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UNA TECNICA SEMPLIFICATA DI CENSIMENTI VISIVI PER MONITORARE L'ABBONDANZA DI SPECIE INDICATRICI DI CAMBIAMENTO CLIMATICO NELLE AREE MARINE PROTETTE DEL MEDITERRANEO: IL CONTRIBUTO DELLA REGIONE SICILIA

A SIMPLIFIED VISUAL CENSUS TECHNIQUE TO MONITOR THE ABUNDANCES OF CLIMATE CHANGE INDICATORS IN MEDITERRANEAN MARINE PROTECTED AREAS: RESULTS FROM THE SICILY REGION

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Riassunto

Il cambiamento climatico sta influenzando rapidamente l'abbondanza e la distribuzione di specie animali e vegetali con importanti ripercussioni sugli ecosistemi marini, in particolare nel mar Mediterraneo, che viene considerato oggi come un hotspot climatico. Considerando i due principali scenari previsti dall'IPCC: RCP8.5 (comunemente associato all'espressione "Business-as-usual", o "Nessuna mitigazione") e RCP4.5 ("Forte mitigazione" , con la messa in atto di iniziative per controllare le emissioni), si assume che entro il 2100, la temperatura superficiale media del bacino aumenterà da 2.7 a 3.8 °C e da 1.1 a 2.1 °C, rispettivamente. I picchi di calore aumenteranno, divenendo più intensi e severi, come recentemente previsto dal rapporto del MEDECC 2020. Questi cambiamenti in atto risultano in una serie di impatti ecologici che interessano molte specie e habitat marini mediterranei, dando luogo a eventi di mortalità di massa, aumento dell'incidenza di epidemie ed un rapido ampiamento della distribuzione geografica di specie termofile ad affinità tropicale e sub-tropicale, rappresentate sia da specie indigene che non indigene (NIS) o aliene.

Il processo di espansione dell'areale geografico di specie native del Mediterraneo, spesso indicato con il termine "meridionalizzazione", è oggi esemplificato da un numero crescente di specie termofile come *Thalassoma pavo, Epinephelus marginatus, Sparisoma cretense, Sphyraena viridensis* che si stanno spostando a latitudini maggiori, occupando aree precedentemente non climaticamente adatte al loro ciclo vitale. Al fenomeno della meridionalizzazione del Mediterraneo si aggiunge l'invasione ed il crescente successo di specie non indigene (NIS) ad affinità tropicale. Queste specie, provenienti principalmente dal mar

Rosso attraverso il Canale di Suez (aperto nel 1869), viene oggi indicato con il termine "Migrazione Lessepsiana", ovvero l'ingresso in Mediterraneo di NIS tropicali, con conseguente "tropicalizzazione" del *Mare Nostrum*.

Il rapido riscaldamento delle acque del Mediterraneo insieme al progressivo aumento della salinità, sta creando condizioni più favorevoli per l'accrescimento e la progressiva espansione geografica di queste popolazioni, lungo un gradiente che va dal bacino orientale del Mar Mediterraneo a quello occidentale e dalle coste del sud del Mediterraneo verso i settori più settentrionali e freddi.

Nel presente studio, sono stati acquisiti nuovi dati di abbondanza riguardanti nove specie ittiche costiere, includendo sia specie native che NIS (*Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis, Serranus cabrilla, Siganus* spp, *Fistularia commersonii*), ritenute indicatori di Cambiamento Climatico. Tali dati sono stati raccolti attraverso un protocollo standard di censimenti visivi (*Underwater Visual Census* o UVC) fornito dal progetto europeo MPA-ENGAGE (Interreg Med programme, https://mpa-engage.interreg-med.eu/), il quale ha permesso di valutare il grado di meridionalizzazione e tropicalizzazione in due Aree Marine Protette siciliane: Ustica (38° 42' N, 13° 11' E) e Linosa (35° 30' N, 12° 51' E).

Nell'AMP di Ustica i campionamenti sono stati svolti da giugno ad ottobre 2020 in tre diverse località e a 4 differenti intervalli di profondità (0-3m; 5-10m; 11-20m; 21-30m), sia attraverso snorkeling (0-3m), sia in immersione con Autorespiratore ad Aria (ARA) (al di sotto dei 5 m di profondità).

L'unità di campionamento utilizzata consiste in transetti di 50m x 5m, eseguiti da osservatori esperti con una lavagnetta in PVC sulla quale viene registrato il numero di individui osservati durante un tempo di 5 minuti. Ai dati raccolti da ricercatori esperti, è stato aggiunto un set di dati raccolto tramite il coinvolgimento di 32 subacquei ricreativi appositamente formati, per un numero totale di 505 transetti (277 realizzati da personale esperto + 228 realizzati da volontari formati). Il coinvolgimento dei volontari è stato realizzato tramite una collaborazione ufficiale con centri di immersione dell'Isola di Ustica e tramite il supporto di PADI/DAN, partner del progetto MPA-ENGAGE. La qualità dei dati raccolti dai volontari, valutata tramite analisi discriminanti (*Hill & Smith function*) ha fornito indicazioni sull'opportunità di filtrare alcune categorie di osservatori per ottenere una matrice dati validata di 393 transetti, che è stata utilizzata per le analisi successive. Ai dati raccolti nell'AMP di Ustica è stato aggregato un secondo dataset estratto da una recente tesi (Aversa, 2020) realizzata presso l'AMP di Linosa (Isole Pelagie), con 905 transetti eseguiti seguendo lo stesso protocollo.

Al fine di determinare il grado di tropicalizzazione delle due AMP (Ustica *vs*. Linosa), l'intera matrice dei dati trasformata in presenza/assenza, è stata utilizzata per definire un *indice di Tropicalizzazione* (i^T) calcolato sulla base della distribuzione relativa di specie a differente affinità termica in tre gruppi di indicatori: 1) *C. julis* e *T. pavo;* 2) gli erbivori *S. salpa* and *S. cretense;* 3) specie invasive di origine tropicale (*Siganus* spp*., Fistularia commersonii*).

I risultati illustrano come gli *indici medi di tropicalizzazione,* calcolati per le diverse fasce batimetriche, risultano sempre più alti per l'AMP di Linosa rispetto a quella di Ustica (Linosa i^T=2.36, Ustica i^T=1.16). L'isola di Linosa, localizzata geograficamente ad una latitudine inferiore risulta quindi fortemente dominata da specie ad affinità sub-tropicale, principalmente

Sparisoma cretense, (media avvistamenti: 9.52 ind/250m^2) che domina in termini di abbondanza tutte le fasce batimetriche esplorate (SIMPER: Contribution % 28.9). Al contrario, nell'AMP di Ustica, la stessa specie è stata osservata solo di rado e limitatamente all'intervallo batimetrico superficiale (media avvistamenti: 0.13 ind/250m^2) e l'erbivoro più abbondante rimane la salpa*S. salpa* (SIMPER: Contribution % 34.8), una specie ad affinità temperata.

Emblematico anche il caso delle due specie di donzella, *T. pavo* e *C. julis,* caratterizzate da una maggiore o minore affinità termica, rispettivamente, che hanno mostrato chiari pattern latitudinali (differenze tra isole) e lungo l'asse verticale. In particolare *T.pavo* risulta tipicamente più abbondante in acque più superficiali (e calde), con abbondanze medie a 0-3m stimate in 9.1 ind/250m^2 ad Ustica 27.9 ind/250m^2 a Linosa. Al contrario, l'abbondanza di *C. julis* aumenta in ambienti più profondi con una media di: 8.6 ind/250m^2 ad Ustica 9 ind/250m^2 a Linosa, alla profondità di 21-30m.

Il pesce coniglio *Siganus luridus*, invasore lessepsiano, risulta comune nell'AMP di Linosa, tuttavia la stessa specie non è stata ancora osservata ad Ustica al momento dello studio.

