (see supplementary data).

Instead, drivers attributable to management, local policies and their enforcement are likely pivotal to the success of an area. Looking at the MPAs that proved to be most effective, Es Freus and Cabo de Palos, specific indicators such as their size, target objective, management and enforcement policies, indicate that additional studies should be performed, focusing transversal characteristics that could help improve the performance of Marine Protected Areas in general, expanding on the findings of Di Franco et al. (2016).

Each Country's policy, particularly the protective measures adopted for the specific designation assigned to their MPAs, are closely linked to the biophysical and socioeconomic characteristics that each area displays.

MPAs were established as a solution to primary target objectives that are specific to each country. In some cases, the principal objective is to conserve biodiversity in order to preserve species and habitats, increasing carrying capacity and system resilience. In most cases overfishing represents one of the principal threats to the ecosystem, acting on species that are vulnerable (VU), endangered (EN) or at risk of extinction (CR) (e.g. *Epinephelus marginatus* (EN), *Scophthalmus maximus* (EN), *Epinephelus costae* (VU), *Argyrosomus regius* (CR); [http://www.iucn.it/pdf/Comitato IUCN Lista Rossa dei pesci ossei marini italiani 2017.pdf](http://www.iucn.it/pdf/Comitato_IUCN_Lista_Rossa_dei_pesci_ossei_marini_italiani_2017.pdf)).

In other cases (e.g. Cape Roux) the designation of the MPA is intended as "Fisheries Reserve", with the main purpose of safeguarding fish stocks.

One of the main problems that MPAs face is attributable to different interests from the managing bodies, generating management conflicts (e.g. Cabo de Palos Marine Reserve that is managed both at the regional and local level) that actually seem to increase policy enforcement.

IUCN management categories can also be linked to MPA effectiveness. For example, in the Bonifacio Natural Reserve (Category IV- Habitat/Species management area), significant differences

for MaxN and the S ratio were found between protected and unprotected areas, and between buffer and external zones, but not between no-take and external ones, where no effect was found. In the Cabo de Palos Marine Reserve (Category VI- Protected Area with sustainable use of natural resources), the primary conservation objective supports the measured results, confirming this MPA's effectiveness in re-establishing balance in the ecosystem, favouring the protection of top predators.

Cases such as Cape Roux Fisheries Reserve differ entirely: there, in line with Seytre and Francour (2009), total abundance and richness did not appear to respond significantly to protection, contrary to expectations. Similarly, Italian, Slovenian, Croatian and Greek MPAs did not show any significant effect on protection, suggesting ineffective management measures and/or enforcement (e.g. in the prevention of poaching). Zakynthos, in particular, being a Category II MPA, simply defined by the IUCN as a National Park, tends to be more lenient with human visitation and its supporting infrastructures. The National Park of Zakynthos is characterized by a seasonal no-take zone where all activities are forbidden, due to the annual migration of the loggerhead sea turtle to its nesting grounds (Dimitriadis *et al.*, 2018; Giakoumi *et al.*, 2018), but this policy, as previously reported by Dimitriadis *et al.* (2018), did not prove to be effective at generating a definitive *reserve effect* on the marine ecosystem.

As reported by Micheli *et al.* (2004) and Claudet *et al.* (2006), the amount of time necessary to restore species richness and density ranges from three years to multiple decades. As reported by Guidetti *et al.* (2006), the joint action between fishermen and MPA bodies, after three years of fishing ban in the Torre Guaceto MPA, led to an increase of fish catch inside the MPA around five-fold the average value obtained outside.

Protecting large top predator fish, with their great reproductive potential, is one of the key factors that can directly lead to spill-over and, indirectly, the *reserve effect* (Birkeland and Dayton, 2005; Garcià-Charton *et al.*, 2007).

The present meta-analysis shows positive general effects on the protection of fish assemblages, particularly on key commercial species. In order to improve the effectiveness of Protected Areas and achieve more effective results, it is necessary to expand on these pointlike findings and to find definitive links between these results, by extend this kind of study to additional seasons. Furthermore, as suggested by Guidetti *et al.* (2002), Salomidi *et al.* (2006) and Giakoumi *et al.*

(2017), it is of fundamental importance to generate a common starting point for management plans in order to implement a proper Before After Control Impact design. Every Mediterranean country employs a different approach to environmental protection, which doesn't bode well with the international span of the Mediterranean basin. A unified approach, with common objectives, expanding protection to larger areas, could provide both ecological and socio-economic benefits (Giakoumi *et al.*, 2011; 2017).

The BUV methods proved to be effective at observing most fish species from all trophic groups, from top predators to herbivores. However, due to the selective nature of these sampling techniques (that use a bait), the abundance of some species didn't necessarily reflect their actual natural occurrence: detritivores and herbivores obviously don't respond similarly to carnivores and omnivores to bait.

This technique is not capable to address each species (e.g. cryptic species), but has proved helpful to monitor a multitude of taxa belonging to almost all trophic groups, although not completely covering herbivores. The BUV technique may represent a useful tool for MPAs managers, capable of providing support for specific policy recommendations and setting up new fishery management models. The results of this study could additionally be useful for improving the integration of principles and recommendations in national and international policies, in order to facilitate the informal and formal engagement of stakeholders in the management of small-scale fishing within MPAs.

Acknowledgements

This study is part of "FishMPABlue2" project funded by Interreg Med. The **FishMPABlue2** project addresses and proposes solutions to existing and potential conflicts between stakeholders. It involves key actors in the planning of conservation measures and fishery regulation processes in the context of sustainable governance involved in Blue Economy. **FishMPABlue2** intends to start from a few successful experiences of co-management between MPAs and fishermen, and develop new means of support for the artisanal fishery, within and around MPAs.

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Supplementary Data

Chapter 2: Testing the ecological role of small scale fishery in Mediterranean marine protected areas

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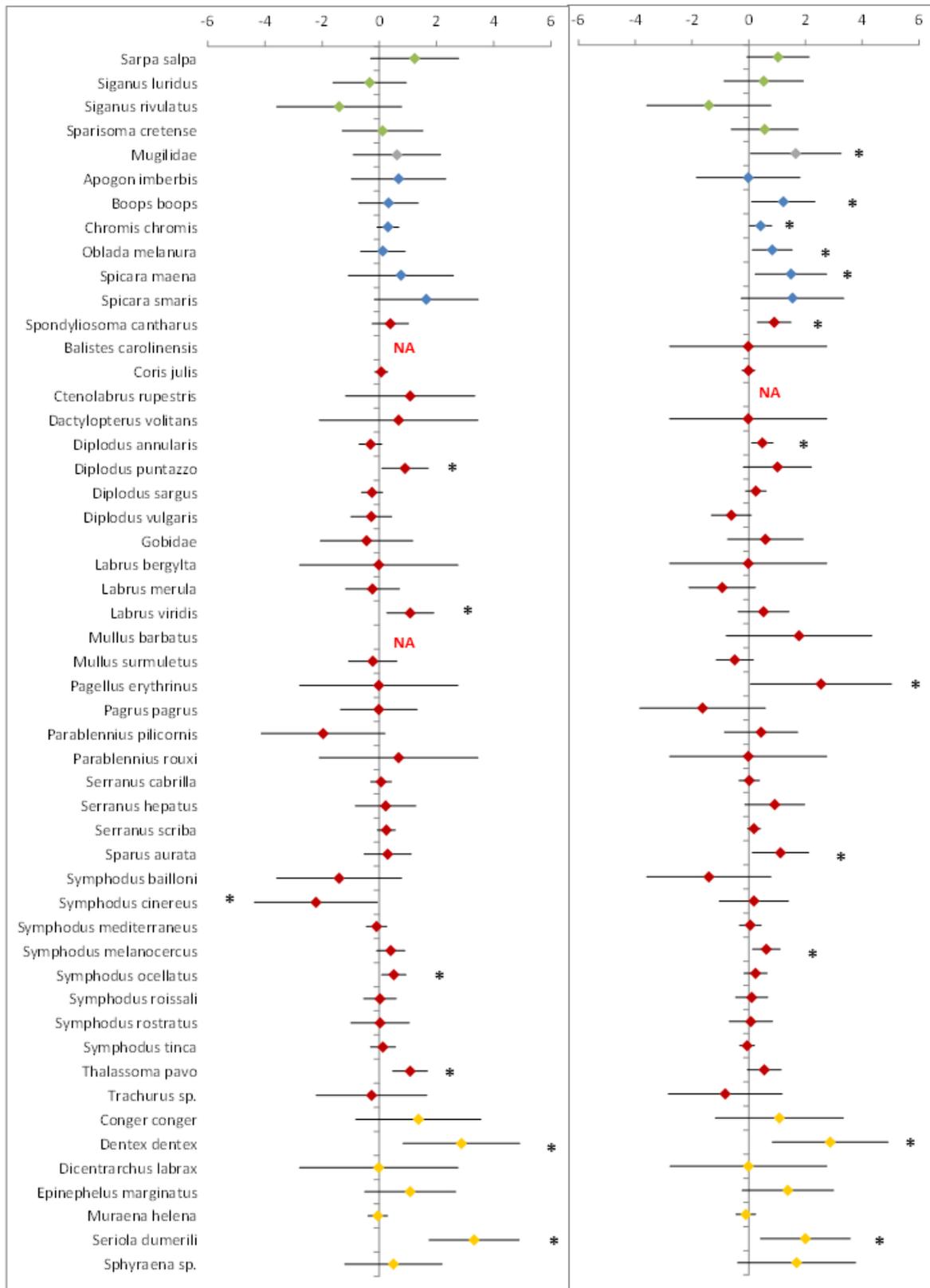


Figure S1. MaxN Trophic Group Ln(NT/Ext) and (Buf/Ext); Response of fish species to protection, reported as the natural log(RR) between no-take and external zone (on left) and buffer and external zone (on right). Bar indicate 95% CI. Species are reported as a trophic group: herbivores (green diamond), detritivores (grey diamond), planktivores (blue diamond), carnivores (red diamond) and top predators (yellow diamond).

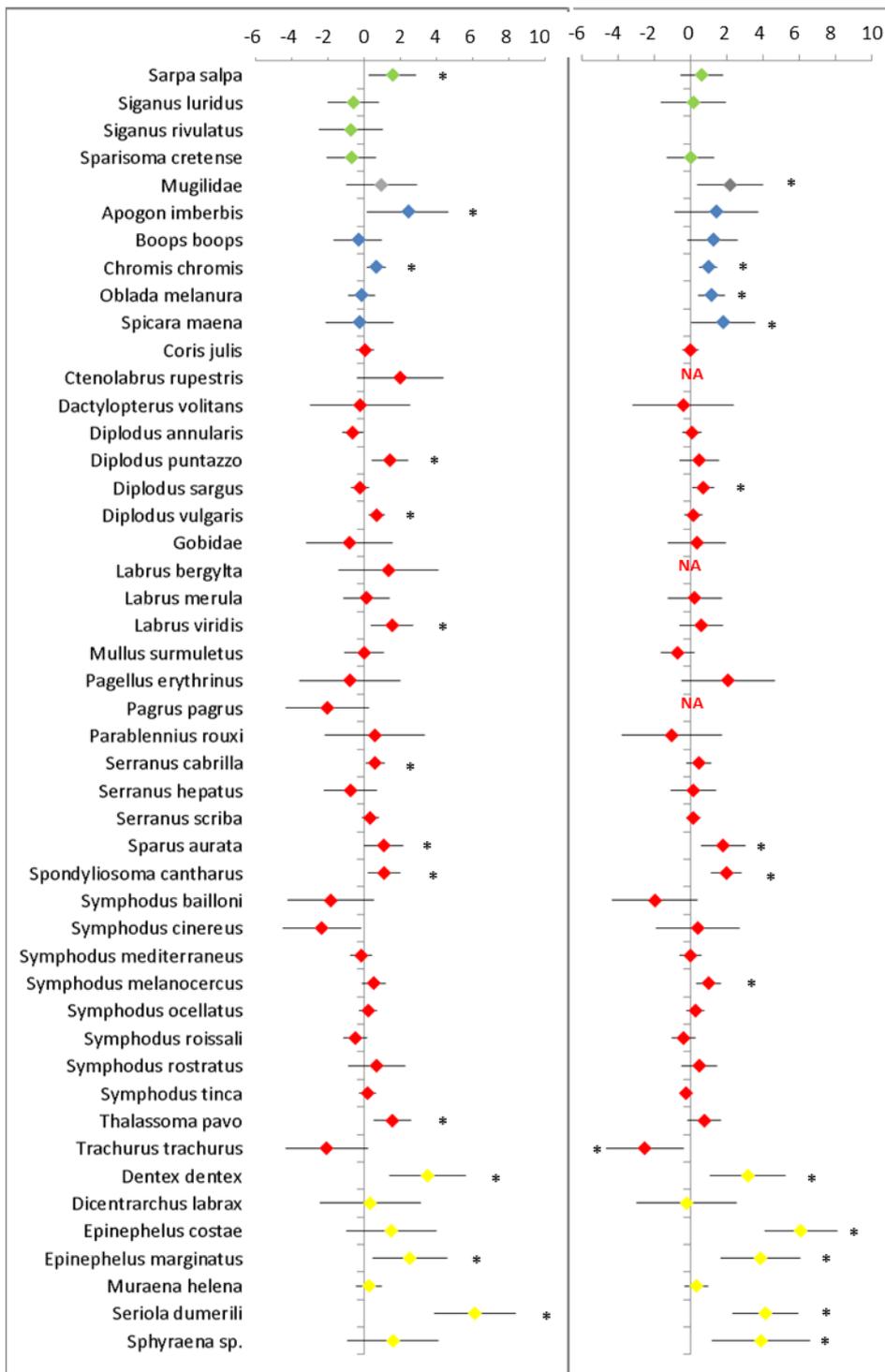


Figure S2. Biomass Trophic Group Ln(NT/Ext) and (Buf/Ext); Response of fish species to protection, reported as the natural log(RR) between no-take and external zone (on left) and buffer and external zone (on right). Bar indicate 95% CI. Species are reported as a trophic group: herbivores (green diamond), detritivores (grey diamond), planktivores (blue diamond), carnivores (red diamond) and top predators (yellow diamond).

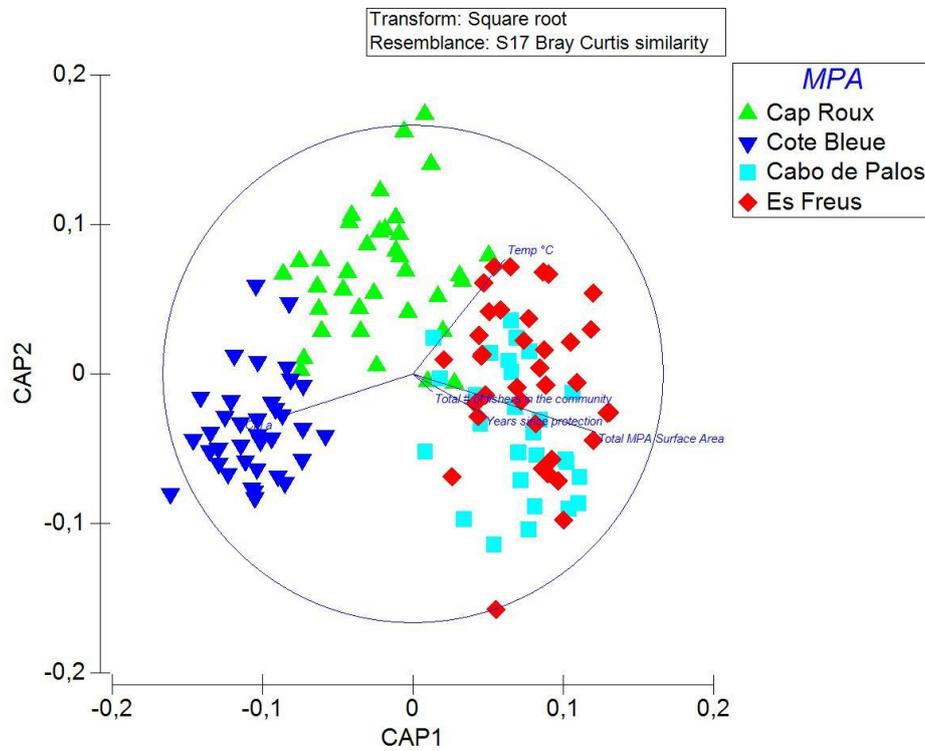


Figure S3. Canonical Analysis of Principal components (CAP) among MPAs belonging to Algero-Provençal Basin (sensu Notarbartolo di Sciara and Agardy, 2010) and predictors: years since protection, average annual chlorophyll (Chl_a), average annual temperature (Temp °C), numbers of fishers (# fishers) and MPA Surface.

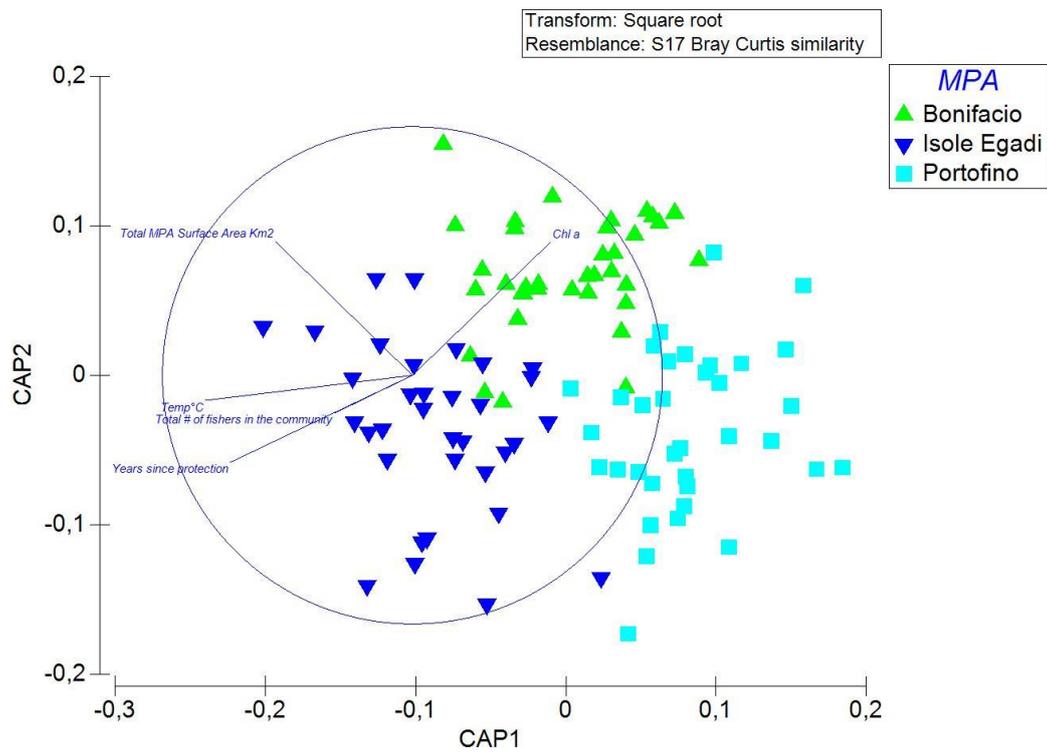


Figure S4. Canonical Analysis of Principal components (CAP) among MPAs belonging to Tyrrhenian Sea (sensu Notarbartolo di Sciara and Agardy, 2010) and predictors: years since protection, average annual chlorophyll (Chl a), average annual temperature (Temp °C), numbers of fishers (# fishers) and MPA Surface.

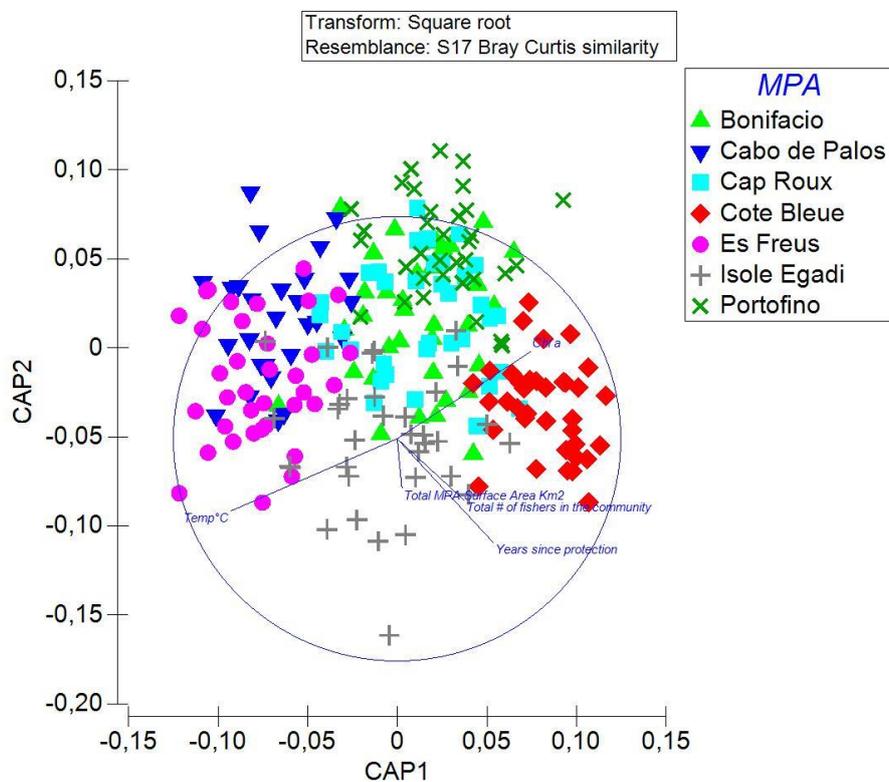


Figure S5. Canonical Analysis of Principal components (CAP) among MPAs belonging to Western-Mediterranean Basin (Algero-Provençal Basin and Tyrrhenian Sea, sensu Notarbartolo di Sciara and Agardy, 2010) and predictors: years since protection, average annual chlorophyll (Chl a), average annual temperature (Temp °C), numbers of fishers (# fishers) and MPA Surface.

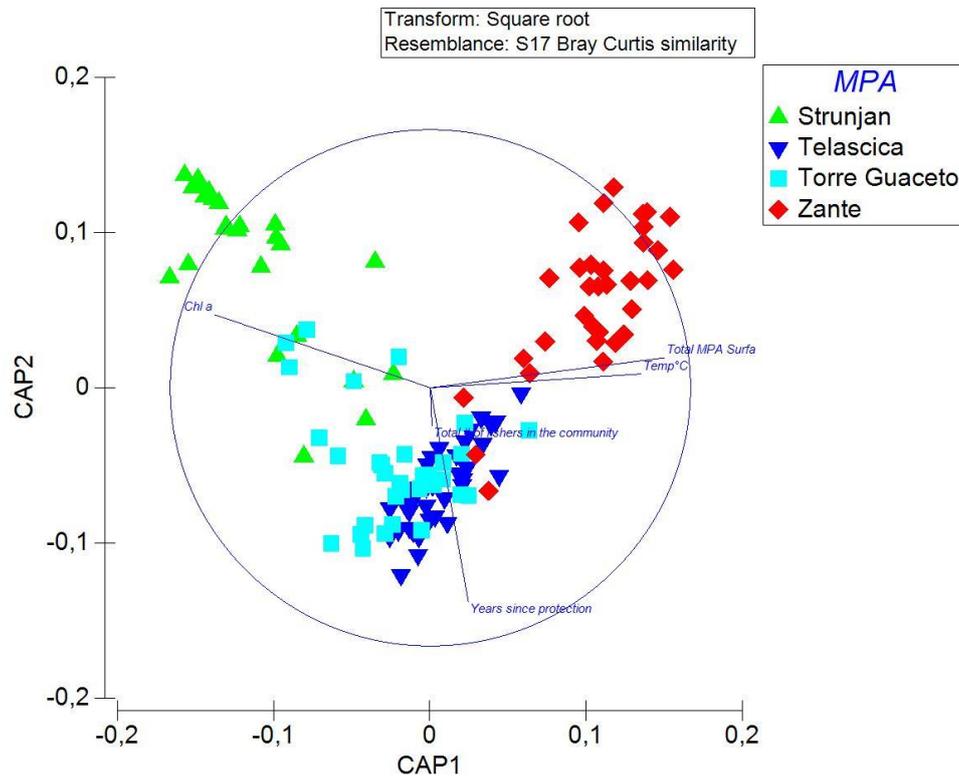


Figure S6. Canonical Analysis of Principal components (CAP) among Eastern-Mediterranean Basin (Adriatic and Ionian Sea, sensu Notarbartolo di Sciara and Agardy, 2010) MPAs' and predictors: years since protection, average annual chlorophyll (Chl a), average annual temperature (Temp °C), numbers of fishers (# fishers) and MPA Surface.

Chapter 3

Is the 'mesopredator release' hypothesis applicable in the Mediterranean context?

INTRODUCTION

One of the main think related to ecology concept is represented by ecosystem structure and function/process (Pasquad *et al.*, 2010; Maitra *et al.*, 2018). Understood the relationship of species becoming from different trophic levels, represents an essential key useful for quantifying the resistance and resilience of a system. During the time, marine ecosystems have been subjected to a pressure of a different nature. In order to deal with a conspicuous market demand, through time, human activity has led to substantial shift on marine ecosystem communities producing (directly on targeted species and indirectly on intra and inter organisms relationship) detrimental effects on ecosystem functioning (Vance-Chalcraft *et al.*, 2007). Alongside resource exploitation use by human pressure (Jackson *et al.*, 2001; Maitra *et al.*, 2018), other drivers as global climate change, pollution and extensive coastal development (Friedlander and DeMartini, 2002), had an undesired effect on one of the ecology milestone concept: the food chain theory (Barbier and Loreau, 2018).

A simplified food chain can be represented with three levels; apex predators (on top of the pyramid), mesopredators as invertebrates feeders, omnivores and small piscivores (at medium-level) and herbivores (on bottom) (Prugh *et al.*, 2009). Obviously, the reality is quite different considering that organisms often feed at more than one trophic level (Pimm and Lawton, 1978; Maitra *et al.*, 2018).

The fishing effect on target species, often apex predators, have been well-studied (Graham *et al.*, 2017; Valdivia *et al.*, 2017). The excessive exploitation of apex predators generates a drastic predation reduction, by promoting the exponential increase of intermediate trophic groups (Ritchie and Johnson, 2009).

This decrease does not allow top-down control but should generate a bottom-up process driven by primary producers, followed by herbivores (Elmhagen *et al.*, 2010). This phenomenon has been firstly studied on terrestrial environments under the name of "mesopredator release", defined as: "the absence or negative change in the density or distribution of an apex predator results in an

expansion in density or distribution, or behaviour of a middle-rank predator in a trophic web” (Crowell 1961; Prugh *et al.*, 2009; Brashares *et al.*, 2010).

These process it is governed by two principal aspects: systems productivity and the existing strength between apex predators, mesopredators and basal levels. It follows that have been proven theories attesting a higher mesopredators presence on less productive systems rather than highly productive levels ruled by apex predators; at intermediate levels, there is a coexistence of these groups (Brashares *et al.*, 2010). A mesopredators release consequences is given by a trophic interactions modification due to the facts that these could replace the apex predators generating trophic chain imbalance and a trophic cascade as a consequence (Prugh *et al.*, 2009).

In this regards Clark *et al.* (1996), Skubel *et al.* (2018) highlight and correlate certain abiotic factors (as sea surface temperature (SST) and Chlorophyll), regarding predator activities in South Africa. Particularly, Clark *et al.* (1996) found that some factors, such as SST, wave height, wind speed and direction, that play a crucial role in the abundance and richness of species in shallow habitats.

Mediterranean context

Due to high levels of coexisting endemisms and specific biotic and abiotic features that uniquely characterize it, the Mediterranean basin is considered a unique terrestrial and marine biodiversity hotspot (Coll *et al.*, 2010), on top of which the above-mentioned global trends of multiple different coastal and marine human activities (tourism, pollutions, aquaculture, maritime transport, fishing) make of the Mediterranean Sea an area of particular ecological interest (Coll *et al.*, 2010; Rodríguez-Rodríguez *et al.*, 2015).

Here, the physics features of the liquid medium, have given rise to an oligotrophic, low-nutrient low-chlorophyll (LNLC) semi-enclosed basin, characterized by low productivity, compared to Oceans. The static water flow does not allow the upwelling phenomenon and consequently, the primary production and biomass tend to be limited (Richon *et al.*, 2018). The western part presents higher nutrient concentrations (P, N and Si) compared to the eastern part (Béthoux *et al.*, 1998; Richon *et al.*, 2018). The principal nutrient inflow is guaranteed by the Atlantic Ocean (Strait of Gibraltar) while the north-eastern part is enriched by river run-off.

Previous studies highlighted the advanced state of degradation of this basin regarding the corroborate state of Mediterranean webs. This was supported by lower trophic height, linkage density, trophic chain length and scarce biomass rate on higher and lower trophic levels (Sala *et al.*,

2004; Coll *et al.*, 2010).

In these regards, Marine Protected Areas (MPAs), represents an able tool to protect habitat and own species (Mosquera *et al.*, 2000; Cotè *et al.*, 2001; Micheli *et al.*, 2004; Sala and Knowlton, 2006; Guidetti and Sala 2007). Here, no-takes areas (where all fishing activities are forbidden) gives rise to pristine scenarios where intra and interspecific relationships among species are not ideally skew. Ideally, because should be taken into account the species home range (Sims *et al.*, 2008). Indeed, the large predators need wide areas and therefore able to move in and out the MPA, rather than a mesopredator, that in most cases, shall carry out its ontogenic period in a defined area.

On MPA context, the harvesting zones (due both as Small-Scale Fisheries (SSF) as recreational fishing) it is restricted to buffer and external zones. Here, the fishing effort plays a crucial role on commercial species (Mosquera *et al.*, 2000; Micheli *et al.*, 2004; Guidetti and Sala 2007; Valdivia *et al.*, 2017). Several authors (i.e. Coll *et al.*, 2008; Woodcock *et al.*, 2016; Valdivia *et al.*, 2017) showed fish communities shift caused by the fishing's action often leading to dispute on MPA protection efficiency.

In this study was tested a controversial hypothesis where mesopredators, either for behavioural aspects or density increases, rise significantly on external zones due to fishing efforts on apex predators.

Through a simple assay, in 11 MPAs, the predation pressure has been tested by means of experimental design which provided the apex and mesopredators comparisons among protected (no-take and buffer areas where all or almost all activities are forbidden) and unprotected (external areas) zones, where SSF plays a crucial role in trophic interactions balance. The goals of this study are to:

- 1) Test the hypothesis that MPAs supports the relation of an almost pristine environment showing higher mean abundances of apex predators in protected compared to unprotected zones.
- 2) Study the mesopredators abundance related to apex predators occurrences (by means of Baited Underwater Video systems and Squidpop techniques) by verifying the strength of top-down controls.