Nel loro complesso, le informazioni raccolte dal presente studio illustrano l'esistenza di chiari pattern di variabilità spaziale nella distribuzione di specie indicatrici in due AMP siciliane caratterizzate da diversi regimi climatici, lungo quattro diverse fasce batimetriche. Questi risultati forniscono un'importante *baseline* utilizzabile in studi futuri per valutare gli attesi cambiamenti della biodiversità nelle AMP siciliane. Il coinvolgimento attivo della subacquea ricreativa nell'implementazione dei protocolli di censimento visivo, realizzato attraverso attività di formazione dei volontari e valutazione dei dati raccolti, fornisce nel complesso un valido supporto alle attività di monitoraggio nelle AMP siciliane. Una partnership tra ricerca, e

stakeholders utile per tener traccia degli impatti dei cambiamenti climatici negli ambienti costieri mediterranei e capace di fornire informazioni utili alla gestione adattativa di questa problematica comune.

Summary

1. Introduction

1.1 What is Climate Change

We often hear about "Climate change", according to United Nations Framework Convention on Climate Change (UNFCCC-Convention Article 1, 1994), this statement means a *modification attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to the natural climate variability observed over comparable periods*.

Climate change has a long-time frame and usually a global scale. It transgresses natural systems as well as societal sectors, and has potentially far-reaching effects on nature and society (Adger and Fischlin, 2007); for this reason requires research-based solutions beyond any single scientific discipline: the problem itself is broader, so the scientific community should draw on extensive knowledge and on interdisciplinary approaches. Interdisciplinarity is considered a scientific activity in which different sub-disciplines are crossed in some way (Bjurström and Polk, 2011b).

The Intergovernmental Panel on Climate Change (IPCC), is assigned to assess this comprehensive body of research, should therefore structure and integrate knowledge which is often heterogeneous and disconnected at different degrees (Birch, 2014a).

Ocean's thermal capacity has led to surface waters warming three times slower than air temperatures over land (Adger and Fischlin, 2007). Nevertheless, isotherms at the ocean surface have migrated at comparable or faster rates than isotherms over land during the past 50 years (1960–2009)(Burrows *et al.*, 2011).

Since the Industrial Revolution in the 1800s, the global temperature has increased at a much faster rate, anthropogenic greenhouse gas emissions have resulted in profound changes in the physical and chemical properties of the ocean that have serious implications for marine species, with concomitant risks to marine industries dependent on those species (Hartmann *et al.*, 2013; Poloczanska *et al.*, 2016). The global ocean has absorbed 93% of the extra energy arising from anthropogenic greenhouse gas emissions, resulting in an increase in average global sea surface temperatures since the beginning of the twentieth century, that approaches 1°C (0.89 °C over the period 1901–2012; (IPCC, 2013). The ocean has also taken up ~30% of anthropogenic carbon dioxide (CO_2) that has been released into the atmosphere, decreasing ocean pH, and fundamentally changing ocean carbonate chemistry in all ocean regions, particularly in the cooler, high latitude waters (Birch, 2014b). Other chemical and physical changes in the ocean attributed to anthropogenic forcing include declines in dissolved oxygen concentrations (Andrews *et al.*, 2013) and alteration of ocean circulation (Cai *et al.*, 2005; Wu *et al.*, 2012). These anthropogenic changes represent risks to marine life and ecosystems (Gattuso *et al.*, 2015; Nagelkerken and Connell, 2015; Poloczanska *et al.*, 2016).

Nonetheless, there are lots of natural causes that also lead to changes in the climate system: natural cycles can cause alternation between warming and cooling. There are also natural factors that force the climate to change, including volcanic aerosols and solar variability (Gent and Danabasoglu, 2011); however, even though these natural forcings contribute to climate change, they are not the leading ones (Meehl *et al.*, 2012).

Greenhouse effects, together with those of other drivers, have been detected throughout the climate system and are extremely likely to have been the dominant cause of the observed warming since the mid-20th century (Fig. 1) (Birch, 2014b; Woodward *et al.*, 2014).

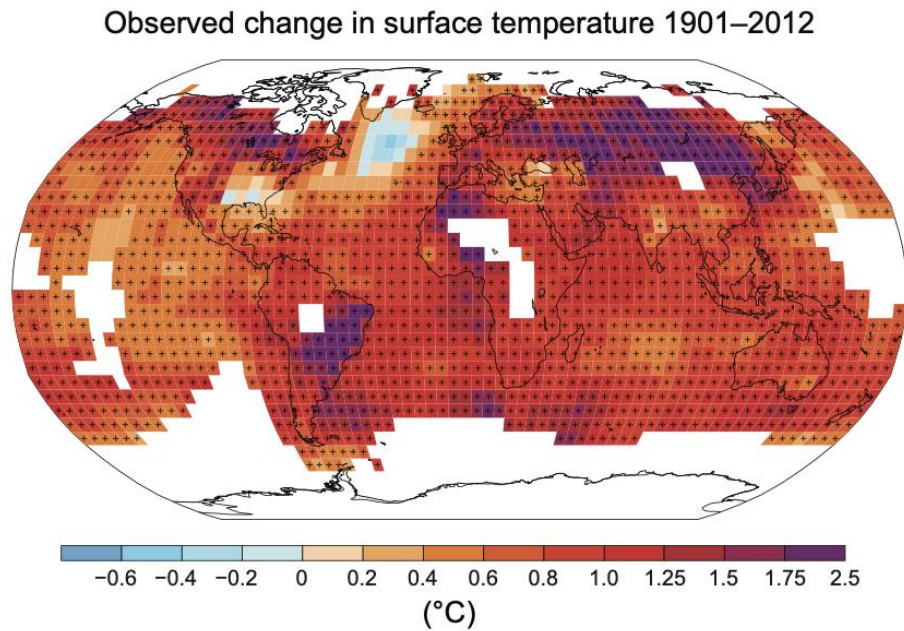**Observed change in surface temperature 1901–2012**

Figure 1- Map of the observed surface temperature change from 1901 to 2012 derived from temperature trends determined by linear regression (IPCC 2013)

Furthermore, the rate of physical and chemical changes in marine ecosystems will almost certainly accelerate over the next several decades in the absence of an immediate effort toward climate mitigation (National Research Council, 2011).

We can affirm that there are multiple stressors on marine ecosystem, occurring at different axes and we should consider their effects as a whole, and not as independent issues (Scott C. Doney *et al.*, 2012).

1.2 Specific axes of change

Climate change in IPCC usage refers to any change in climate over time, whether due to natural variability or because of human activity.

Warming of the climate system is unequivocal, and since the 1950s, many of the observed changes are unprecedented over decades to millennia. The atmosphere and ocean have warmed, also the amounts of snow and ice have diminished (Birch, 2014b). These changes occur on different axes including temperature, salinity, sea level, carbon, pH and oxygen, each of these representing the physical properties of the Oceans (IPCC, 2013).

The following paragraphs synthetize the major changes observed   :

1.2.1   Changes in Temperature

The primary direct consequence is the increase in ocean temperatures (Boero and Bonsdorff, 2007). The upper ocean layer (above 700 m) has warmed from 1971 to 2010 and likely also before (from the 1870s to 1971). The strongest warming is near the sea surface (0.11 ± 0.2 °C per decade in the upper 75 m between 1971 and 2010), while at 700 m we find a warming of about 0.09°C per decade (Birch, 2014a). Warming is not spatially uniform owing to ocean circulation, spatially variable changes in winds, and interaction with natural modes of climate variability (Ishii *et al.*, 2006).

In some marine regions the average Sea Surface Temperatures (SST) have already risen faster than the global average (Boero and Bonsdorff, 2007).

There is an important correlation between wind speed change and spatial deviations of SST warming, with wind speed modulating SST warming in the tropics. The equatorial maximum in

SST warming appears in both the Pacific and Atlantic with reduced warming in the subtropics, this is especially clear in the subtropical Atlantic, where the subtropical SST minimum on either side of the equator is associated with little change in wind speed (Bjurström and Polk, 2011a). In the European Sea we there is another warming zone: from daily monitoring surface data, since 1985, summer SST in the North and Baltic Seas increased at a rate 3 times the global rate, and 2–5 times faster than other seasons' SST (Mackenzie and Schiedek, 2007); in the Mediterranean Sea rose at a rate of 0.56 °C/decade or 4 times the global rate (Belkin, 2009). The 1982–2003 warming magnitude increased eastward, from 0.5–1.0 °C in the Gulf of Lions and Ligurian Sea up to 2–3 °C in the Levantine Basin (European Environment Agency, 2007). In the Black Sea the coldest year of 1993 was a turning point after which SST rose through 2002 at a rate of >1.5 °C/decade (Ginzburg *et al.*, 2008)

1.2.2   Change in Salinity and ocean circulation

Seawater salinity is an important indicator for the global hydrological cycle. In the past few decades, most researchers have studied the climate system based on its thermodynamics, such as using changes in sea surface temperature to indicate climate change. In the last one or two decades, researchers started to focus on oceanic dynamics because a new observational method was developed, such as using sea surface height data observed by satellites to calculate ocean currents (Vineis *et al.*, 2011). A key advantage of using the ocean salinity field to study the global water cycle is that over the period of observational coverage, spatial patterns and climatological mean values are considered stable (Holland *et al.*, 2008). However, research on

the role of freshwater/seawater salinity in climate change has been relatively slow because of the absence of historical salinity observations due to the difficulty in obtaining salinity them (Durack *et al.*, 2012).

The main source of fresh water in the open ocean is precipitation in the atmosphere. The basic structure of atmospheric circulation determines the distribution of precipitation over the ocean that is mainly affected by the vertical circulation of the atmosphere. Another element that controls freshwater flux is the evaporation term, which is mainly related to the wind field at the surface, as determined by the turbulent flux exchange between the air and sea. Large-scale surface wind is mainly controlled by the distribution of sea surface pressure (Du *et al.*, 2019).

Previous studies have shown that Evaporation minus Precipitation and ocean dynamic processes, including Ekman transport caused by wind fields, geostrophic transport, and vertical entrainment, jointly determine the mean distribution of Sea Surface Salinity (SSS) (Wang *et al.*, 2019).

Under the influence of global warming, there are significant changes in the global climate system as well as in water circulation and SSS responses (Hartmann *et al.*, 2013). According to the *Clapeyron-Clausius equation*, $dp/dT = L/(T(V_v-V_l))$, where L is the latent heat of evaporation, and V_v and V_l are the specific volumes at temperature T of the vapor and liquid phases, respectively. For every 1 degree increase in temperature, water vapor increases by 6–7% and rainfall increases by 2–3% (Birch, 2014b), this means that the thermodynamic and kinetic constrained climate system follows the rules under global warming: SSS will become higher in salty areas and lower in fresher area; according to this, the mean contrast between high and low salinity regions increased by 0.13 ±0.4 from 1950 to 2008. (IPCC, 2013)

The salinity in the upper layers of the ocean, in the northern hemisphere, is significantly fresher (Boyer *et al.*, 2005) while the tropical western Pacific, which is the center of the global deep convection, is the core area of the warm pool and freshwater pool. Changes in the tropical western Pacific reflect the theory of "warm gets warmer, wet gets wetter" (Durack *et al.*, 2012). Salinity changes play a role in sea level change due to the addition or removal of fresh water and due to the hyaline contraction factor in sea level (density) calculations (Du *et al.*, 2019).

1.2.3 Change in Sea level

"Mean sea level" at the coast is defined as the height of the sea with respect to a local land benchmark, averaged over a period of time, such as a month or a year, long enough that fluctuations caused by waves and tides are largely removed. Changes in mean sea level as measured by coastal tide gauges are called "relative sea level changes", because they can come about either by movement of the land on which the tide gauge is situated or by changes in the height of the adjacent sea surface (Gornitz *et al.*, 2001).

Eustatic sea level change results from changes to the density or to the total mass of water, both relate to climate. Density is reduced by thermal expansion occurring as the ocean temperature rises. Observational estimates of interior temperature changes in the ocean reported by Huybrechts et al. (1996) were limited and estimates of thermal expansion were made from simple ocean models. Thermal expansion (or steric sea level rise) occurs at all ocean temperatures and is one of the major contributors to sea level changes during the 20th and 21st centuries and because of the large heat capacity of the ocean, thermal expansion would continue for many centuries after climate had been stabilized (Doney *et al.*, 2012; Hartmann *et al.*, 2013).

Over the 20th century the rate of sea level rise has roughly tripled in response to 0.8°C global warming (Jevrejeva *et al.*, 2010) due to this the Global Mean Sea Llevel (GMSL) estimated in 1993 from IPCC to be $1,9 \pm 0.2$ mm yr^{-1} over the period 1901–2010 (Nielsen and D'haen, 2014), it is very likely that the mean rate was increased to 3.2 ± 0.4 mm yr^{-1} between 1993 and 2010. Sea level change is not expected to be geographically uniform; the pattern depends on ocean surface fluxes, interior conditions, and circulation. The most serious impacts are caused not only by changes in mean sea level but by changes to extreme sea levels especially storm surges and exceptionally high waves, which are forced by meteorological conditions. Climate-related changes in these therefore also must be considered (Doney *et al.*, 2012).

1.2.4 Changes in Ocean Biogeochemistry

Resulting of increased oceanic CO_2 uptake are the predominant factors driving ocean acidification (Dore *et al.*, 2009). Ocean acidification reflects a series of chemical changes: elevated aqueous CO_2 and total inorganic carbon as well as reduced pH, carbonate ion, and calcium carbonate saturation states (Doney *et al.*, 2009). The pH of surface seawater has decreased by 0.1 since the beginning of the industrial era corresponding to a 26% increase in acidity over the past 150 years, mostly in the past several decades. Future projections suggest declines of an additional 0.2–0.3 pH units over this century (Upton and Folger, 2014).

Concerning change in oxygen concentration, globally, oceans have lost around 2% of dissolved oxygen since the 1950s and are expected to lose about 3–4% by the year 2100 under a business-as-usual scenario (Representative Concentration Pathway 8.5).

The number of coastal hypoxic regions has grown dramatically with time (Diaz and Rosenberg, 2008), in large part because of human activities that increased the amount of bioavailable nitrogen arriving to the coastal ocean. Future levels of coastal hypoxia will be modulated by climate change via alterations in vertical stratification (temperature, precipitation, freshwater runoff), winds, and water mixing (Rabalais *et al.*, 2010). Projected declines in ocean oxygen levels reflect the combined effects of reduced oxygen solubility from warming and reduced ventilation from stratification and circulation changes (Keeling *et al.*, 2010).

All these events affect also the Arctic Sea Ice extent, being the rate of the annual decrease very likely between 3.5 and 4.1% per decade. Moreover, glaciers are persistently shrinking: the time series of measured changes in glacier length, area, volume and mass revealed that glaciers will continue to shrink in the future even without further temperature increase (Woodward *et al.*, 2014). According to this, we can say that the Arctic is expected to be sea-ice free during summer starting in the mid- to late twenty-first century, if not earlier, and thick (Holland *et al*, 2008). Multiyear Arctic ice is being replaced with thin, first-year ice (Maslanik *et al.*, 2007).

These physical and chemical changes have strong direct and indirect effects on the physiology and behavior of marine organisms, which can translate to population- and community-level changes. Consequently, changes in ocean conditions can alter key biological interactions and ecosystem structure and functioning.

1.3   *Response of marine organisms to climate change*

Ecological responses to climate change are varied and many (Parmesan, 2006; Woodward *et al.*, 2014).

First climate change can affect biodiversity through a combination of:

(i) a direct effect on the organisms (temperature causes changes in survival, reproductive success, dispersal pattern and behavior);

(ii) effects mediated by biotic interactions (conferral of competitive advantage to one of a pair of overlapping species); and

(iii) indirect effects through ocean currents. Sanford (1999) showed that small changes in climate may generate large changes in marine communities through regulation of keystone predation (Casey *et al.*, 1999) demonstrated that environmental warming alters food-web structure and function of aquatic ecosystems.