- 3) Assess the mesopredator release hypothesis in the Mediterranean context exploiting 11 pilot MPAs and external controls where both intense industrial and small-scale fisheries (SSF) remove top predators, potentially leading to a density/biomass increase of mesopredator fishes.

METHODS

MPAs, sampling sites and spatial replication



Figure 3.1. Map of the 6 countries and 11 MPAs involved in the study

Fish assemblages were surveyed in June-September 2017 (Fig. 3.1, Table 3.1) at 11 Mediterranean Protected Areas located in six different countries (France, Spain, Greece, Italy, Croatia and Slovenia) with different management/protection schemes. Each Marine Protected Area (MPA) is governed by a specific national policy which results in different zonation schemes. As an example, Italian and Spanish MPAs (Isole Egadi, Torre Guaceto, Portofino, Es Freus and Cabo de Palos) share a similar three-level protection scheme: Zone A (i.e. no-take, no-access), Zone B (buffer zone with local artisanal fishery permitted) and Zone C (general/buffer zone usually with only local artisanal fishery permitted). By contrast, the Greek MPA I worked on (Zakynthos) has a 6-month, non-permanent protection scheme, Slovenian and Croatian MPAs (Strunjan and Telašćica) have a two-

level protection scheme (Take vs No-Take Zone), whilst French ones are quite mixed. This variability considered, a common, experimental design with 2 levels of protection was employed: Protected (No-Take, Buffer) and Unprotected (External zones), with two random sites within each zone.

Table 3.1. Scheme of the selected MPAs with considered descriptors

DESIGNATION	LOCATION	COUNTRY	Latitude	Longitude	Total # of fishers in the community	Years since protection
Natural Reserve	Bonifacio	France	38°47,116	001°29,114	38	18
Marine Reserve	Cabo de Palos	Spain	37°39,095	000°39,238	27	22
Fisheries reserve	Cap Roux	France	43°27,338	006°55,479	6	14
Marine Park	Côte Bleue	France	43°19,479	005°09,648	19	35
Marine reserve	Es Freus	Spain	41°20,189	009°14,812	18	18
MPA	Egadi islands	Italy	44°18,821	009°09,938	40	26
MPA	Portofino	Italy	45°32,314	013°36,332	22	18
Landscape park	Strunjan	Slovenia	40°42,794	017°48,624	10	13
Natural park	Telascica	Croatia	43°52,047	15°11,057	7	29
MPA	Torre Guaceto	Italy	37°59,033	012°02,115	5	26
Marine national park	Zakynthos	Greece	37°42,450	20°56,828	35	18

To achieve the desired outcome, two sampling techniques have been used, which are suitable for cross-sectional studies. Instead of more common visual census techniques, two novel techniques were employed to study fish assemblages composition and processes: standard *Baited Underwater Video* (BUV) and *Squidpop* surveys. These methods manage to rule out the influence that might be caused by the presence of the diver, which could otherwise lead to biased estimations relative to fish behaviour.

To assess the spatial variability of shallow water fish assemblages in the MPAs, field surveys were carried out by deploying BUV systems in two different zones (protected and unprotected zones) at two random sites within each zone. Thirty-six replicate samplings were collected for each MPA for a total of 378 deployments.

It is well-known in literature that rocky reefs represent the bottom type that is most commonly subject to protection in MPAs, hosting most of the species targeted by fishing (Boero *et al.*, 2005; Guidetti *et al.* 2008). For this reason, samplings were carried out on rocky bottoms at depths between 5 and 15 meters at the same daylight hours of 8:00 A.M. and 3:00 P.M. In addition, GPS coordinates were marked down for each replicate sampling.

In order to assess fish composition, abundance, and biomass of different trophic guild levels, (including pelagic species that usually tend to be shy and hardly detectable by scuba divers), fish data were collected using Stereo-Baited Underwater Video system technique (BUVs).

To assess and obtain a quantitative and standardized estimate of predation pressure, and to characterize predatory fish metrics (species composition and abundance) along the gradient of protection and exploitation by small-scale fisheries, a total of 144 Squidpops have been deployed within the eleven MPAs. For each protection level (no-take-buffer and external), replicates were randomly located within 2 sites (with about fourteen replicates within each MPA). Like the BUVs, each Squidpop's replicate sampling was deployed during daylight hours and positioned 500m in rocky bottoms at depths between 5 and 15 meters, at a safe distance from the BUV systems, in order to avoid interference between the two bait' types.

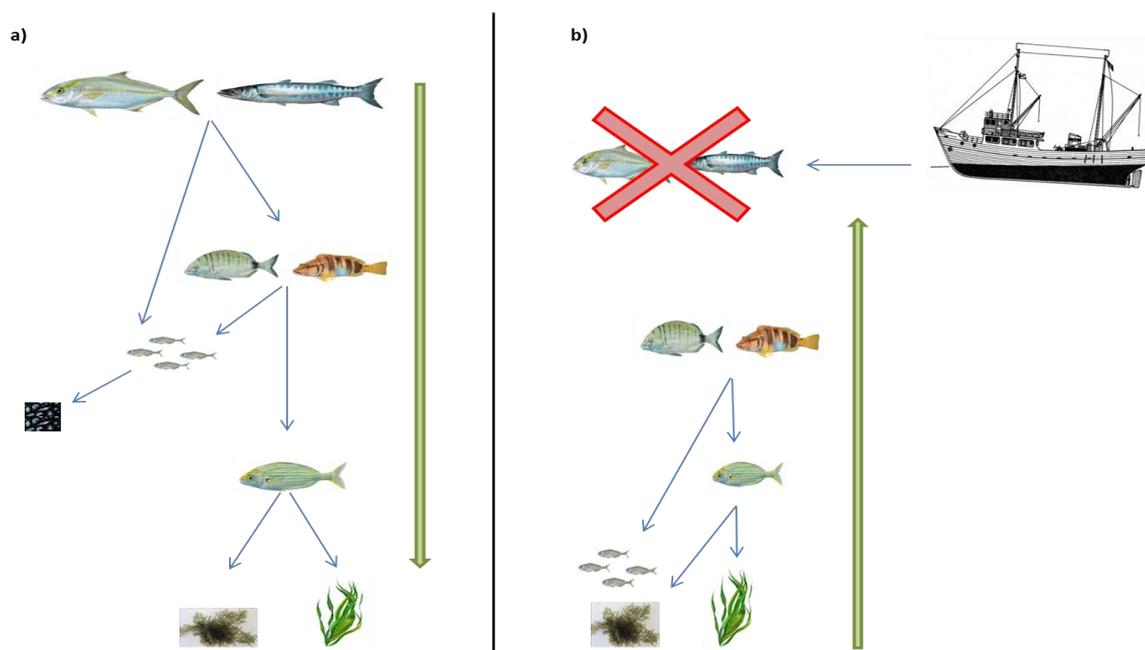


Figure 3.2. A schematic representation of top-down control promoted by apex predators (a) and bottom-up effect due to the fishing effort consequence.

Stereo Baited Underwater Video system

The Baited Underwater Video system is a novel technique that consists of a stainless steel frame (120x80 cm, respectively the primary and secondary axis) equipped with two GoPro Hero 3 high definition cameras (720p@60 fps) with a wide-angle lens, continuously recording for one hour. In front of the cameras, at 1.20 m distance, a mesh bag with 400 g of crushed *Sardina pilchardus* was placed to attract fish inside the field of view. Due to their high oil dispersion, this bait type ensured the arrival of different species belonging to different trophic levels. As reported in multiple studies, sardines represents the most effective type of bait at attracting fish thanks to Odour-plume dispersion (Westerberg *et al.*, 2010; Wraith *et al.*, 2013). To ensure proper stabilization during descent, a lead has been fixed at each extremity of the secondary axis of the frame.

BUVs were lowered from the boat to the sea floor and recovered using a rope attached to a surface buoy marking the location.

Each replicate was left recorded for 60 min around, and the start and end time were checked. To avoid the pseudo-replication, each BUV system were deployed 150 meters apart in the same location.

Up to 12 BUV replicates were dropped in a single day with freshly crushed sardines filled for each deployment. A total of 378 were deployed during the survey campaign. Only in Strunjan's MPA were made four replicates for each random site due to the small extension of MPA. Due to the low visibility or the instrument sideways dropped, some replicates were deleted from the total count. Particularly 370 was used in total for the analysis.

BUVs Analyses

For each 60min video, species richness (S) and MaxN (a conservative measure of abundance calculated as the maximum number of fish of the same species identified over the observation period) and size were successively analyzed and chronicled. To evaluate species richness, every fish sighted in the field of view was considered, regardless of proximity, whilst $MaxN$ was calculated taking only species present within 2 meters around the bait in consideration.

All length measurements were made by using Myrtus (M.I.T. GmbH company) after prior calibration. Footage from each camera (left and right) was synchronized and the length of fishes was considered as total length (TL) and measured at time of MaxN (Watson *et al.*, 2010; Barley *et al.*, 2017).

For each individual was used the allometric relationship between weight (W) and length (L).

$$W=aL^b$$

where “a” and “b” are species-specific constant available on FishBase (<https://www.fishbase.de>).

The total biomass of a species per sample was calculated by multiplying the maximum number of individuals with the maximum average size of the same multiplied by the conversion coefficients.

Squidpop

The Squidpop consists of a standard prey item (Duffy *et al.*, 2016); this method uses a piece of dry squid mantle, 1, 3 cm in diameter, cut by a cork borer, tethered by a monofilament line to the end of a fibreglass plant stake (60 cm x 0.60 cm) and secured with electrical tape (Fig. 3.3). One assay consisted of 25 Squidpop replicates, which get fixed to the rocky bottom, usually by using cable ties to secure the stakes to the substratum. Each of the 25 stakes was implanted on the bottom roughly along the same depth contour, at a distance of 1-2 m, arranged in one or two rows (Fig. 3.4). To assess the relative rates of bait consumption across the different zones of protection, a diver annotated by binary code (0 absence, 1 presence) the number of baits eaten after one and twenty-four hours and retrieved the equipment. The amount of squid eaten tell us how much predation has occurred. Furthermore, a GoPro Hero 3 camera was positioned alongside every Squidpop replicate, during the first hour of deployment, to better quantify mesopredator richness and abundance in the area (which can be subject to different levels of protection).



Figure 3.3. Step of squidpop deployments

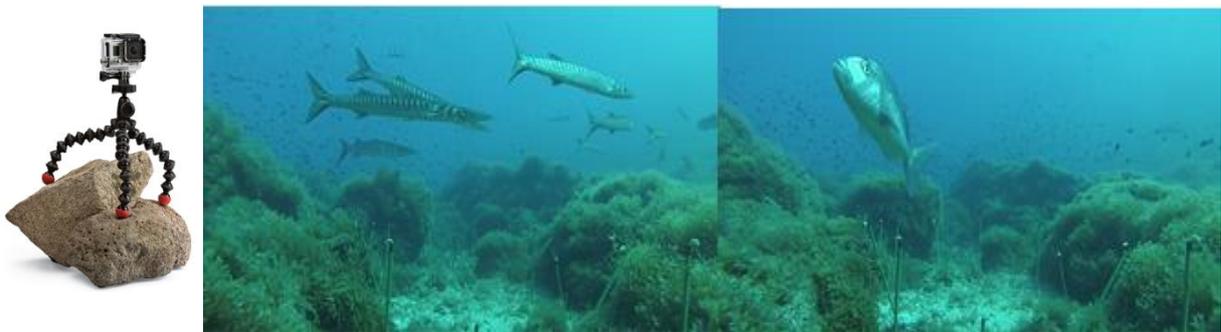


Figure 3.4. A GoPro cameras deployed to a row of squidpops during the first hour of deployment.

Squidpop video analysis

From each Squidpop replicate, again a one-hour video was recorded and abundance and species richness marked down. All data was inputted in a calculation matrix from which the percentage of predation frequency and intensity were extracted, at the one and twenty-four-hour mark.

Analysis

Meta-analytical approach on fish assemblages

A meta-analytical approach has been used to summarize data collected from eleven MPAs, providing results of independent experiments. Results are reported as Effect-size analysed in the metric of the natural logarithm of the ratio among the values of the response variable (i.e. species richness, MaxN) between protected (inside MPA) and external zones, in order to examine the

potential role of fisheries in areas where fisheries is strictly forbidden or local artisanal fisheries is allowed as response ratio (Micheli et al., 2004; Guidetti et al., 2008). A positive response ratio means an individual abundance or species richness greater within experimental groups rather than in controls (i.e. higher values in no-take rather than external zones). A negative response means the opposite trend (i.e. the abundance and/or species richness is higher in fished zones than unfished). Null effect means that there are no differences between the experimental and control groups.

The Effect size estimate was made by determining the log response ratio (Ln(RR)) calculated as:

$$\text{Ln} (\bar{Y}_E/\bar{Y}_C) \rightarrow (\bar{Y}_{\text{MPA}}/\bar{Y}_{\text{External}})$$

where \bar{Y}_E and \bar{Y}_C were the mean values of a variable (species richness or MaxN) in experimental (\bar{Y}_{MPA}) and control areas ($\bar{Y}_{\text{External}}$) (protected and unprotected zones respectively). To test how far samplings are spread out from their average value, the variance associated with the estimate means of different groups were analyzed.

$$S_{\text{LRR}} = \frac{S^2_E}{n_{(E)} \bar{Y}_E^2} - \frac{S^2_C}{n_{(C)} \bar{Y}_C^2}$$

These were provided by the ratio between the mean square for the protected zones and the sample size for each group. The equation represents the variance of Ln(RR), where S^2_E and $n_{(E)}$ constitute the variance and number of replies in experimental groups and S^2_C and $n_{(C)}$ are the variance and number of replies in control groups. The methods “continuous fixed-effect inverse variance” was utilized. This method performs a fixed-effect meta-analysis with inverse variance weighting.

It is important to note that a significant effect occurs when the 95% confidence limit around the average response ratio value does not fit into zero (Guidetti and Sala 2007).

Confidence Interval:

$$\text{CI} = \text{Ln}(\text{RR}) \pm (t_{\alpha/2}(n-1)) * S_{\text{LRR}}$$

where t is a critical value determined from the t n–1 distribution.

Furthermore, the responses to protection have been also measured as effect size on a functional level (i.e. trophic groups) based on Guidetti (2014).

Meta-analytical approach on top-down control

In order to assess the predation intensity along a gradient of protection/exploitation by SSF has been followed a meta-analytical approach (as reported for principal variables analyzed by BUVs). Especially, the Ln (RR) has been obtained comparing the ratio between average percentage of bait loss after the first hour of deployment between protected (no-take and buffer zones) vs unprotected (external zones) ($\bar{Y}_{\text{MPA}}\%/\bar{Y}_{\text{External}}\%$) among all eleven MPAs (both as overall effect as single “MPA-response”).

By means of video during the first hour of deployment, the main species approaching the squidpop stake has been recorded. The behaviour of all species (including target and no target) for each zone was reported as the ratio of total number of interactions with bait, expressed as a percentage, within all levels of protection among MPAs.

ANOVA

Univariate analyses of variance (ANOVA) of untransformed data were performed to assess differences, in each MPAs, for both protected (no-take, buffer) and unprotected (external) groups of Apex and Mesopredators abundances. The asymptotic significance was calculated using 9999 unrestricted permutations and type III based on Euclidean distance matrix performing a Monte Carlo tests (Anderson, 2001). In order to give a more accurate sense to the statistical hypothesis, significant results were considered using Bonferroni adjustment.