One of the most documented impacts of temperature changes in marine organisms is the change in distribution and abundance, which has been demonstrated many times in different fish species (Cheung *et al.*, 2013), shifts in species distributions in relation to climate change are widely-reported (41% of MCID) with observational evidence from leading (poleward) and trailing (equatorward) edges of species' distributions and from measurements at the centers of species distributions (Poloczanska *et al.*, 2016).

One of the predicted effects of the global increase of temperature is the poleward range expansion of plant and animal species, both on land and in the sea (Burrows *et al.*, 2011).

Poleward range expansions are commonly attributed to global change, but could alternatively be driven by rapid evolutionary adaptation (Krehenwinkel *et al.*, 2015).

In the world's ocean, evidence of this phenomenon is accumulating especially for warm-temperate areas (Bianchi and Morri, 1998). Adaptation might even allow a species to emerge as profiteer from global change by enabling rapid range expansions into formerly unsuitable areas (Clements and Ditommaso, 2011; Franks *et al*., 2012).

Rising temperatures coordinated with other kind of impacts such as biological invasions, marine overexploitation, and pollution (Walther *et al.*, 2009) synergic impact is usually greater for marine environments because of their high environmental connectivity (Burrows *et al.*, 2011) and because of the pivotal role of water temperatures that strongly influence growth, survival and reproduction (Crozier and Hutchings, 2014). In fact, even modest changes can trigger a rapid cascade of multiple pressures over marine organisms, including altered species interactions and trophic pathways from primary producers to upper-trophic-level fish, seabirds, and marine mammals. The redistribution of Earth's species is among the most evident consequences of global warming (Poloczanska *et al.*, 2016) and a critical aspect for the health of both natural ecosystems and human populations worldwide (Pecl *et al.*, 2017). Investigating the response of an individual species to single forcing factors, although essential, provides an incomplete story and highlights the need for more comprehensive, multispecies- to ecosystem-level analyses. Species interactions are among the most important forces structuring ecological communities and information on how a changing climate affects the extent of density changes is considered central to building more accurate predictions, climate change impacts on species distribution, biodiversity and ecosystem functioning are probably underestimated by current forecasts mostly neglecting such processes (Tylianakis *et al.*, 2008; Gilman *et al.*, 2010).

1.4   *The Mediterranean Sea and climate related impact*

1.4.1 Physical domain

The *Mare medi terraneum* (in Latin) describes the Mediterranean as a ''sea in the middle of the land''. This basin is the largest (2,969,000 km^2) and deepest (average 1,460 m, maximum 5,267 m) enclosed sea on Earth (Figure 2a). Situated at the crossroads of Africa, Europe, and Asia, the

Mediterranean coasts have witnessed the flourishing and decline of many civilizations. The region was an important route for merchants and travelers of ancient times, allowing for trade and cultural exchange, and today it is notable for contributions to global economy and trade (NOAA, 2009).

The Mediterranean Sea connects, through the Strait of Gibraltar, to the Atlantic Ocean in the west and, through the Dardanelles, to the Sea of Marmara and the Black Sea, in the northeast. In the southeast, the Suez Canal links the Mediterranean to the Red Sea and the Indian Ocean (Figure 2a). In the Strait of Sicily, a shallow ridge at 400 m depth separates the island of Sicily from the coast of Tunisia and divides the sea into two main subregions: the western (area = 0.85 million km^2) and the eastern (area = 1.65 million km^2).

It is an "evaporation" basin: evaporation is higher in its eastern half, causing the water level to decrease and salinity to increase from west to east. The resulting pressure gradient pushes relatively cool, low-salinity water from the Atlantic across the Mediterranean basin. This water warms up to the east, where it becomes saltier and then sinks in the Levantine Sea before circulating west and exiting through the Strait of Gibraltar. (Coll *et al.*, 2010). The climate in

the region is characterized by hot, dry summers and cool, humid winters. The annual mean sea surface temperature shows a high seasonality and important gradients from west to east and north to south (Figure 2b) (Cartes, 1994). The basin is generally oligotrophic, but regional features enrich coastal areas through changing wind conditions, temporal thermoclines, currents and river discharges, and municipal sewage (Figure 2c) (Estrada, 1996; Zavatarelli *et al.*, 1998; Bosc *et al.*, 2004). The basin is characterized by strong environmental gradients, in which the eastern end is more oligotrophic than the western. The biological production decreases from north to south and west to east and is inversely related to the increase in temperature and salinity (Danovaro *et al.*, 1999).

The Mediterranean has narrow continental shelves and a large area of open sea. Therefore, a large part of the Mediterranean basin can be classified as deep sea (Figure 2d) and includes some unusual features:

(1) high homothermy from 300–500 m to the bottom, where temperatures vary from 12.8 °C–13.5 °C in the western basin to 13.5 °C–15.5 °C in the eastern, and

(2) high salinity of 37.5–39.5 psu. Unlike in the Atlantic Ocean, where temperature decreases with depth, there are no thermal boundaries in the Mediterranean deep sea (Emig and Geistdoerfer, 2004). Shelf waters represent 20% of the total Mediterranean waters, compared with the 7.6% of the world oceans, and therefore play a proportionally greater role here than in the world's oceans. Shelves in the south are mainly narrow and steep (e.g., Moroccan, Algerian, and Libyan coasts, with the exception of the Gulf of Gabés), while those in the north are wider (e.g., the north and central Adriatic Sea, the Aegean Sea, and the Gulf of Lions) (Rixen *et al.*,

2005)(Figure 2d). These features influence the morphology and constrain the connections to the Atlantic, the Red Sea, and the Indian Ocean (Bethoux *et al.*, 1999).
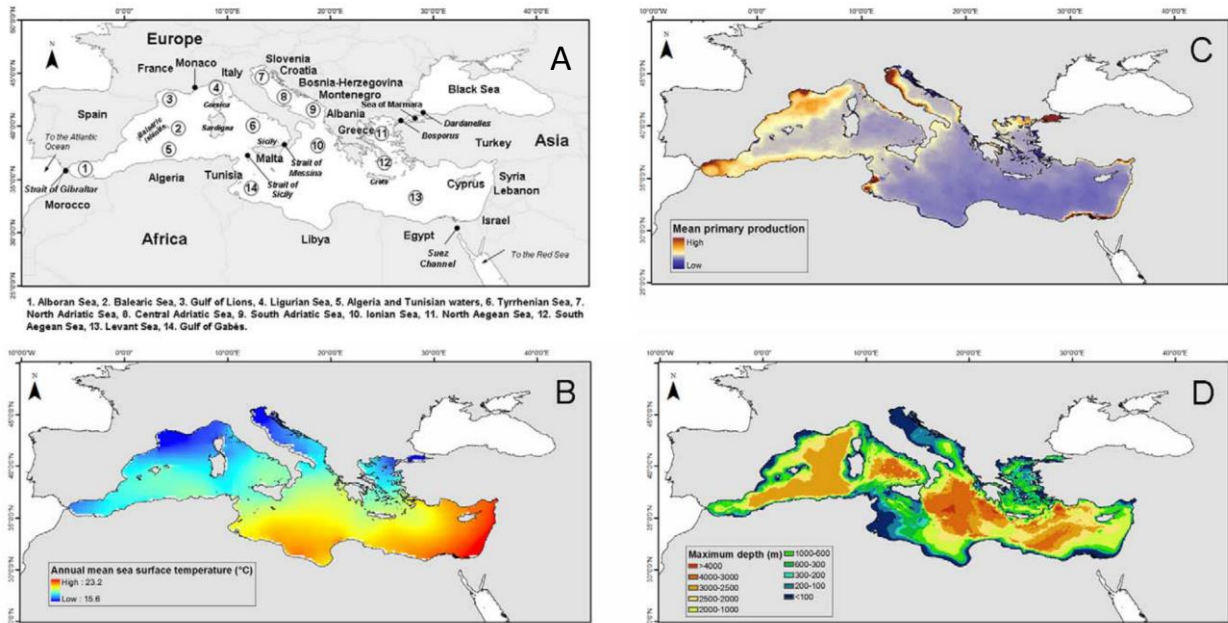

Figure 2- Biogeographic regions and oceanographic features of the Mediterranean Sea. (A) Main biogeographic regions, basins, and administrative divisions of the Mediterranean Sea, (B) Annual mean sea surface temperature (C) (2003, NOAA), (C) Annual mean relative primary production (2002, Inland and Marine Waters Unit, Institute for Environment and Sustainability, EU Joint Research Centre, Ispra, Italy), and (D) maximum average depth (m) (NOAA).