Species identification

Species were clustered and then divided into five trophic levels following Guidetti *et al* (2014) and matched with the literature available data and FishBase web site (<http://www.fishbase.org/search.php>). This categorization was made considering the different Mesopredator and Apex trophic value (up to 3.8 and from 4 or more respectively)

Mean relative abundance of apex and mesopredator were estimated by BUV surveys. Biomass of each species, belonging to each trophic group, was extracted on the maximum value of MaxN.

Results

A total of 370 BUV deployments and 154 Squidpop were carried out in 11 MPAs from June to September 2017.

When considering all 11 MPAs together, the protected zones exhibits the highest number of mesopredators species (43 fish taxa) whereas External zones showed lower values (35 fish taxa). The same trend was found for apex predators showing higher value in protected zones compared to unprotected (10 and 8 species respectively).

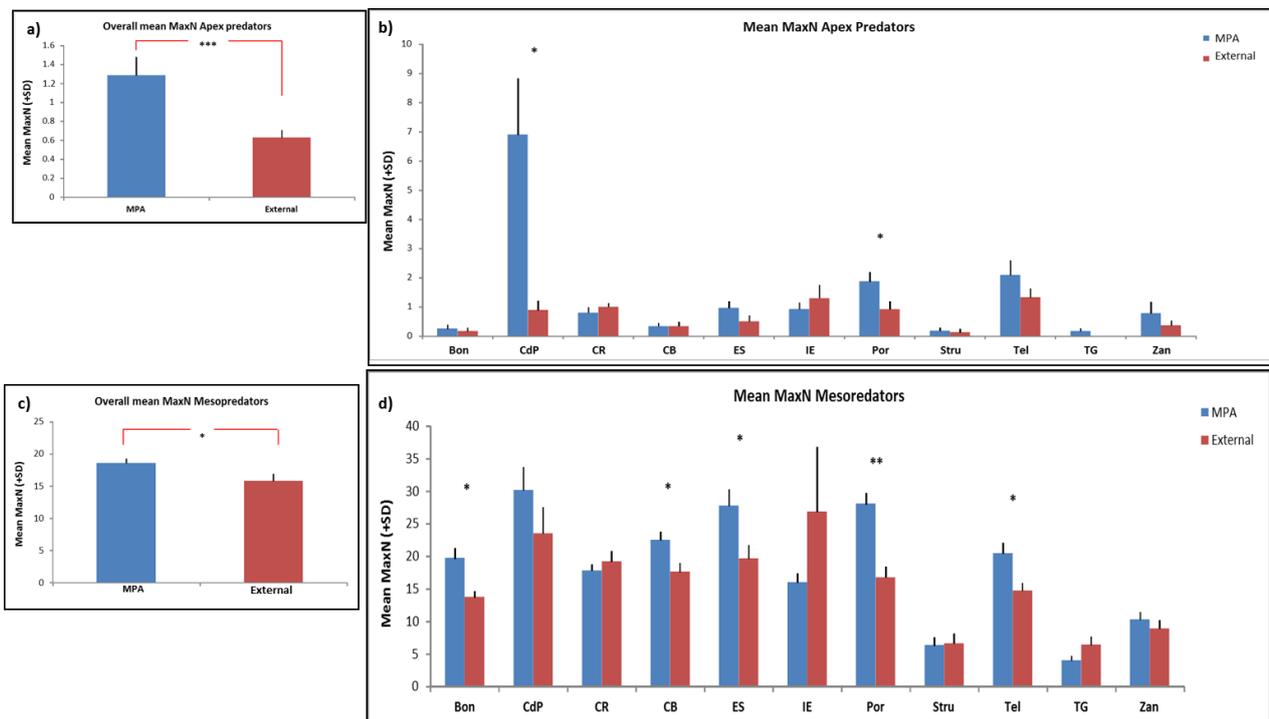


Figure 3.5. Mean relative abundances (+ SD) between protected and unprotected zones for a) Apex predators b) in each MPA and c) Mesopredators d) in each MPA. The asterisks highlight statistical significant differences between protected and unprotected zones

The overall mean relative number (\pm SE) of apex predators, recorded during 370 BUV surveys, was significantly higher in protected zones (1.286 ± 0.188) compared to unprotected (0.63 ± 0.07) ($p < 0.0008$). Considering the single MPA's response, only Cabo de Palos (protected 6.88 ± 1.92 ; unprotected 0.88 ± 0.3) and Portofino (protected 1.86 ± 0.3 ; unprotected 0.91 ± 0.25) showed significant differences (between MPA inside and outside ($p < 0.0376$ and $p < 0.048$ respectively).

In order to assess differences in mesopredators mean relative abundances (\pm SE), comparing protected (18.53 ± 0.72) and unprotected zones (15.82 ± 1.06), an ANOVA was carried out. Results showed significant differences between the two degrees of protection ($p < 0.0106$).

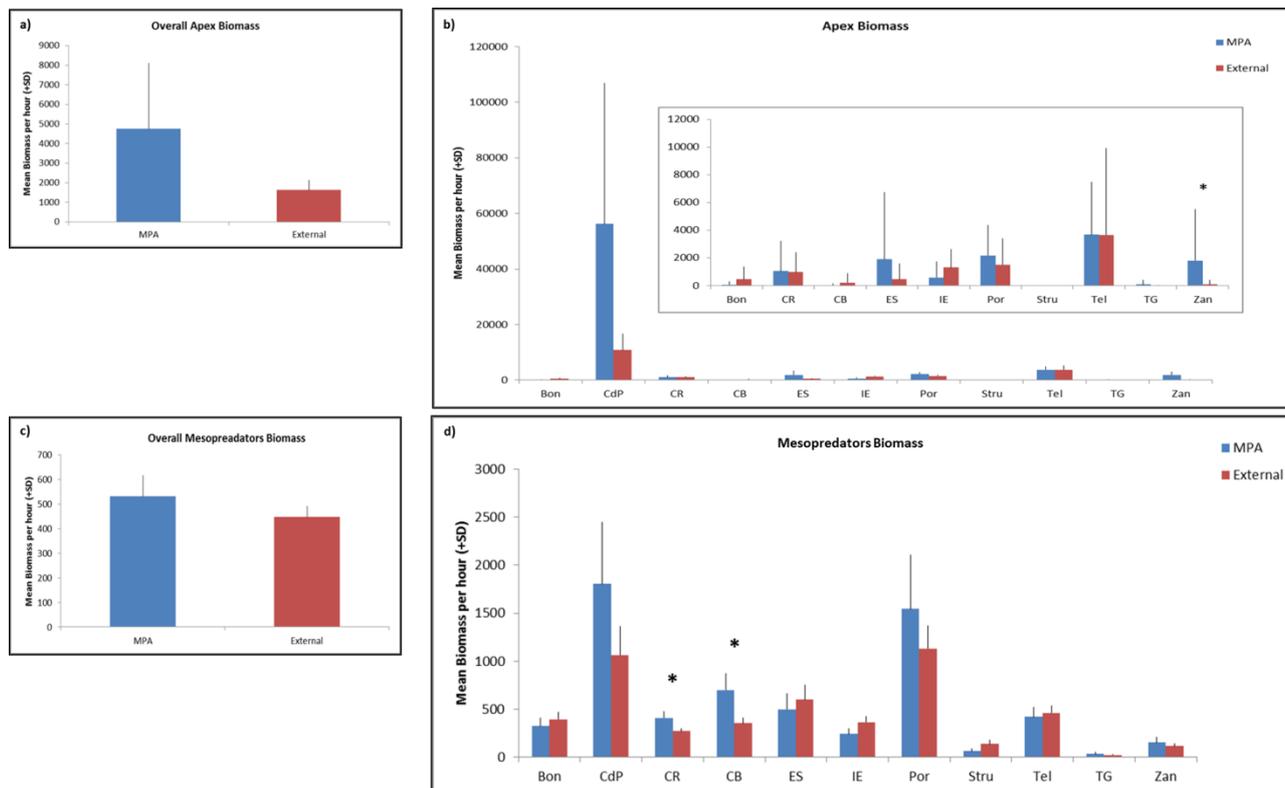


Figure 3.6. Mean biomass (+SD) between protected and unprotected zones for a) Apex predators b) in each MPA and c) Mesopredators d) in each MPA. The asterisks highlight statistical significant differences between protected and unprotected zones

Overall, both MaxN of Apex and Mesopredators was higher inside than outside the MPAs (Fig. 3.5.a,c; $P(\text{MC})=0.0008$ and $P(\text{MC})=0.036$, respectively). When focusing on single MPA responses, Bonifacio ($P(\text{MC}) = 0.0171$), Cote Bleue ($P(\text{MC}) = 0.0263$), Es Freus ($P(\text{MC}) = 0.0444$), Portofino ($P(\text{MC})= 0.0004$) and Telasciça ($P(\text{MC})= 0.0313$) showed a similar trend (Fig. 3.5 .d). Average biomass did not show any difference among protected and unprotected zones both for Apex and Mesopredators (Fig. 3.6.a, .c). Differences in the biomass of Apex predators were only detected in the Zakynthos MPA ($P(\text{MC}) = 0.0335$) (Fig. 3.5.b), while mesopredators exhibited higher biomass inside the MPa only at Cape Roux ($P(\text{MC}) = 0.0429$) and Cote Bleue ($P(\text{MC}) = 0.0237$) (Fig. 3.6.d).

Apex biomass vs Mesopredator biomass

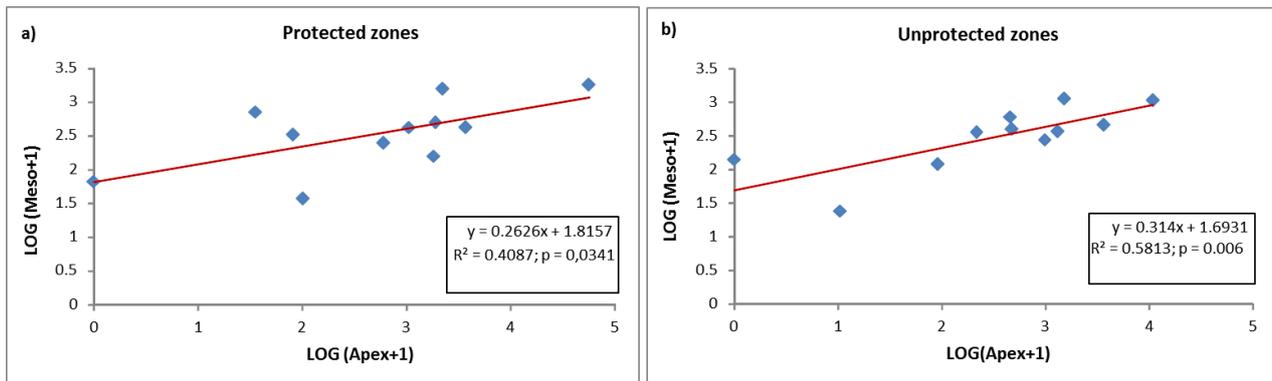


Figure 3.7. Linear regression a) of apex vs mesopredators biomass in protected zones and b) unprotected, N=11.

The allometric relationship between apex and mesopredator biomass was also assessed to evaluate whether the apex predators might affect the mesopredators biomass both inside and outside the 11 MPAs (Fig. 3.7.a; 3.7.b). The regression analyses revealed that the higher the apex predator biomass the higher was the mesopredators' biomass, thus rejecting once again any hypothesis of mesopredator release ($R = 0.63$ and $R = 0.762$, respectively).

Apex and Mesopredator frequency of predation

Here we have presented the results from 144 squidpop replicates deployed in 11 MPAs during the first monitoring year (from June to September 2017).

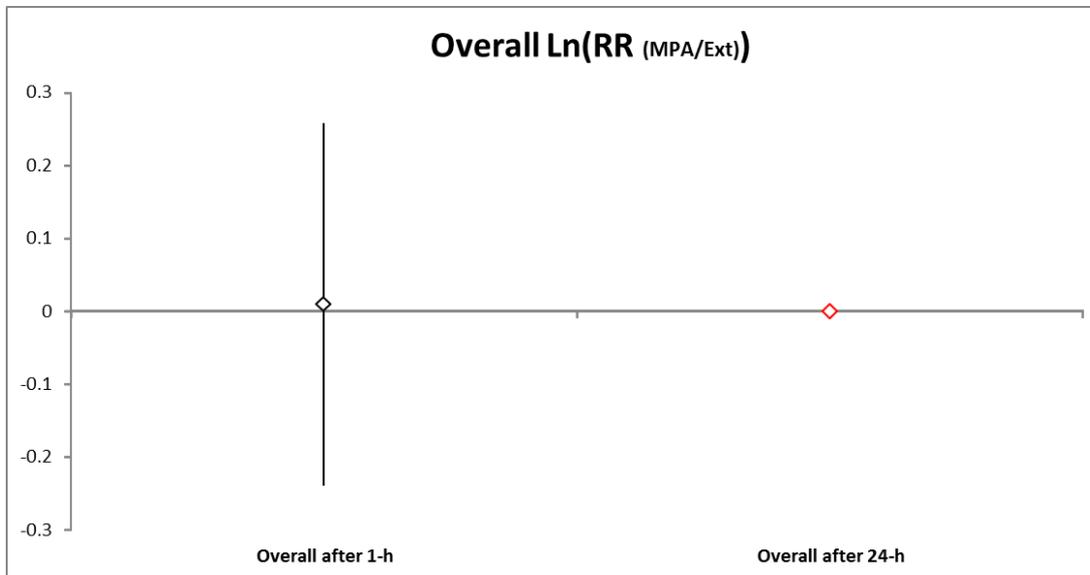


Figure 3.8. Overall log-response ratios showing predation intensity under different levels of protection (comparing inside and outside MPA) in the 11 MPAs after 1-hour and 24-hour squidpop deployments. Bars indicate 95% confidence interval.

In order to assess how predation rate (as number of bait lost after 1 and 24 hours of deployments) differs between protected and unprotected zones, an effect size was calculated.

In both cases, the overall effect did not show significant differences when pulling together the 11 MPAs (Fig. 3.8). That means that predation intensity was quite the same in and out MPAs.

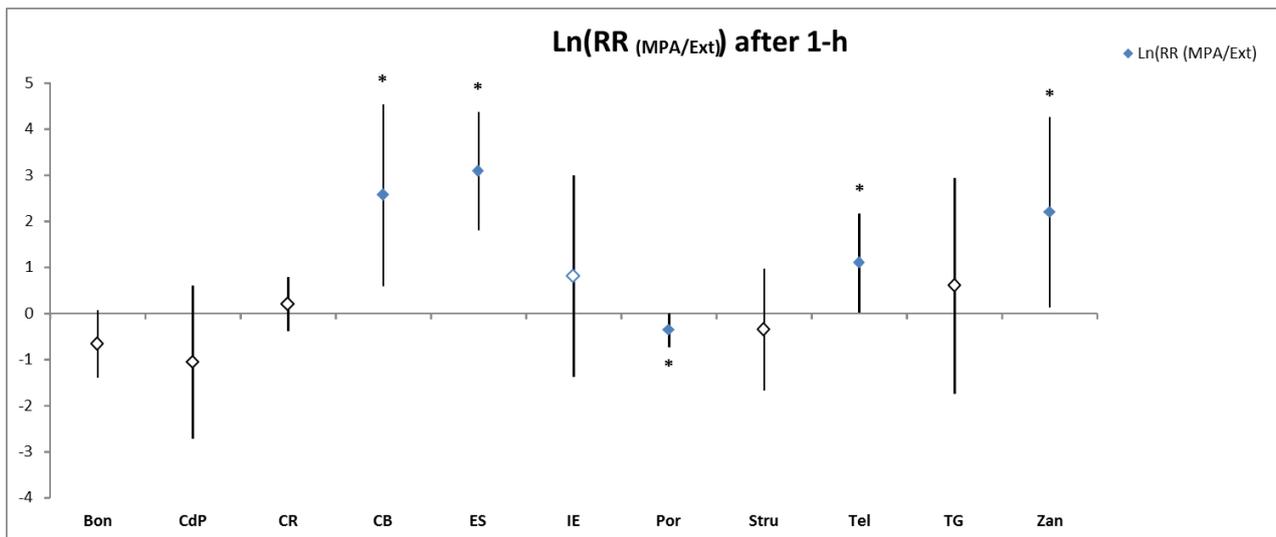


Figure 3.9. Log-response ratios showing predation intensity after 1-hour squidpop deployments under two levels of protection (inside MPAs vs external zones) in the 11 MPAs. Bars indicate 95% confidence intervals. Asterisks indicate significant effects. NA: not available. The 11 MPAs are reported in alphabetical order (Bon: Bonifacio; CdP: Cabo de Palos; CR: Cap roux; CB: Cote Bleue; EF: Es Freus; Ega: Egadi Islands; Por: Portofino; Stru: Strunjan; Tel: Telascica; TG: Torre Guaceto; Zak: Zakynthos).

Focusing on the intensity of predation between protected and unprotected within each MPA, after one hour of deployment, a different behaviour of each MPAs has been found (Fig. 3.9). Particularly, Cote Bleue, Es Freus, Telasciča and Zante showed positive effect comparing the number of baits lost in and out MPA after one hour of deployment ($RR_{Cote\ Bleue(MPA/External)} = 2.565$, $CI = 1.969$; $RR_{Es\ Freus(MPA/External)} = 3.091$, $CI = 1.281$; $RR_{Telasciča(MPA/External)} = 1.099$, $CI = 1.068$; $RR_{Zante(MPA/External)} = 2.193$, $CI = 2.057$).

Contrary to what expected, Portofino MPA showed a negative effect of bait lost, after one hour ($RR_{Portofino(MPA/External)} = -0.362$, $CI = 0.352$), meaning that there was a higher intensity of predation in external compared to MPA zones.

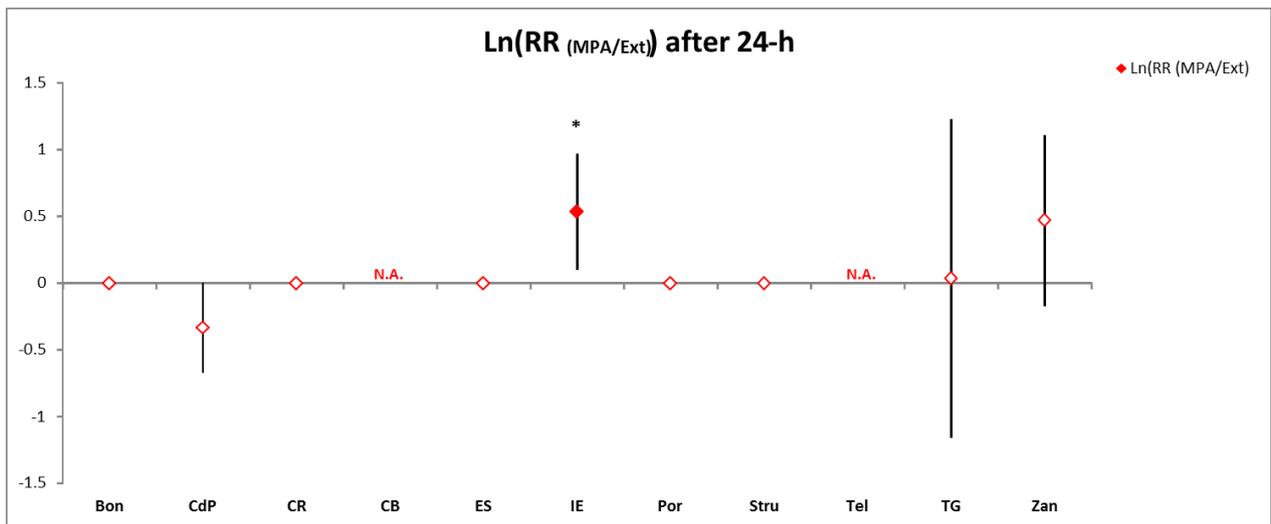


Figure 3.10. Log-response ratios showing predation intensity after 24-hour squidpop deployments under two levels of protection (inside MPAs vs external zones) in the 11 MPAs. Bars indicate 95% confidence intervals. Asterisks mark a significant effects. NA: not available. The 11 MPAs are reported in alphabetical order (Bon: Bonifacio; CdP: Cabo de Palos; CR: Cap roux; CB: Cote Bleue; EF: Es Freus; Ega: Egadi Islands; Por: Portofino; Stru: Strunjan; Tel: Telascica; TG: Torre Guaceto; Zak: Zakynthos).

No differences in predation intensity between protected and unprotected zones were found after 24-hour of deployments in all MPAs except for Isole Egadi MPA ($RR_{(MPA/External)} = 0.535$, $CI = 0.436$) where a higher percentage of squidpop in unprotected zones not had been eaten (Fig. 3.10). In some cases, a logarithm of the response ratio was not applicable (Cote Bleue and Telasciča), due to the missing value of replicates in external zone (mean and standard error zero).

Trophic groups contribution of predation

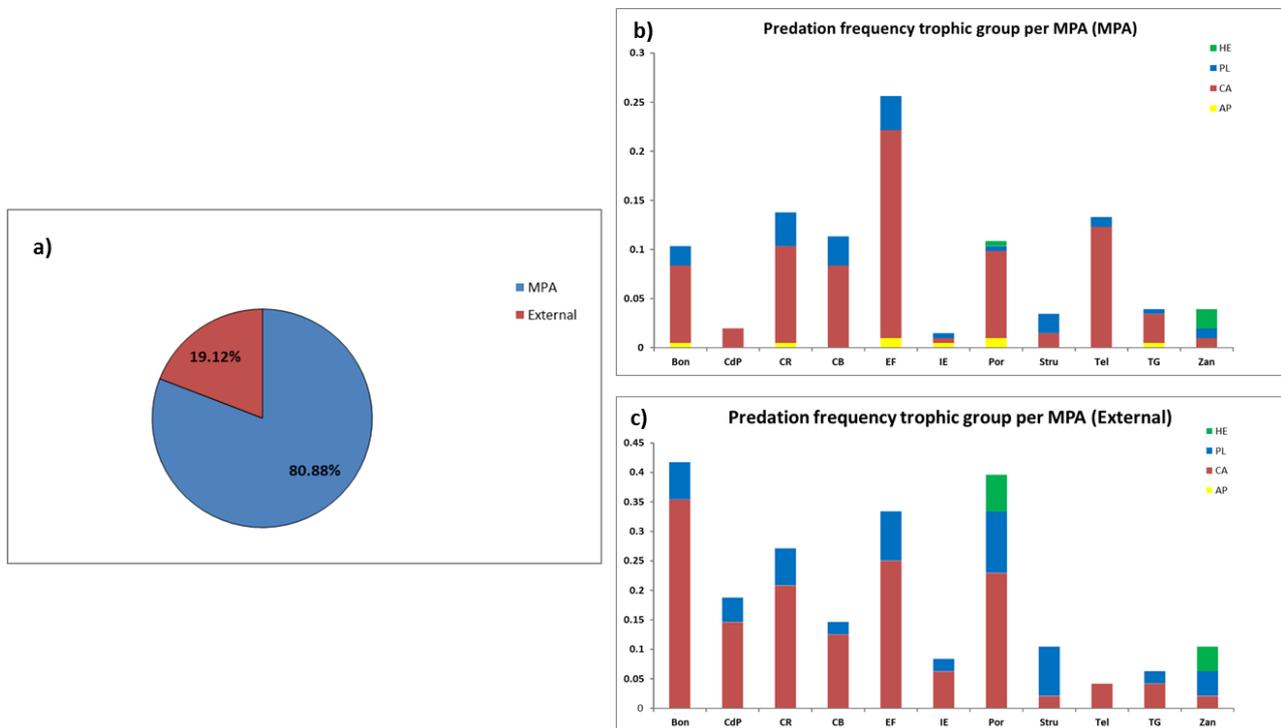


Figure 3.11. Overall percentage contribution of predation of all species per protection's levels (a). Predation frequency for each trophic group per MPA (b) and external zones (c). Trophic groups are reported as herbivores (green), planktivores (blue), carnivores (red) and top predators (yellow).

The analysis of the videos recorded during the first hour of squidpop deployments allowed us to identify a total of 22 fish taxa interacting with baits. Considering the levels of protection, species richness was higher inside MPA (21 species) than in External zone (14 species). Figure 3.11. a provides the predation percentage contribution of all species along a gradient of protection. Furthermore, the predation frequency interaction's with bait for each trophic group, per protected zones, among all MPAs was assessed (Fig. 3.11.b; 3.11.c). As expected, comparing the response ratio results, the number of predators (AP) interacting with bait was exclusive inside the MPA where ideally, all fishing activities are forbidden.

To give a more accurate sense, during the first hour of deployment, the species interacting with bait were checked. In the graph above, the sum of each species and their contribution is reported. The different species were clustered by trophic group and the sum of each species was related within the total interactions for each zone (Fig. 3.11 .b; 3.11 .c). This was done in order to assess and quantify the number of bait interactions out by different trophic groups.

DISCUSSIONS

This study is one of the first attempts to verify the hypothesis of Mesopredator release in the context of Mediterranean MPAs. Contrarily to what previous studies carried out in marine (De Martini *et al.*, 2008; Friedlander and DeMartini, 2002) and terrestrial (Crowell 1961; Prugh *et al.*, 2009; Brashares *et al.*, 2010) environments suggested, these findings reject the mesopredator release hypothesis. The results highlighted a different but consistent relationship between apex and mesopredators among protected (experimental condition) and unprotected (control conditions) zones. These findings reinforce earlier evidence on MPAs' role in maintaining higher abundance and biomass of fish species often threatened by fisheries.

The overall mean abundance of apex predators and mesopredators were highly different between protected than unprotected zones, underlying the detrimental role of fishing activities (Claudet *et al.*, 2006; Guidetti and Sala, 2007; Claudet *et al.*, 2008; Di Franco *et al.*, 2009).

Looking at overall mean biomass, the hypothesis that mesopredators would increase in unprotected zones was not verified. Both as the apex and mesopredators biomasses, showed no significant differences among the interested zones, with the exception of a few MPAs (Fig. 3.6 .b, .d). Moreover, mesopredators biomass increases proportionately to apex biomass, both inside and, even more so, outside MPAs, supporting a refusal of the mesopredator release theory (Fig. 3.7.a, .b).

Furthermore, predation intensity did not differ between protected and unprotected zones in all the MPAs under consideration. The overall effect size showed no statistical difference both after one and twenty-four hours of exposition to predators in the wild (Fig. 3.8), indicating no variations in top-down control through space. Whilst overall predation intensity did not differ among groups, for some MPAs this trend seems to be not verified. In fact, in Cote Bleue, Es Freus, Telascica and Zakynthos, significant differences were found in predation rates inside and outside MPAs boundaries.

The overall percentage of predation contribution, resulted to be much higher (80.88%) in protected than unprotected areas (19.12%), the first of which were also characterized by a more structured community (Fig. 3.11. a), with the second witnessing a near absence of top predators in predation contribution.

Interestingly, this study revealed an opposite trend in mesopredator predation frequency relating to MPA efficiency. In fact, in well-functioning MPAs, the intensity of predation by mesopredators is lower than less efficient ones, possibly because well-functioning MPAs have more resources available that make squidpop bait less attractive (see Fig. 3.9, 3.10; CdP After 1 and 24 h), or because mesopredators tend to be shyer due to the high abundance of top predators (Brown and Kotler, 2004; Bologna, 2007; Gusmao *et al.*, 2018).

The higher abundance of some apex predators, such as *Seriola dumerili* and *Dentex dentex*, was observed in the almost pristine and well-enforced MPAs (Cabo de Palos, Bonifacio, in line with Garcia-Charton *et al.* (2007)) which also showed, almost paradoxically, one of the lowest rates of predation frequency. In Cabo de Palos Marine Reserve, we witnessed an MPA where the food chain shows an upward shift in trophic levels, displaying changes in the fundamental ecological functioning of the protected ecosystems (Micheli and Halpern, 2005; Garcia-Charton *et al.*, 2008).

Moreover, to support the findings related to MPA efficiency, in Zakynthos Marine National Park (well-enforced but fully protected for just six months a year), we witnessed an ecosystem altered by the exposure to invasive alien species (Giakoumi *et al.*, 2018) (belonging to *Siganus* Genus). Firstly recorded in 1972 (Tortonese, 1970; Bariche, 2006), both species (*Siganus luridus* and *Siganus rivulatus*) have had, in a couple of decades, a significantly detrimental effect on the Eastern-Mediterranean algal community. The almost total removal of the algal canopy fundamentally modified the rocky bottom, indirectly removing support to the invertebrates associated with it. This cascade-effect produced a modification in resources that generated a barren situation. For instance, this could explain the Zakynthos Marine Park results, where consumption rate was higher in protected zones (characterized by *Posidonia oceanica* canopy) after the first hour of deployment compared to external zones (outlined by barren condition) (Dimitriadis *et al.*, 2018).

Furthermore, the obtained results could be supported by behavioural factors. Although the fishing activity mostly influences apex and mesopredator abundance among protected and unprotected areas, predation carried out by apex predators themselves could promote behavioural modifications at lower trophic levels. As reported by Liberman *et al* (1995), predator activity is one of the main factors influencing sheltering behaviour in prey, contrary to what was reported by Gauff *et al* (2018). This could explain the obtained results, since increases in predation activity seem to also increase mesopredator sheltering behaviour. In this respect, Cabo de Palos and Portofino MPAs have shown higher apex predator mean abundances, and lower predation

frequency at the same time. Conversely, the Isole Egadi MPA showed significant rates of predation in protected zones after twenty-four hours of squidpop deployment. Comparing this behaviour with other MPAs results in an inverse proportion between the number of apex predators and predation intensity. The greater the number of predators, the lower the predation, and vice versa.

This study evidences the successful role that MPAs have on maintaining high fish abundance in protected zones rather than outside, where the activity of fisheries is allowed. Comparing protected and unprotected zones there were no mean biomass differences among the two principal trophic groups analyzed, showing no spatial segregation of apex and mesopredator size.

It should be noted that marine species are not spatially confined and therefore free to move across, differently from terrestrial ones. This study was carried out in coastal shallow environments between five and twelve meters of depth, on rocky bottom, neglecting areas of algal coverage that, indirectly, could support higher mesopredators richness and abundance. No mean trophic levels differences were found among protected and unprotected zones, suggesting a possible functional species redundancy between MPAs and their boundaries.