Geological history, biogeography, ecology, and human history have contributed to the Mediterranean's high cultural and biological diversity (Danovaro and Pusceddu, 2007; Marris, 2010). The recent marine biota in the Mediterranean Sea is primarily derived from the Atlantic Ocean, but the wide range of climate and hydrology have contributed to the co-occurrence and survival of both temperate and subtropical organisms (Bianchi and Morri, 1998). High percentages of Mediterranean marine species are endemic (Bianchi and Morri, 1998; Danovaro and Pusceddu, 2007)

1.4.2 Mediterranean biodiversity

During Miocene orogeny (around 10 Ma), the Isthmus of Suez was formed, separating the Mediterranean from the Indo-Pacific. Towards the end of the Miocene (about 6 Ma), the connection with the Atlantic also closed, and the Mediterranean become an isolated sea (Bianchi and Morri, 2000). Its negative water balance should have nearly desiccated the Mediterranean, which was probably transformed into a series of large evaporitic lakes during the so-called `salinity crisis' at the Messinian Stage Desiccation of the isolated Mediterranean should have driven the Tethyan biota to extinction: this is probably true for the deep water fauna (Bouchet and Taviani, 1992), but at least part of the shallow-water biota may have survived, perhaps in refuge areas, through the Neogene (Myers, 1996).

Anyway, the once high biodiversity was at least severely reduced and only with the re-opening of the Straits of Gibraltar, at the dawn of the Pliocene (5 Ma), the Mediterranean Sea was repopulated by species of Atlantic origin. This made the biogeographic physiognomy of the Mediterranean Sea to become that of an Atlantic province (Bianchi and Morri, 2000)

The alternation of the ice ages with the warm interglacial periods during the whole Quaternary resulted in different immigration waves of Atlantic fauna of boreal or subtropical origin, respectively. From this complex history, it can be concluded that the high species richness of the Mediterranean is largely due to both its long evolutionary history through the Tertiary and the post-Pliocene diversity pump from the Atlantic (Bianchi and Morri, 2000).

As a result, the present marine biota of species belonging to several biogeographic categories:

(i) temperate Atlantic-Mediterranean species;

(ii) cosmopolitan/panoceanic species;

(iii) endemic elements, comprising both paleoendemic species (possibly of Tethyan origin) and neoendemic species (mainly of Pliocenic origin);

(iv) subtropical Atlantic species (interglacial remnants, especially of the Tyrrhenian Stage);

(v) boreal Atlantic species (ice-ages remnants, especially of the Würm glacial period);

(vi) Red Sea migrants (especially into the Levant Sea);

(vii) eastern Atlantic migrants (especially into the Alboran Sea).

These categories tend to occur more or less abundantly in different parts of the Mediterranean, so that ten biogeographic sectors are distinguished within this sea (Fig. 3) (Bianchi and Morri, 2000).
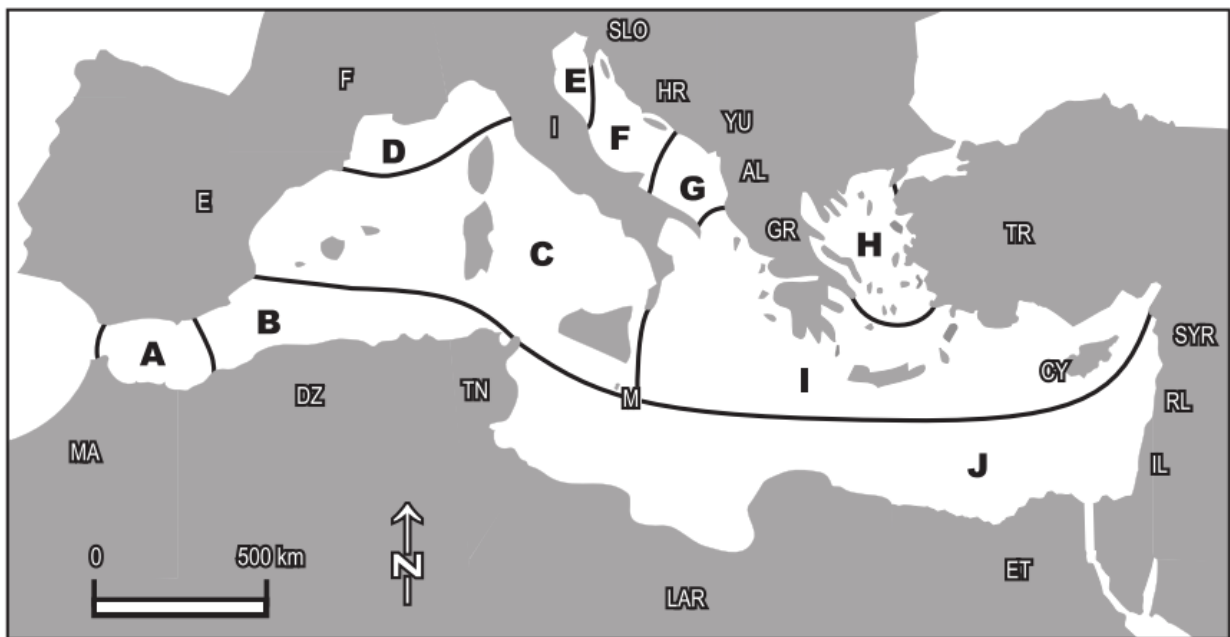

Figure 3- Biogeographic sectors within the Mediterranean: (A) Alboran Sea; (B) Algeria and southern Spain; (C) Balearic Sea to Tyrrhenian Sea; (D) Gulf of Lyon and Ligurian Sea; (E) North Adriatic; (F) Central Adriatic; (G) South Adriatic; (H) North Aegean; (I)

The most `typical' Mediterranean flora and fauna obviously occur in the central parts of this sea, and especially in the western basin. The Alboran Sea, located immediately east of Gibraltar, exhibits stronger Atlantic affinities, due to the continued penetration of Atlantic flora and fauna with the incoming influx of water (Harmelin and D'Hondt, 1993). On the contrary, the Levant Sea is experiencing an important influx of Red Sea species after the opening of the Suez Canal a phenomenon known as `Lessepsian migration' in recognition of Ferdinand de Lesseps, the French diplomat who promoted the cut of the Canal (Gücü and Bingel, 1994).

Mediterranean region has been referenced as one of the most responsive regions to climate change and was defined as a primary "hot-spot" by (Giorgi, 2006), based on the results from global climate change projection scenarios. The context of global warming stresses the necessity to assess the possible consequences of climate change on this sensitive region which would become warmer and drier (Adger and Fischlin, 2007; Birch, 2014a)

Because local scale processes are acting over the basin and drive the Mediterranean circulation, the use of regional models is required for climate studies over this region (Niu *et al.*, 2011).