Natural disturbance events can alter the trajectories of coastal systems, causing variable responses in fish populations via erratic recruitment, ecological plasticity, changes in growth, feeding behaviour and conditions (Nyström *et al.* 2000; Barley *et al.*, 2017). Additionally, it is possible that the higher apex competition, in protected zones, could change the choices and habits of the competitors, which are forced to move towards the edges to find additional resources. The existence of two groups of competitors, besides fishing activities, could promote mesopredator species richness reductions in unprotected zones. This could justify the different mesopredator richness rates among the areas (results not shown: the absolute value of forty-three and thirty-five species in protected and unprotected zones respectively).

The reported surveys have been carried out during summer 2017 and we don't know if the analysed trends are constant or undergo temporal changes. Moreover, the experimental design provided a sampling between five and fifteen meters of depth, ignoring deeper waters.

Even if ethically forbidden due to the very concept of MPA, it would be valuable to take experimental catch to evaluate, through stable isotopes, effectively "who eats what".

It is a fact that top-down processes driven by apex predators can have a significant influence on ecosystem function, through their effects on mesopredator populations (Ritchie and Johnson, 2009).

However, these interactions may be affected by bottom-up processes bolstered by artisanal and industrial fisheries.

Furthermore, it is necessary to consider species behaviour in order to evaluate the mechanisms that can cause misrepresented responses (*e.g.* fear and loathing out by apex on mesopredator). Finally, the synergic effect of predators and fishing activity could generate behavioural changes on mesopredator predation mode (*e.g.* from wait-chase to ambush modality or from cruise-chase to dwelling).

Marine systems appear underrepresented in comparison to terrestrial ones due to the different nature of the medium and the absence of an ecotone that eliminates spatial limitations. Fulfilling these gaps could be crucial for MPA management planning in order to restore the original trophic state in these ideally pristine systems.

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Chapter 4

Assessing ocean acidification effects on fish assemblages composition and function via altered habitat provisioning

INTRODUCTION

Ecological communities are constantly responding to environmental change, and the diversity among functionally similar taxa broadens their range of responses (Loreau et al., 2002). Ocean acidification (OA) – a global environmental perturbation in the form of seawater CO₂ enrichment and changes in carbonate chemistry due to rising CO₂ emissions – is occurring at an unprecedented rate and is predicted to further act over time (Honish et al. 2012; Portner et al. 2014). Laboratory studies, often carried out on single species in isolation, have documented detrimental biological consequences of OA levels expected by the end of this century and beyond, with marine molluscs and scleractinian corals identified as OA-intolerant taxa, and crustaceans and fishes considered as more tolerant groups (Kroeker et al. 2013; Wittman & Portner 2013).

Since difficulties occur when the results of laboratory experiments have to be scaled up to assess community wide responses to increasing CO₂ (Riebesell & Gattuso 2015), recently, the OA research field has moved into large community experiments to ensure that the effects are not just stress-responses and are more ecologically realistic.

In this context, shallow marine CO₂ vents may be used as natural analogues to further our knowledge of OA at the community level. Recent evidence from ecosystem-scale studies suggests that many different OA effects might be at play by ultimately affecting community emergent properties (Hall-Spencer et al. 2008; Fabricius et al. 2011, 2014; Inoue et al. 2013; Enochs et al. 2015). For instance, (a) ocean acidification mediates biodiversity shift altering competitive interactions and changing structural complexity, in four different major biogenic habitats (i.e., coral reefs, mussel beds, seagrass meadows and macroalgae) (Sunday et al. 2017). (b) The increase of primary producers boosted by elevated CO₂ might counter-intuitively drive – via more food/habitat provisioning – greater abundances of grazer species (Connell et al. 2017). (c) The reduced abundance of OA-sensitive species can be compensated by the increased abundance of more OA-tolerant species (Fabricius et al. 2011; Kroeker et al. 2011), therefore fostering compensatory dynamics and likely maintaining ecosystem function (Gonzalez & Loreau 2009).

Indeed, such processes do not act in isolation and can concomitantly operate. Under these circumstances, ecological facilitation, species interactions and counteracting tolerant/intolerant population responses to CO₂ enrichment/pH lowering may prevent clear predictions about the impact of OA at ecosystem level (Gaylord et al. 2015; Kroeker et al. 2017). To date, how OA-associated benthic community changes affect their taxonomic and functional properties awaits further study.

Here, I aim at documenting both direct and indirect OA-driven benthic community shifts, habitat complexity changes and fish diversity and functional responses. Using Baited-Underwater-Videosystems (BUVs) and tethering experiments to assess fish predation intensity, I focussed my study on fish associated with benthic habitats along three different CO₂ gradients of well-studied temperate (Vulcano Island, Italy), subtropical (Shikinejima, Japan) and tropical (Normanby island, Papua New Guinea) volcanic seeps. Firstly, I assessed whether CO₂ enrichment can reverse competitive dominance in benthic habitat forming species by acting as a stressor for reef building and/or OA sensitive species and as a resource for canopy algae and turf forms, hence releasing their dominance. Secondly, I hypothesised that community properties (i.e. overall associated fish species richness) would vary as a consequence of habitat provisioning changes in relation to OA, and I anticipate fish communities may respond in different ways in the different systems investigated. Specifically, the richness of fish taxa will decrease where reef-building species (i.e., scleractinians) presence or heterogeneity is affected by elevated CO₂ levels, while turf algae are boosted. By contrast, where CO₂ enrichment positively affects primary producers (i.e. boosting algal or seagrass productivity and their complexity), that could be the case of vegetated temperate habitats, this will allow the fish richness to increase. Finally, I expect community function, evaluated as predation intensities in different CO₂ conditions, will be maintained despite fish composition changes with OA.

METHODS

Description of the different CO₂ seeps sites investigated

Vulcano Island

In Vulcano Island, benthic community surveys, BUV deployments to assess fish assemblages and tethering experiments were carried out on May 2017 at four shallow sites (1-3 m depth) in Levante Bay (38°22'53.27"N; 14°56'25.07"E; NE Sicily; Italy), two exposed to high-CO₂ and two to ambient-CO₂ levels. The southern part of the bay is characterized by active shallow-water volcanic CO₂ seeps that generates a pH/pCO₂ gradient at depths ranging from 1 to 6 meters along its north-eastern coast. Winds coming from west generate a circular current within the bay that moves the water mass eastward, shaping a pH/pCO₂ gradient ranging from 5.2-5.5 pH at the emission sites, to 8.1 pH at >500m distance. Previous geochemical studies monitored the concentration of the main gases in Levante bay shallow waters, including the main seep emission spots which exhibit on average 98% CO₂, 1.5% N₂, 0.21% O₂, 1700 ppm CH₄, <5 ppm H₂ e 400 ppm H₂S (Boatta *et al.*, 2013). One of the main issues related to experiments at hydrothermal sites is represented by the presence of toxic gases such as H₂S. In Vulcano Island, as a consequence of high O₂ concentrations at the emission sites, this gas rapidly oxidises to sulphates and is absent a few meters apart from emission spots (Boatta *et al.*, 2013). To avoid any confounding effect, the surveys were performed at 300 meters (high-CO₂ condition sites with on average 7.8 pH units) and at 600 meters (ambient-CO₂ conditions, i.e. mean 8.1 pH units), at well-characterised pH/pCO₂ sampling stations along the Vulcano gradient (e.g. Arnold *et al.*, 2012; Johnson *et al.*, 2013; Boatta *et al.*, 2013; Calosi *et al.*, 2013; Vizzini *et al.*, 2013; Milazzo *et al.*, 2014; Cornwall *et al.*, 2017; Brown *et al.*, 2018).

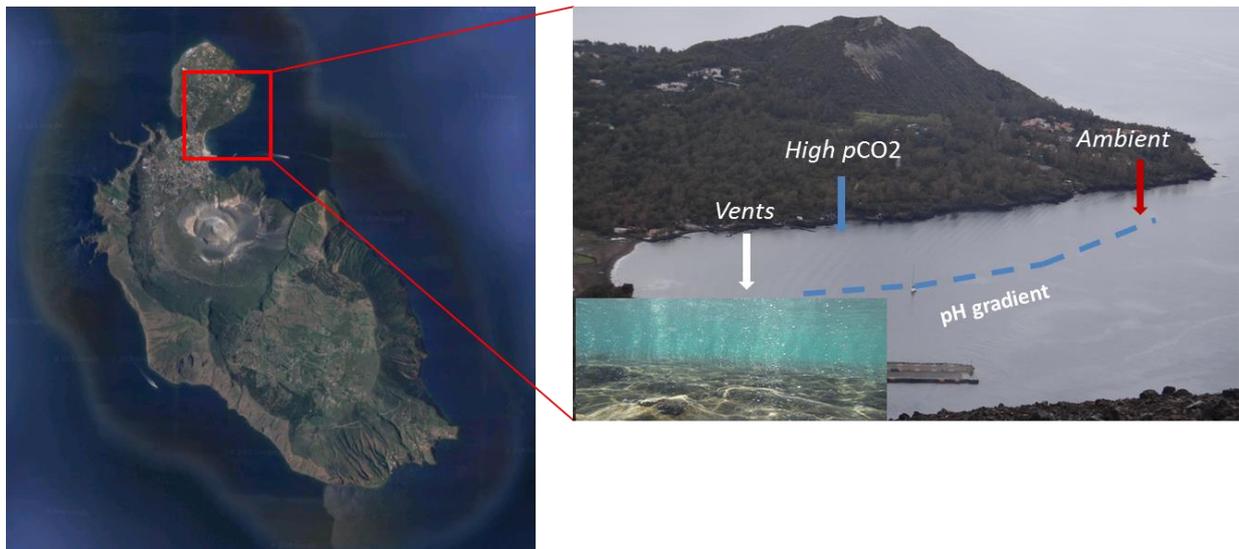


Figure 4.1. Satellite (left) and aerial (right) images of Levante Bay in Vulcano Island. The white arrow points to the main seep, from which the pH gradient originates moving outside the bay. The two surveyed locations are marked by the blue and red arrows.

Shikine Island

Shikine-jima is a volcanic island east of the Izu peninsula in Japan ($34^{\circ} 19' 9''$ N, $139^{\circ} 12' 18''$ E) with many CO_2 seeps in its shallow waters. Benthic and fish surveys were carried out on Sept-Oct 2016 at both high- CO_2 and ambient- CO_2 sites located around Mikama Bay ($34^{\circ}19'34''\text{N}$; $139^{\circ}12'36''\text{E}$). The island coast is invested by the Kuroshio warm current, which promotes the presence of many different tropical scleractinian and fish species in shallow waters (Agostini *et al.*, 2018). In this study, surveys were conducted on rocky bottom at depths ranging from 3 to 10 meters in two locations exposed to different pH/ $p\text{CO}_2$ conditions (high- CO_2 and ambient- CO_2). Both the geo- and the carbonate chemistry of these locations were extensively characterised by Agostini *et al.* (2015). More recently, Agostini *et al.* (2018) have documented benthic community shifts both in the intertidal and the upper subtidal zones.



Figure 4.2. Satellite and aerial images of Mikama Bay in Shikine Island (Japan) where different seep areas originate pH gradients moving outside the bay. The two surveyed locations are marked by the blue and red arrows.

Normanby Island

In January 2017, benthic surveys, BUV deployments and Squidpop tethering experiments were carried out in Upa Upasina Bay (9°49'27.21"S; 150°49'4.31"E; Normanby Island, Papua New Guinea). Two locations and four random sites within each location were selected in another well-established active CO₂ emission area, one with current ambient CO₂ concentrations exhibiting on average 400 $\mu\text{atm pCO}_2$ and the other with elevated CO₂ levels (~1300 $\mu\text{atm pCO}_2$). In addition to detailed carbonate chemistry data, the main biogenic characteristics of such locations were deeply documented by previous studies (Fabricius *et al.*, 2011; 2014), revealing spatial shift from from branching, foliose and table corals to hard massive corals in the seeping area. Surveys were carried out at depths ranging from 3 to 10 meters.



Figure 4.3. Satellite (left) and aerial (right) images of Upa Upasina in Normanby Island. The pH gradient originates from the Upa Upasina village (blue arrow). The red arrow indicates the control zone.

Carbonate chemistry analyses

Seawater pH, temperature and salinity were monitored using and YSI multi-parameter logger in Italy and a WQC24 multi-parameter logger coupled to a depth sensor (DKK-TOA Corporation, Tokyo, Japan) and a GPS (eTrex30x, Garmin) in Japan and Papua New Guinea. Total alkalinity (TA) was measured from water samples collected at each subtidal site ($n = 6$ for each site). Water samples were immediately filtered at $0.45 \mu\text{m}$ using disposable cellulose acetate filters (Dismic, Advantech, Japan) and stored at room temperature in the dark until measurement. TA was measured by titration (785 DMP Titrino, Metrohm) with HCl at 0.1 mol l^{-1} , and calculated from the Gran function between pH 4.2 and 3.0. The titrations were cross-validated using a working standard (SD: $\pm 9 \mu\text{mol kg}^{-1}$) and against certified reference material purchased from the A.G. Dickson laboratory. Temperature, pH, salinity and total alkalinity were used to calculate $p\text{CO}_2$ levels, which in the case of Vulcano island and Shikine-jima were also controlled by continuous *in situ* measurements of $p\text{CO}_2$ deploying a Hydro-II CO_2 sensor (Contros System & Solutions GmbH, Germany). Direct

measures of pCO₂ were taken every 10m along linear 100m transects in the different sampling sites. Four and six 100 m transects were positioned in Vulcano and Shikine respectively, both in the High and the Ambient CO₂ sites. The measured pH, TA, temperature and salinity were used as the input variables, alongside the dissociation constants from Mehrbach et al. (1973), as adjusted by Dickson & Millero (1987), KSO₄ using Dickson (1990), and total borate concentrations from Uppström (1974). The summary of the results of carbonate chemistry analyses are shown in Table 4.1. Due to the large mobility of some fish species and the spatial extent of more acidified sites around the seeps, the carbonate chemistry data are reported as an overall average for each CO₂ location in each study site.

Table 4.1 - Summary of the seawater carbonate chemistry of the sampling locations off Vulcano Island (ITA), Shikine Island (JAP) and Papua New Guinea (PNG) CO₂ gradients. Measurements were taken on different days using both a YSI logger or a DKK-TOA logger every 5 or 10 meters along 50 and 100m transects, respectively.

	n	Salinity	T (°C)	pH	Calculated pCO ₂	Measured pCO ₂
ITA_High CO ₂	102	38.11 (±0.03)	19.38 (±0.06)	7.77 (± 0.19)	1122 (± 592)	986 (±482)
ITA_Amb CO ₂	108	38.07 (±0.02)	19.21 (±0.06)	8.08 (±0.02)	395 (± 22)	430 (±59)
JAP_High CO ₂	298	34.71 (±0.05)	19.54 (±0.66)	7.87 (±0.15)	987 (±740)	1098 (±618)
JAP_Amb CO ₂	280	34.72 (±0.05)	18.89 (±0.31)	8.24 (±0.01)	325 (±41)	356 (±108)
PNG_High CO ₂	205	35.27 (±0.11)	30.85 (±0.3)	7.83 (±0.27)	397 (±67)	n.a.
PNG_Amb CO ₂	216	35.31 (±0.09)	30.49 (±0.17)	8.17 (±0.06)	1299 (±1113)	n.a.

SUBSTRATE RUGOSITY, BENTHIC CANOPY AND PERCENTAGE COVER

In each study site, a standard chain method (Luckhurst & Luckhurst, 1978) was used to estimate the substrate rugosity. This method consists in using a 30 meter chain with 1 cm long links, distended at the bottom (across different physical substratum) in order to map out the topography of each transect. The chain is stretched out on rocks, pebbles and boulders to typify the real substrate heights avoiding encompassing the most complex hard biotic features (e.g. massive corals). A transect line is positioned under the chain to verify difference between the linear transect and the chain. The ratio between contoured and linear distances provides the chain-transect rugosity.

The percentage coverage (%C) of the benthic morphologies was calculated using the photoquadrat method. Ten replicates were performed along 100m transects (Fig. 4.4). Each quadrat was

randomly positioned about every ten meters along the transect line and photographed with an Olympus Stylus Tough TG3 camera with a PT056 housing. In order to trace the contours of all benthic features, the Image-J software was used to calculate the percentage coverage.

Finally, the biotic canopy was estimated at every meter by measuring the height of the features present at the bottom, using the point-intercept standard method. The main benthic features are reported below (Tab. 4.2).

Table 4.2. *Benthic features within the climatic zones considered: canopy forming algae (height >5 cm), non-canopy forming algae (height ≤5 cm), turf (unidentified small filamentous algae and diatoms aggregations), crustose coralline algae (CCA).*

Vulcano Island (Italy)	Shikinejima Island (JAPAN)	Normanby Island (PAPUA NEW GUINEA)
Seagrass		Seagrass
Canopy	Canopy	Macroalgae
Non Canopy	Non Canopy	
Turf	Turf	Thick Turf
		Thin Turf
CCA	CCA	
Anemone	Anemone	Anemone
Other	Table Coral	Table Coral
	Soft Coral	Soft Coral
	Encrusting	Encrusting
	Massive Corals	Massive Corals
		Small Branch Coral
		Big Branch Coral
		Foliate Coral
		Solitary Corals
		Erected Sponge
		Encrusting Sponge

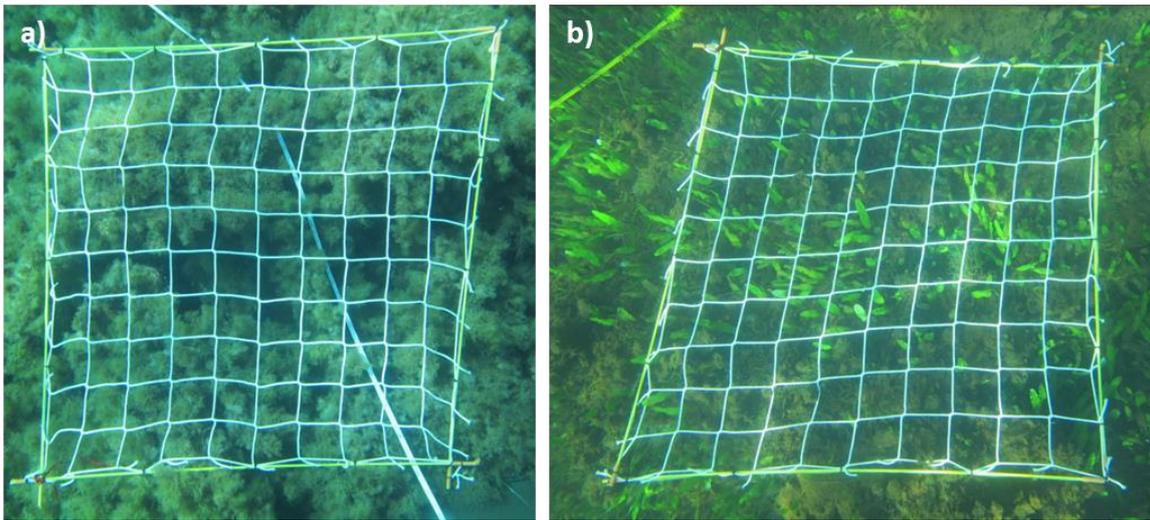


Figure 4.4. Examples of the quadrats used to calculate percentage coverage in Vulcano at ambient (a) and elevated (b) pCO_2 levels.

Assessing fish assemblages' composition and structure: description of the Stereo Baited Underwater Video system technique and of the video analyses employed

The Baited Underwater Video system is a remote technique that consists of a stainless steel frame (120x80 cm, respectively the primary and secondary axis) equipped with a GoPro Hero 3 high definition camera (720p@60 fps) with a wide-angle lens, continuously recording for one hour. In front of the cameras, at 1.20 m distance, a perforated box with 400 g of crushed *Sardina* spp. was placed to attract fish inside the field of view. Due to their high fish oil dispersion, this bait type ensured the arrival of different species belonging to different trophic levels.

As reported in multiple studies, sardines represents the most effective type of bait at attracting fish thanks to Odour-plume dispersion (Westerberg *et al.*, 2010; Wraith *et al.*, 2013). To ensure proper stabilization during descent, a lead has been fixed at each extremity of the secondary axis of the frame. BUVs were deployed from the boat and recovered after 1-h using a rope attached to a surface buoy marking the location.

Each replicate was left recorded for 60 min, and the start and end time were checked. To avoid the pseudo-replication, each BUV system were deployed different meters apart in the same location. During the same day replicates were dropped in different pH/ pCO_2 sites to avoid the odour plume dispersion and attractions of the previous replicates. In order to assess the spatial variability of shallow water reef fish assemblages, seventeen BUV deployments were carried out in Vulcano Island (nine in high- CO_2 and eight in ambient- CO_2 condition), fourteen in Shikine-Jima Island (seven

in high-CO₂ and seven in ambient-CO₂ condition) and nineteen in Normanby Island (nine in high-CO₂ and ten in ambient-CO₂ condition).

For each 60min video, species richness (S) and MaxN (a conservative measure of abundance calculated as the maximum number of fish of the same species identified over the observation period) were successively analysed. To evaluate species richness, every fish sighted in the field of view was considered, regardless of proximity, whilst *MaxN* was calculated taking only species present within 2 meters around the bait.

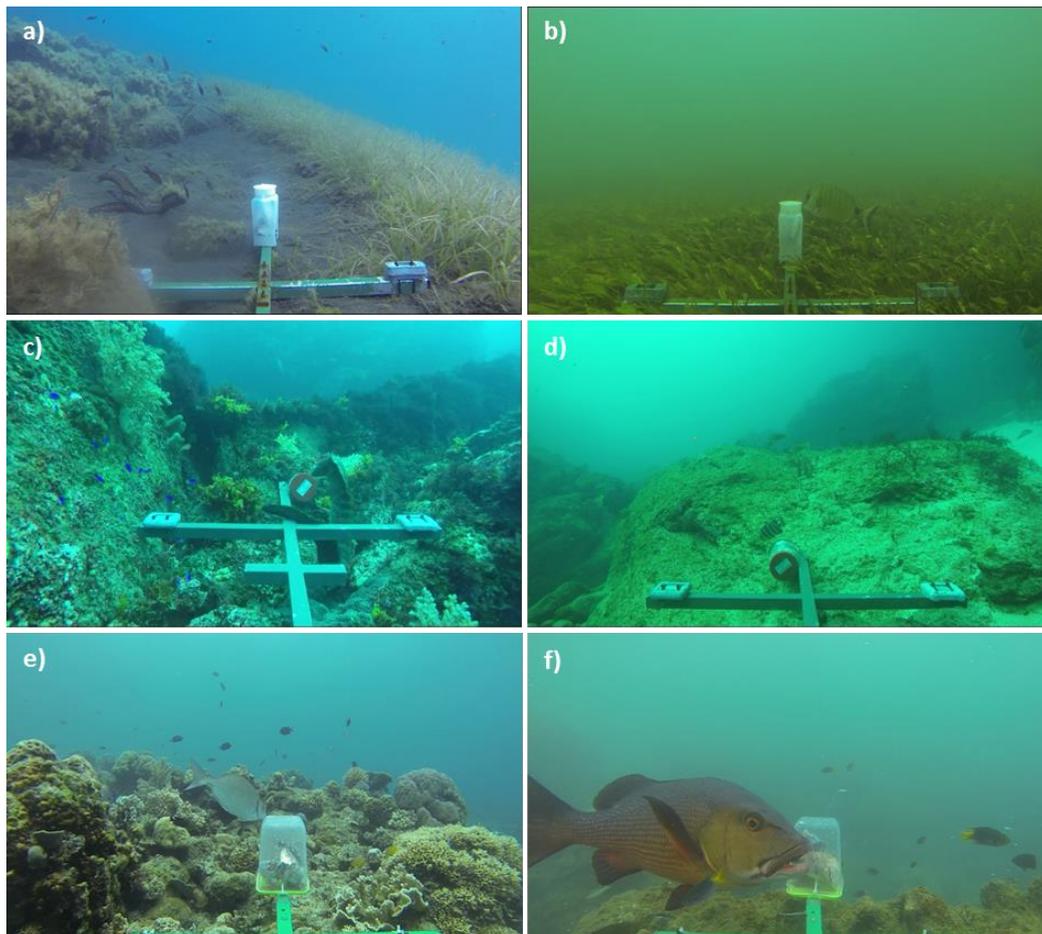


Figure 4.5. Still frames taken from the BUVs surveys at each CO₂ seep location at ambient (left) and high CO₂ (right) locations in Vulcano Island (a, b), Shikine Island (c, d), Normanby Island (e, f).

Assessing fish predation intensity by a standard tethering experiment

The tethering experiment consisted of 1-h and 24-h exposures of standard prey items to fish predation, namely the Squidpop technique (Duffy *et al.*, 2016). This method uses as a bait a 3 cm diameter disk of dried squid mantle tethered by a monofilament line to the end of a fibreglass plant stake (60 cm x 0.60 cm). One assay consisted of 25 Squidpop replicates arranged in one or

two rows and positioned on the sea bottom roughly along the same depth contour. Stakes were spaced each other by 1-2 m (Fig. 4.6). To assess the relative rates of bait consumption across the different $p\text{CO}_2$ conditions, a diver annotated by binary code (0 absence, 1 presence) the number of baits eaten after one and twenty-four hours, after which stakes were retrieved. During the first hour of deployment, a GoPro Hero 3 camera was positioned alongside every Squidpop replicate, to qualitatively assess predator richness.

To investigate how predator pressure may vary across the different CO_2 seeps, predation frequency of the two CO_2 locations within each seep. Twelve replicates were randomly deployed in Vulcano Island (Italy) (6 in high- CO_2 and six in ambient- CO_2 conditions), fourteen in Shikine Island (Japan) (seven in high- CO_2 and seven in ambient- CO_2 conditions) and eighteen (nine in high- CO_2 and nine in ambient- CO_2 conditions) in Normanby Island (PNG).

From each Squidpop replicate, again a one-hour video was recorded and abundance and species richness marked down. All data was inputted in a calculation matrix from which the percentage of predation frequency and intensity were extracted, at the one and twenty-four-hour mark at considered $\text{pH}/p\text{CO}_2$ sites.

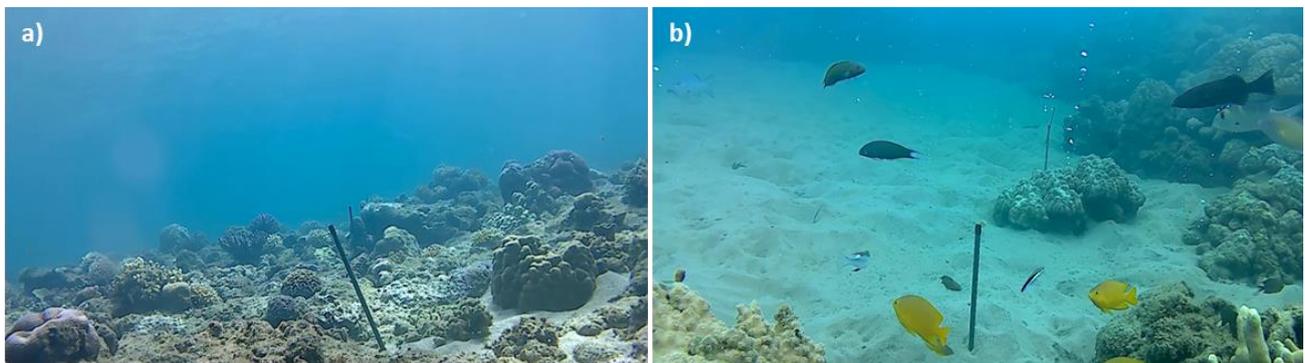


Figure 4.6. Still frames from squidpop surveys in Papua New Guinea at ambient (a) and elevated (b) $p\text{CO}_2$ levels.

Experimental designs and statistical analyses

In order to compare reef fish assemblage composition and predation frequency, I employed an orthogonal fixed experimental design for each seep site, where a fixed location factor with two levels (high- CO_2 and Ambient- CO_2 conditions) was chosen. Replicates were randomly positioned in each location both for BUVs and Squidpops at a depth between two and six meters in Vulcano and up to ten meters in Shikine and Normanby Islands.

To investigate whether benthic assemblages would change and how they might be grouped among a specific pH/pCO₂ condition, the different operational taxonomic units (OTU) were ordered using a non-metric dimensional scaling technique (nMDS). A Permutational Analysis of Variance, PERMANOVA (Anderson and Ter Braak, 2003) was performed using the “PRIMER and PERMANOVA + package” software, in order to assess the significance of each OTU among different pH/pCO₂ conditions. A Triangular similarity matrix was calculated using the Bray-Curtis similarity coefficient and the data was square root transformed using 9999 permutations. Fish data were ordered in a multivariate matrix, transformed using a square root and a subsequent resemblance matrix was calculated using the Bray-Curtis coefficient. The PERMANOVA analysis was performed using 9999 permutations. A principal component ordination (PCO) was run to show changes in fish assemblages as a result of the main biogenic habitat changes at the three volcanic seeps. Finally, a linear regression analysis was performed in order to test more thoroughly the relation between benthic taxa and reef fish species richness.

META-ANALYSIS

In order to assess the predation intensity along a gradient of pH/pCO₂ has been followed a meta-analytical approach. Especially, the Ln (RR) has been obtained comparing the ratio between average percentage of bait loss after the first hour of deployment between experimental condition (high-CO₂) vs control (ambient-CO₂) ($\bar{Y}\%_{\text{HIGH } p\text{CO}_2} / \bar{Y}\%_{\text{Ambient-CO}_2}$) among all climatic zones (both as overall effect as single “climatic zone-response”).

By means of video during the first hour of deployment, the main species approaching the squidpop stake has been recorded. The behaviour of all species for each condition was reported as the ratio of total number of interactions with bait, expressed as a percentage.

RESULTS AND DISCUSSION

The substrate rugosity, expressed as the ratio of the contoured distance to the linear distance along the benthic transects, did not change (T tests; p>0.05) between the different CO₂ locations with an average rugosity index of 1.33 (±0.05 SD) in the High CO₂ sites and of 1.26 (±0.12 SD) in the control sites. This suggests that the topography of the physical substratum (e.g. rocks, pebbles and boulders, sand) of the investigated seep sites was similar between CO₂ conditions.

conditions. The increased prevalence of low profile algae at High CO₂ aligns with results from other natural analogues. In general losses of scleractinians and canopy-forming algal species resulted in much reduced habitat complexity with an overall loss of canopy height in elevated CO₂ conditions (data still to be processed).

In general, table (Acroporids), massive and encrusting (Favids and Porites) corals were dominant or quite common at control sites in Papua New Guinea and Japan respectively. In this latter case, scleractinians thrived at this high latitude (34° N) in the NW Pacific due to the warm northward flow of the Kuroshio Current (Midorikawa et al. 2005). Hard corals other than boulder corals of the genus *Porites* spp. in Papua New Guinea were absent at higher CO₂ levels. In contrast to other Pacific CO₂ seeps (Inoue et al., 2013), soft corals were missing at elevated CO₂ areas in Japan and PNG, while anemones were common in Vulcano as previously observed by Suggett et al. (2012) and turfs covered most of the rocky substrata in Japan, Italy and PNG.