1.4. Physical forcing

The circulation of the Mediterranean Sea is forced by water exchanges through various straits and channels (e.g., Gibraltar, Sicily, and Otranto), wind stress, and buoyancy at the surface due to freshwater and heat fluxes (Figure 4) (Moschella, 2008).
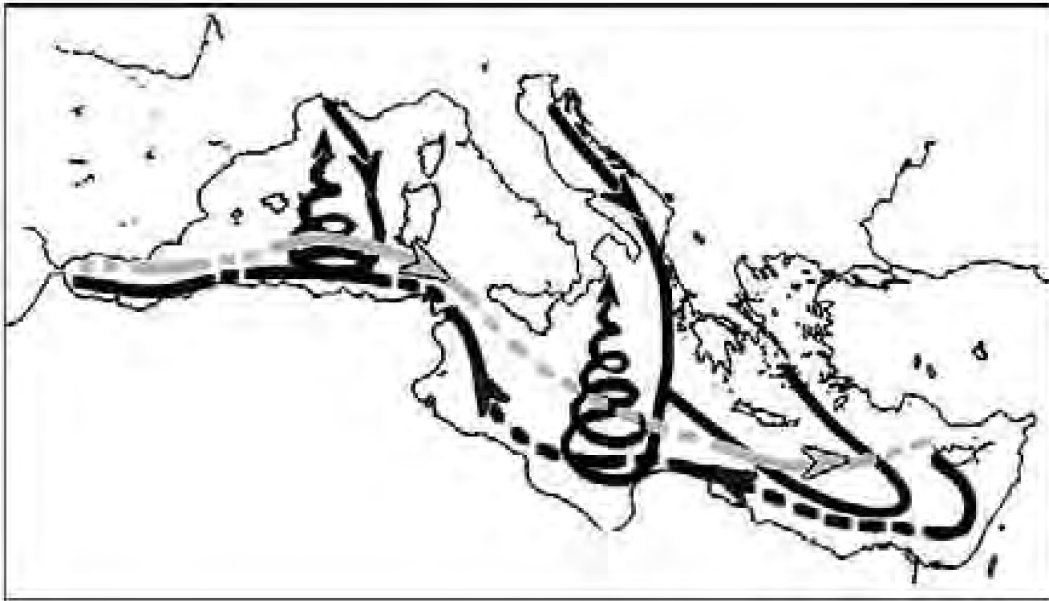

Figure 4- Scheme of the Mediterranean Sea thermohaline circulation. Grey lines represent the surface/intermediate water mass circulation forced by Gibraltar-Atlantic inflow and Levantine Intermediate Water (LIW) formation processes occurring in the northern Levantine basin. Black lines indicate the meridional vertical circulation in western and eastern Mediterranean forced by the deep-water formation processes occurring in the Gulf of Lions and in the Southern Adriatic (after Bianchi et al., 2006; redrawn after Pinardi et al., 2005).

The basin has its own thermohaline circulation (called MTHC hereafter) driven by deep and intermediate dense water convection taking place in the Gulf of Lions, the Adriatic, the South Aegean, and the North-East Levantine. These processes lead to a short residence time of the water masses compared to the wide ocean (≈100 years: Malanotte-Rizzoli *et al.*, 2014) and provide oxygen to the deepest part of the water column. However, the MTHC has varied over

time through changes of the surface fluxes acting over the basin. Sediment layers, called sapropels, reflect for instance past circulation states when the MTHC had strongly weakened, preventing the deep ventilation of the basin (Rohling, 1994). More recently, some circulation changes have been observed in the eastern Mediterranean. This so-called Eastern Mediterranean Transient (EMT) happened in the 1990's and is characterized by a switch of the main location of eastern deep water formation from the Adriatic to the Aegean, and was first reported by (Roether *et al.*, 2007); it showed from observational evidences that very dense waters were formed during that period in the Aegean Sea until the basin was filled with dense water, which then overflowed in the Levantine and Ionian basins through the sills of the Cretan Arc straits to form Eastern Mediterranean Deep Water (EMDW). The EMT has been attributed to important meteorological anomalies in the area, as well as to changes in circulation patterns (Roether *et al.*, 1996; Lascaratos *et al.*, 1999). Other long-term processes, such as the damming of main rivers resulting in salinity increase, may further influence the thermohaline circulation and the properties of sea water. Furthermore, salinity is increasing in the intermediate/deep layer of the Mediterranean Sea, whereas temperature is increasing in the surface layer (Roether *et al.*, 2007; Rubino and Hainbucher, 2007).

1.4.5   Biological response

The main question stemming from the observed modifications in the physical forcing of the Mediterranean Sea concerns their possible impact on both biodiversity and ecosystem functioning (BEF). Many signals of a response of the biotic component to large- scale physical changes are being identified (Moschella, 2008).

The successful geographical spread of species of warm water affinity is the most evident phenomenon correlated with global warming. The increasing importance of thermophilic biota in the Mediterranean Sea can be described by two major processes of change, involving both indigenous ("meridionalization") and non-indigenous ("tropicalization") species (Boero *et al.*, 2009).

Climate warming is predicted to drive species ranges northwards in the Northern Hemisphere and southwards in the Southern Hemisphere (Parmesan *et al.*, 1999; Walther *et al.*, 2002) and this tendency is broadly confirmed in the Mediterranean realm (Bianchi, 2007). This phenomenon has been named "meridionalization" (Feliatra and Bianchi, 1993), since "meridional" species, typical of the southern and usually warmer sectors of the Mediterranean Basin, are spreading northwards. More than 30 Mediterranean warm-water indigenous fish species have now been recorded north of their original geographical distribution. For some of these fishes, similar poleward extensions have been also recorded in extra-Mediterranean areas, thus reinforcing the consistency of this pattern. Moreover, our capacity to detect these changes is often unbalanced: it is reasonably easier to find a new species in a new area than to demonstrate its disappearance. As a result, these shifts usually result in the perception of increasing diversity at the local and regional level (Moschella, 2008).

Much attention is being devoted to this topic due to the increasing importance of Non Indigenous Species (NIS) in the Mediterranean Basin, the arrival and establishment of NIS in the Mediterranean Sea is a continuous process which seems to have accelerated in the last decades (Golani *et al.*, 2007; Moschella, 2008). Today, more than 500 NIS are listed from the Mediterranean Sea (Golani *et al.*, 2007), mostly of tropical and subtropical origin. The

increasing number, abundance, and success of thermophilic NIS reinforces the signal of climate trends towards warming and it has often been termed "tropicalization" (Bianchi and Morri, 2000; Bianchi, 2007) . Clearly, climatic forcing is enhanced by non-climatic reasons, such as the increase of marine traffic and the opening of the Suez Canal, resulting into an unprecedented form of basin-wide change, leading to a general biotic "homogenization" (Ricciardi and Cohen, 2007) of the Mediterranean. Even though some cases of replacement of Indigenous Species (IS) by NIS have been recorded, no final extinctions of Mediterranean IS can be registered. This led to adding NIS to IS, rapidly enriching Mediterranean species lists (Boudouresque, 2004; Boero and Bonsdorff, 2007). In the eastern Mediterranean, especially along the coasts of Israel, many NIS replaced, albeit not completely, IS performing similar ecological roles (Galil, 2007). The lack of historical datasets prevents a proper evaluation of community changes, but evident replacements likely happened (Goren and Galil, 2005) showed for many species. These replacements are obvious for commercial fish: *Siganus rivulatus*, for instance, might have substituted the native herbivores *Sarpa salpa* and *Boops boops*.

The replacement of an IS by a NIS can occur due to multiple causes, sometimes even not mutually exclusive. The arrival of NIS, indeed, might lead to a complex network of interactions with IS (Figure 5) (Moschella, 2008).
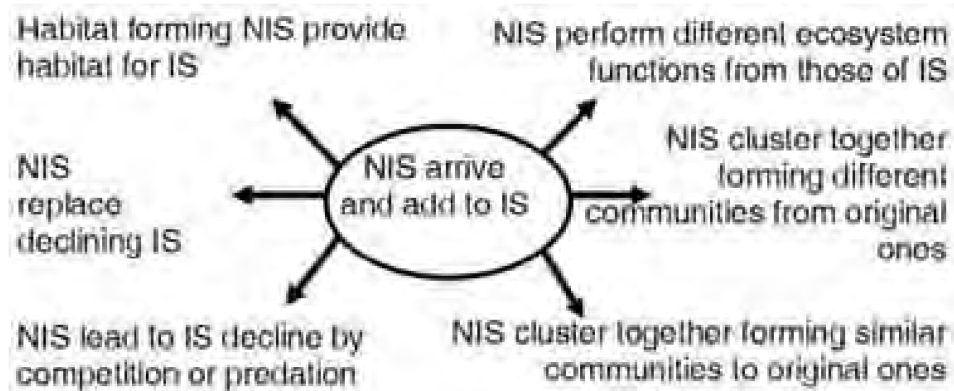
Figure 5- Possible scenarios deriving from the arrival and establishment of Non Indigenous Species (NIS) and their relationships with Indigenous Species (IS). If a NIS is a habitat former (e.g., a canopy-forming alga) it can provide habitat space for NIS. A NIS might replace a IS whose populations are declining for other reasons than the arrival of the replacing species. A NIS can cause the decline of a IS by using local resources more efficiently (decline by competition), or by directly feeding on it (decline by predation). A NIS might increase ecosystem efficiency by adding novel functions due to features that are not shared by any IS. A group of NIS might cluster together and form either NIS assemblages that replicate those of the area of origin of NIS or, also, new NIS assemblages deriving from the clustering of NIS coming from different original areas.

1.4.5   Extreme events

Temperature anomalies and higher sea surface temperatures (SST) have severely impacted entire shallow coastal ecosystems, causing the elimination of sensitive species as well as mass mortalities. The large-scale loss of biodiversity at the ecosystem level can turn diverse and structurally complex benthic and pelagic communities into simpler microbial ones (Sala and Knowlton, 2006). Increasing frequency, severity and expansion of mass mortalities related to seasonal stratification (hypoxia/anoxia) or to temperature anomalies were observed in different parts of the Mediterranean. The first evidences of this phenomenon dated from the first half of'80 years affecting the Western Mediterranean and the Aegean Sea (Garrabou *et al.*, 2019); the most impressive phenomenon happened in 1999 when an unprecedented large scale mass mortality event (MME) impacted populations of more than 30 species from different Phyla

along the French and Italian coasts (Cerrano *et al.*, 2000). Following this event, several other large scale MMEs have been reported, along with numerous other minor ones, which are usually more restricted in geographic extend and/or number of affected species (Garrabou *et al.*, 2009, 2019).

New MMEs are expected during the coming years. To date, despite the efforts, neither updated nor comprehensive information can support scientific analysis ofmortality events at a Mediterranean regional scale. Such information is vital to guide management and conservation strategies that can then inform adaptive management schemes that aim to face the impacts of climate change. The reported mass mortality events mainly concerned the Western Mediterranean ecoregion, with 55.5% of observations (i.e., Liguro-Provençal: 25.4%, Balearic: 16.6%, and Tyrrhenian: 13.5% sub-ecoregions); followed by the Adriatic Sea with 23.5%), the Aegean Sea (12.7%), and the Ionian Sea (0.4%) (Garrabou *et al.*, 2019).

1.5   Marine Protected Areas

In the Mediterranean Sea, and for the specific purpose of inclusion of sites in MAPAMED, the generic term «Marine Protected Area» is understood as «a clearly defined marine geographical space - including subtidal, intertidal and supratidal terrain and coastal lakes/lagoons connected permanently or temporarily to the sea, together with its overlying water - recognised, dedicated and managed, through legal or other effective means, to achieve the longterm conservation of nature with associated ecosystem services and cultural values» (Gabrié *et al.*, 2012). The Aichi Target 11 also mentions «Other Effective area-based Conservation Measures» (OECMs). This

denomination seems to also indicate protection designations, even though there is yet no clear international guidance as to how it applies.

In Mediterranean Sea we count 1215 Marine Protected Areas (MPAs) and Other Effective area-based Conservation Measures (OECMs) now cover 6.81 % of the Mediterranean through a large variety of conservation designations, with national designations accounting for only 1.27% and no-go, no-take or no-fishing zones for 0.04%. Over 72.77% of the surface covered is in the Western Mediterranean. Designations cover 9.79% of European waters mostly due to the Natura 2000 at sea network which rarely affords strict restrictive measures. To reach the 10% quantitative part of the Aichi Target, an additional 71,900 km^2 (2.86 % of the Mediterranean). Considering the high pressures exerted on the Mediterranean marine environment with growing trends, it is clear that willingness to invest in marine conservation needs to be boosted up.

The IUCN (International Union for the Conservation of Nature) proposes a classification system for terrestrial and marine protected areas based on their management objectives. This system is recognized worldwide (although not applied by all countries or for each site) and comprises six management categories; not all Mediterranean MPAs have been assigned an IUCN category so far and some have not been assigned the most fitted category. However, IUCN maintains its efforts to fill this gap and brings support to MPAs and Governments so that categories are properly assigned.

In recent years, some attempts have been made to develop additional MPA classification systems that integrate the diversity of MPAs and bring precision on their actual level of protection, thus complementing the IUCN system (NOAA, 2011; Horta e Costa, 2016).

The US National MPA Center (NOAA, 2011) has developed a classification system that provides straightforward means to describe MPAs in purely functional terms using five objective characteristics common to most MPAs:

• Conservation Focus

• Level of Protection

• Permanence of Protection

• Constancy of Protection

• Scale of Protection

1.5.1 Climate related impacts in Mediterranean MPAs

The majority of Mediterranean MPAs have been classified as multiple-use MPAs, which seek a balance between biodiversity conservation and some sustainable level of human use. Less than 0.1% of the Mediterranean's total area is covered by strict protection and/or no-take zones, and MPAs in Categories IV (Management areas for habitats and species) and II (National Parks) with some level of human use are the most common management types (Gabrié *et al.*, 2012) Even with management, many MPAs may be exposed to several simultaneous stressors such as commercial and recreational fishing, pollution, habitat degradation and climate change, often acting in synergy and thereby amplifying their individual impacts on the marine ecosystem and its communities (Harley and Rogers-Bennett, 2004); this accumulation of stressors can considerably decrease the resilience of MPA ecosystems to an additional stress such as climate change. That is, their natural capacity to absorb, resist or recover from disturbances or to adapt to these changes without causing community shifts or local population extinctions.

The former measure will require collaboration with other coastal users and planners as well as ensuring that good monitoring information is gathered so as to be able to take action and identify targeted approaches to specific pressures. The second type of measure will require the identification of areas that were resilient to past climate change impacts as well as the collection of information on the responses of different communities and sites in these locations (Otero *et al.*, 2013).

Conducting a vulnerability assessment for a specific site can focus attention on management actions that can be useful for MPA managers and other users. The assessment should evaluate impacts caused by climate and non-climate drivers, such as changes in sea level, storms, temperature, and sedimentation patterns. The approach to be used will ultimately depend on the objective of the vulnerability assessment (EEA Report No 12/2012).

1.5.2 Monitoring approaches in MPA

Different tools may be used for vulnerability assessments at different spatial and temporal scales, in different regions and for different management purposes, based on the information and data available. A range of methods (including indicator-, index-, GIS- and model-based methods) for assessing vulnerability to climate change are outlined in the ETC CCA Technical Paper 1/2011. Index- and indicator-based approaches (including related GIS applications) are simple methods that can provide a 'first-look' assessment to identify priority vulnerable coastal areas and can also be useful for informing stakeholders. GISbased decision support systems (DSS) can be used to investigate multiple climate change impacts on coastal areas, with prioritization of vulnerable locations and analysis of data uncertainties; while methods based on

dynamic computer models are important tools for analysing and mapping the likelihood of climate change and the associated vulnerability of coastal systems. MPA managers rarely use many of these tools and could therefore benefit from assistance from research institutions, consultancy companies or universities.