If in one hand, such community responses are in agreement with previous surveys available in the literature from two out of the three study sites (Fabricius et al., 2011; Agostini et al., 2018; but see Nagelkerken et al., 2015 for the Vulcano site), on the other hand and as expected, the type of transitions occurring under OA seems not to be as deterministic as previously hypothesised (e.g. from calcifying to non-calcifying species; Hall-Spencer et al., 2008). The magnitude and the direction of the observed community changes varied between the three CO₂ seep sites, likely being mostly led by a dual role of CO₂ as a stressor (e.g., for those habitat forming organisms building up carbonatic structure such as scleractinian corals) and as a resource for low profile primary producers (e.g. turf-forming or other low-profile algae). Therefore, my data further provide evidence that OA-related shifts may be due to a combination of positive/negative physiological effects on subordinate and dominant species and, thus, to changes in the competition strengths between them (Connell et al., 2013; Gaylord et al., 2015; Sunday et al., 2017). In this regard, extensive experimental and theoretical work suggest that many types of phase shifts may occur in marine ecosystems, with isolated or interacting drivers (e.g. global change, overfishing, pollution) leading to abrupt changes in the structure of ecological communities (e.g. Scheffer et al. 2001; Rocha et al., 2015). More specifically, this findings add on to our knowledge of ecosystem-level responses to OA, with many documented shifts in temperate, warm-temperate and tropical CO₂ seeps systems worldwide (e.g. Hall-Spencer et al., 2008; Fabricius et al. 2011, Connell et al., 2018, Agostini et al. 2018). For instance, dominance shifts from hard to soft corals or macroalgae were observed in the tropical and the temperate NW Pacific Ocean (Inoue et al., 2013; Enochs et al.,

2015; Agostini et al., 2018). Loss of coral reef complexity and species composition changes were documented in Papua New Guinea and Palau (Fabricius et al., 2014; Barkley et al., 2015); whilst kelps, other canopy- or mat-forming macroalgae substitute calcifying taxa (e.g. scleractinian corals, rhodolith beds and coralline algae) off shallow and deep CO₂ seeps in the Mediterranean Sea (Hall-Spencer et al., 2008; Kroeker et al., 2013b; Baggini et al., 2014; Linares et al., 2015). Likely, physiological plasticity mechanisms promoting species-specific resistance under elevated pCO₂ (Rodolfo-Metalpa et al., 2011; Inoue et al., 2013; Barkley et al., 2015, Kenkel et al., 2017), alongside nutrient/food levels and other local environmental conditions may play a role in shaping species resilience and the direction of community shifts (Camp et al., 2018).

Interestingly, the observed benthic community shifts observed in the three CO₂ seep sites ultimately resulted in cascading effects on associated fish assemblages (Fig. 4.7. b, e, h) and importantly led to mixed diversity responses (Fig. 4.7. c, f, i).

Despite potentially stressful abiotic conditions under increasing CO₂ concentrations, species richness was higher at ambient pH/pCO₂ levels in Japan and PNG (which exhibited higher benthic heterogeneity levels), whereas peaked at high CO₂ levels in Vulcano Island (Italy) where a mosaic of seagrasses, turfs and non canopy-forming algae (i.e. high heterogeneity) occurred under CO₂ enrichment conditions. In this case, both the seagrass *Cymodocea nodosa* and many low profile brown algae may have a large effect on the associated fish community by providing a complex 3-dimensional framework and increase the fish diversity. Indeed this findings may raise concerns, as the mixed, complex, and unexpected responses may limit predictability the community responses under OA as well as posing obvious limitations to linear extrapolations at global scale (Sunday et al., 2017; Kroeker et al., 2017).

Changes in the structure and composition of benthic assemblages can lower community resilience, but if there is ecological redundancy among the compensating species, then compensation could maintain ecosystem function even with the loss or reduced abundance of some vulnerable species under OA. In this study, predation intensity did not differed between High pCO₂ and Control sites in all the CO₂ seeps considered. Overall effect sizes showed no statistical difference both after one

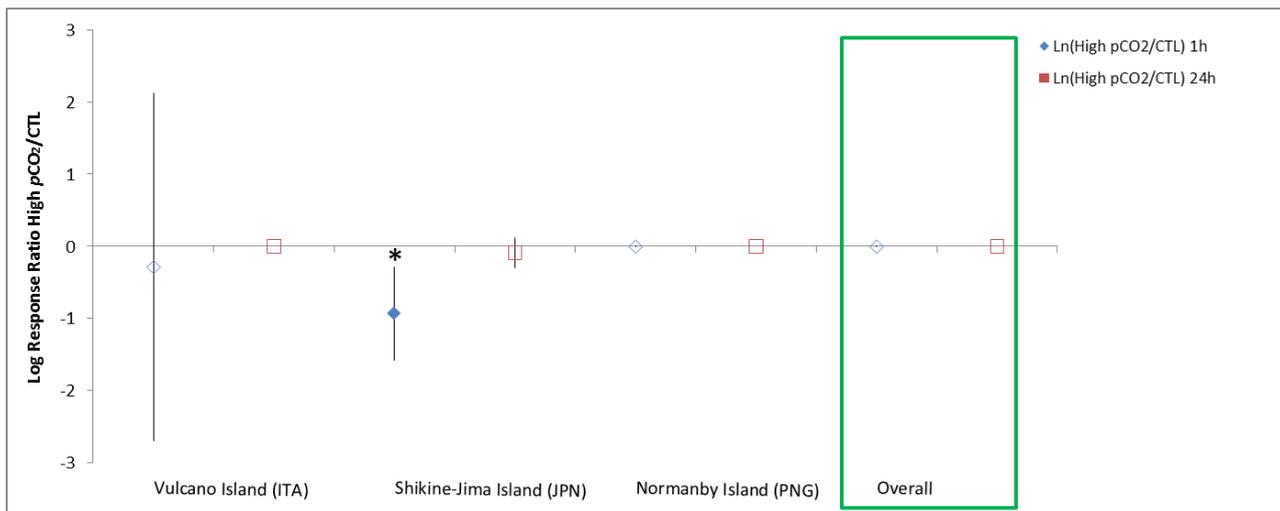


Figure 4.8. Predator pressure response at different CO_2 gradients along three climatic zones: Shikine-jima Island (Japan), Vulcano Island (Italy) and Normanby Island (Papua New Guinea). Measured as the natural log ratio of the amount of bait lost after one (blue diamonds) and twenty-four hours (red square) of deployment, standardized to the number of replicates. Bars indicate 95% confidence intervals; Diamonds and Squares represents the log Response Ratio (RR) between High CO_2 and Control conditions. Null effects are represented by empty symbols.

and twenty-four hours of exposition to predators in the wild (Fig. 4.8.). Only in Shikine Island the tethering experiment revealed a significant negative effect after 1-hour ($\ln(\text{High } pCO_2/\text{CTL}) = -0.934$, $CI = 0.641$).

Despite intrinsic differences in the exploitation levels between the regions investigated, such findings rather support the hypothesis of unaltered food web dynamics under OA conditions despite fish community and diversity changes. My findings contrast with Nagelkerken et al. (2015) which documented a reduced number of predator species in High CO_2 locations off Vulcano Island. The present study rather highlights similar (and quite high) predation rates between Ambient and High CO_2 conditions. Such functional redundancy does not support the general hypothesis of CO_2 -driven phase-shifts and food web simplification observed for invertebrate taxa in coral reefs or temperate seagrass systems (Fabricius et al., 2014; Vizzini et al., 2017) and again suggests the use of multiple natural CO_2 gradients in providing quantitative data on changing community responses to long-term exposures to elevated CO_2 .

Although natural analogues provide many benefits when investigating higher ecological levels of organization, there are also some key caveats to their use for ecosystem level assessments, such as limited degree of spatial replication, movement of animals in and out of the experimental sites, or confounding effects of other drivers. Moreover, their use for understanding ecosystem level

processes such as larval dispersal, postsettlement migrations or habitat selection maybe be limited because the spatial scales at which these processes occur are usually larger than the surface areas of these natural laboratories. Despite this, I found rather consistent changes in the fish community and diversity, which are indeed linked to biogenic habitat shifts occurring as a result of chronic exposure to elevated CO₂ levels. Importantly these findings add on recent evidence suggesting that ocean acidification mediates biodiversity shift via biogenic habitat changes (e.g. reduced complexity) (Sunday et al. 2017). In addition to general indirect fish diversity changes (e.g. losses in subtropical and tropical locations and increases in temperate areas) this study also demonstrates unaltered top-down controls under OA conditions. Despite this, whilst the direct effects of ocean acidification are readily detectable on many marine organisms, indirect effects of ocean acidification may leave a stronger imprint on marine communities.

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Chapter 5

Concluding remarks

This thesis represents an attempt to inform on the potential responses of fish communities to different drivers of change. Particularly, the first section emphasises the role played by SSF on the composition and structure of fish assemblages in the context of MPAs and external controls. The results show a positive response to protection of the main descriptors analysed. In fact, species richness, relative abundances and biomass revealed a positive overall trend. As a whole, the data show a positive trend on protection efficiency, even though specific MPA responses were heterogeneous. The positive response of species targeted by fisheries (in terms of diversity, relative abundance and biomass) points out a promising picture, although we are a long way from being able to assert MPAs are the right tool for resource and diversity protection. The observed variability of fish species to different protection levels suggests that some MPAs does not benefit from adequate protection measures. In this context, increasing enforcement and management actions could re-establish the balance for some of the observed response variables. Moreover, as it has been already documented in other MPAs (*i.e.* Guidetti *et al.*, 2010), a way to increase the participatory process is to utilise fishermen themselves as the MPA's guardians. Increasing stakeholder awareness and ownership can lead to higher surveillance levels and enforcement indirectly.

Overall, Western Mediterranean MPAs responded positively, showing mixed but overall increasing values related to protection measure. However, some MPAs display far from acceptable protection standards (*e.g.* Cape Roux, Isole Egadi and Strunjan) since their outcome did not show any positive response in any of the analysed variables. This response could be explained in multiple ways such as: areas' dimensions and subdivisions (Claudet *et al.*, 2008; Di Franco *et al.* 2016; Giakoumi *et al.*, 2017), adopted enforcement typology, systems productivity (Béthoux *et al.*, 1998; Richon *et al.*, 2018), surveillance (Di Franco *et al.* 2016) and pressures from stakeholders (including the SSF) (Food and Agriculture Organisation of the United Nations, 2014), may be predictors of the observed response.

Expanding to other areas and adopting similar management plans should lead to an increased carrying capacity in the Basin. This would ensure benefits to both MPA's managers and higher fishing activity income. Failing to meet protection objectives, would generate a collapse of the

already precarious Mediterranean ecosystem's resistance and resilience. In addition, the rising of the average temperature, the tropicalisation and meridionalisation of Mediterranean Sea, and the Lessepsian migrants (*i.e.* fishes of Indo-Pacific origin that have been introduced into the Mediterranean Sea through the Suez Canal) (Giakoumi *et al.*, 2018) are driving ecosystem modifications (*i.e.* *Zakynthos Marine National Park* and the spread of Genus *Siganus*) (Dimitriadis *et al.*, 2018). Overall, proper functioning of MPAs can represent a buffer from different stressors, which on a time scale of decades could negate the deleterious effects on fish communities and marine ecosystems.

Chapter 3 of this thesis tested a controversial hypothesis, by now verified on terrestrial systems: *i.e.*, the so called "Mesopredator release". This study findings reject the validity of this hypothesis, conversely to what had been previously seen on some marine ecosystems (*i.e.* Ritchie *et al.*, 2009; Barley *et al.*, 2017). In order to analyse the top-down process, this study compared apex and mesopredator abundance and biomass in protected and unprotected zones in the context of Mediterranean MPAs. Protected zones (as pristine areas where hypothetically, apex predators operate with top-down control on mesopredators) were compared with the unprotected ones (where SSF plays a crucial role in trophic balance). The results rejected the mesopredator release hypothesis due to the higher abundance of mesopredators inside than outside the MPAs, contrary to expectations. Moreover, mesopredator predation pressure was higher inside rather than outside MPAs. Tethering experiment results underlined that the more structured the environment, the lower mesopredator predation intensity ultimately is. In environments with higher apex biomass and abundance, different mechanisms can be triggered to directly or indirectly (hunting and loathing) may generate reductions in mesopredators predation. This is the case of the Cabo de Palos and Portofino MPAs, where to high apex and mesopredator abundance and biomass follow lower predation rates in protected than unprotected zones. However, in the latter, the lower apex occurrence (due to fishing effort) encouraged higher mesopredator predation frequency. The results of this study have pointed out that both fishing and apex occurrence play a form of top-down control on mesopredators. Future analysis on the temporal scale may confirm or reject these initial results.

Chapter 4 supports and validates previous studies focusing on OA effects on habitat provisioning. With some mixed responses, overall the results show similar trends for fish assemblages to those documented for benthic invertebrates. Specifically, chronical exposures to elevated CO₂ levels did affect habitat forming species likely in a direct way (Kroeker *at al.*, 2013), and indirectly modified

associated fish assemblages (Sunday *et al.*, 2014; Nagelkerken *et al.*, 2015, 2017; Sunday *et al.*, 2017) both in terms of diversity and relative abundance. Fish species may be able to acclimatise to elevated CO₂ conditions, however changes in the levels of habitat provisioning did alter their community response. Despite this, predation rates along the different $p\text{CO}_2$ gradients were unaltered, being similar among zones with $p\text{CO}_2$ values similar to what is predicted at the end of the century ($\sim 1000 \mu\text{atm}$) and current levels ($\sim 400 \mu\text{atm}$). These results confirm and support the hypothesis that food web dynamics under OA conditions are unaltered despite community shifts and fish diversity changes, contrasting with previous literature documenting unbalanced food webs both for benthic and fish communities. Therefore, the present results support a high trophic resiliency of fish communities, likely as a result of a functional redundancy of fish predator species.

Ultimately, a few considerations can be made from the findings of this thesis.

Marine Protected Areas can be extremely effective when their protection measures are well-enforced, resulting in large increases in fish biomass, abundance and diversity that spill over beyond their boundaries, providing benefits to the local economy. Strong protection of the zones is paramount to their success, and their success directly benefits all the players who have a stake in marine economy, particularly the small-scale fisheries characteristic of the Mediterranean Sea. These constitute 83% of fishing boats in the basin, employing more than 150.000 fishermen, but currently generating only 14% of the total catch. Thanks to their practices and smaller scale, SSFs are not only compatible with the protective measures necessary around MPAs, but can also constitute an important tool to provide additional supervision and management, interpreting a particularly convenient dual role of both enforcers and beneficiaries of the measures necessary to the sustenance of protected areas. MPAs that already employ small fisheries in such way have demonstrated improved efficacy, and it should be considered of fundamental importance to integrate fishermen in future management planning.

All protective measures though, even when ideal, will only be effective in a liveable sea, and we cannot disregard the impending adverse conditions caused by Ocean Acidification. Even though certain organisms, particularly fish, can acclimatize to decreases in pH in the medium term, habitat formers have not demonstrated this ability, which could indirectly yet fundamentally affect fish communities and their presence along coastal areas, impacting activities revolving around them even in otherwise protected areas.

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