For any potential climate change monitoring program to be implemented in these sites, the individual MPAs already need to be carrying out general monitoring and surveillance of their habitats and species (including such variables as area, cover, quality and population numbers) in order to keep track of the condition of their marine environment. Building on this information, the monitoring program should incorporate climate change and its impacts within the standard procedure. The results of climate change monitoring supplemented by observations of coastal and marine condition (i.e. physical and biochemical parameters) can then be used to determine causes and effects when changes in marine biodiversity are found (Otero *et al.*, 2013). Organisms provide some of the most sensitive indicators of climate change and evolutionary responses are becoming apparent in species with short generation times**.**

1.5.3 The contribution of Citizen science to monitoring programs

Recently there has been a significant increase in public involvement in scientific research, now referred to as "Citizen Science". Alan Irwin was one of the first to use the term "Citizen Science" in 1994 in the context of describing expertise by lay people (Irwin *et al.* , 1994). This term was soon modified to describe a research technique using members of the public to gather or analyse scientific data (Bonney *et al.*, 2009). Citizen science is defined by the European Commission Green Paper as "general public engagement in scientific research activities where citizens

actively contribute to science either with their intellectual effort, or surrounding knowledge, or their tools and resources" ('Green Paper on Citizen Science: Citizen Science for Europe', 2013). Citizen science engages the public in scientific projects that are difficult to conduct solely by scientists who lack the resources to gather or analyze data on a large scale ('Green Paper on Citizen Science: Citizen Science for Europe', 2013), it engages interested volunteers in a wide variety of projects including monitoring wildlife (Wiersma, 2010; Alipanah *et al.*, 2020) and the environment , as well as classifying images (Smith *et al.*, 2011; Williams *et al.*, 2015)transcribing old records and annotating images from past biodiversity collections (Elizabeth, 2015). Project objectives range from supporting scientific investigations within academic institutions to increasing the interest and knowledge of the general population on science.

Citizen science projects can be classified in several different ways. An initial classification for these projects was based on the type of volunteer involvement, dividing them into (Domroese, 2013):

• Contributory, where the participants contribute to data collection, and sometimes help analyse the data and disseminate results.(Domroese, 2013);

• Collaborative, where citizens also analyse samples, data and sometimes help design the study, interpret the data, draw conclusion, and disseminate the results;

• Co-created, where citizens participate at all stages of the project, including defining the questions, developing the hypotheses, right through to discussion of the results and answering new questions.

An alternative classification for specific citizen science projects is based on the goals of the study and identified the following five mutually exclusive and exhaustive types of projects (Wiggins and Crowston, 2011):

• Action projects are initiated by volunteers designed to encourage intervention in local concerns such as improving water quality in their local stream;

• Conservation projects address natural resource management goals, for example a project to monitor the type and abundance of beach litter (Rosevelt *et al.*, 2013);

• Investigation projects focus on scientific research goals in a physical setting, for example a detailed study of otter demographics in California (Brzeski *et al.*,2013);

• Virtual projects also focus on scientific research goals, but are entirely based on information technology with all volunteer interaction occurring on-line such as in Galaxy Zoo, where volunteers find and classify galaxies (Schawinski, 2011);

• Education projects that are often performed in the classroom or school grounds as part of the science curriculum, for example a butterflies and ground squirrel monitoring study (Kelemen-Finan *et al.*, 2018).

CS has some limitations, one of which was found in the impossibility of obtaining a detection effort uniformly distributed in time and space (Fore *et al.*, 2001). From the point of view of data quality (Kremen *et al.*,2011), important sampling bias can occur (Dickinson *et al.*, 2012). The involvement of non-specialist volunteers is often criticized on the grounds that the information gathered may be unreliable due to insufficient training (Darwall and Dulvy, 1996). To overcome these obstacles, appropriate sampling protocols and methods must be developed. The reliability and relevance of data generated by CS programs are viewed with some skepticism by the

scientific community (Darwall and Dulvy, 1996). However, if properly trained, volunteers, can collect data that are reliable and perform evaluations comparable to those performed by researchers. In the CS some studies of marine biodiversity were conducted to evaluate the accuracy and consistency of the data recorded by the volunteers (Goffredo *et al.*, 2010). The results show that trained recreational divers achieve an acceptable level of accuracy and consistency.

To assess the reliability of the data collected, comparisons are made between the data collected by the volunteers and those of the reference researchers. At the end of the dive, a questionnaire is usually completed independently and separately, without any interference between the volunteers. Once the data recorded in the questionnaires have been processed, they are analyzed and compared: if they are consistent with each other, the entire dataset can be used. In this way the level of coherence achieved by the volunteers during the validation tests is comparable to that performed by CS projects where the volunteers undergo specific training for sampling activities on transects (Mumby *et al.* , 2011).

1.5 Aim of the study

As previously highlighted one of the main effect of climate change and specifically, global warming, on fish species is a displacement of native species towards habitat offering more suitable condition for their life, thus they generally migrate either to higher latitudes or move to greater depths in search of colder waters with less competition. In this context, thanks to the implementation of specifically "climate change indicators", changes in fish biodiversity and abundance were observed in three different sites of the Ustica island and at four different ranges and data from Ustica were then compared with a similar dataset from a southernmost island, located in the Sicily Strait, i.e. Linosa, also a MPA. Thus, the specific aims of this thesis were:

1. to assess differences in the degree of tropicalization of the two islands, evaluated through the comparison of a "tropicalization index" developed *ad hoc*;

2. to determine differences in the degree of tropicalization along a bathymetric gradient within each island and between the two islands, and

3. ss part of the data were collected with the help of recreational divers, to assess the goodness of this information for the purposes of the protocol implementation.

2. Material & Methods

2.1. Study Area

Fieldwork was carried out within the Ustica Marine Protected Area, Sicily, and additional data were obtained by Aversa (2020), from the island of Linosa. The Ustica MPA is located in the Southern Tyrrhenian Sea (Western Mediterranean, 10°43'43'' E–38°42'20'' N), 60 km North of the Sicilian coast (Fig.6a-1). This MPA, created in 1986, is the first of the Mediterranean Sea and encompasses a total area of 16000 ha. Like other MPAs, protection measures are implemented according to three different levels: a "no-take zone" (zone A), that covers 65 ha along the western part of the island, a "general reserve" (zone B) and a "take zone" (zone C), share the remaining area equally.

According to the institutive decree of the MPA (D.I. 12∕11∕1986), only research activities are authorized in the no-take zone, whereas recreational activities, such as SCUBA diving, boat anchoring, swimming, and angling, are strictly prohibited. There are no restrictions on recreational activities in either the B or the C zones (Gianguzza *et al.*, 2009).

Within the Ustica MPA, three sampling locations, located in the north, east and west sides of the island were selected as permanent monitoring locations (Fig. 6b).

Further data were obtained from the MSc Thesis of Aversa (2020), which applied the same methodology in Linosa (35°85'N, 12°85'E), a small volcanic island located in the middle of the Sicily strait. Linosa belongs to the MPA of Pelagie islands (Lampedusa, Linosa and Lampione), which was created in 2002, covering a total sea surface of 4367 ha. It is located in the Southern part of the Sicily Strait (Mediterranean Sea) (Fig.6a-2), 165 km away from the African coast and 167 km off Sicily (Italy). Its geographical extension is very narrow with a surface of 5.4

km^2 and a roughly square-shaped contour of 11 km, with each side oriented toward a cardinal point (Fig. 6c)(Azzurro *et al.*, 2017). Within the island of Linosa, Aversa (2020) selected three permanent monitoring locations in the morth, east and west side of the island. The availability of the Linosa dataset allowed to compare the data collected from Ustica with the ones of an other volcanic island located at a different latitude and exposed to a different climate regime. In Ustica, three different sampling locations separated by a minimal distance of about 0.5 km, were chosen, considering the characteristics required by the protocol of the MPA-ENGAGE project, i.e. rocky bottoms with moderate slope, not dominated by meadows of *Posidonia oceanica.*
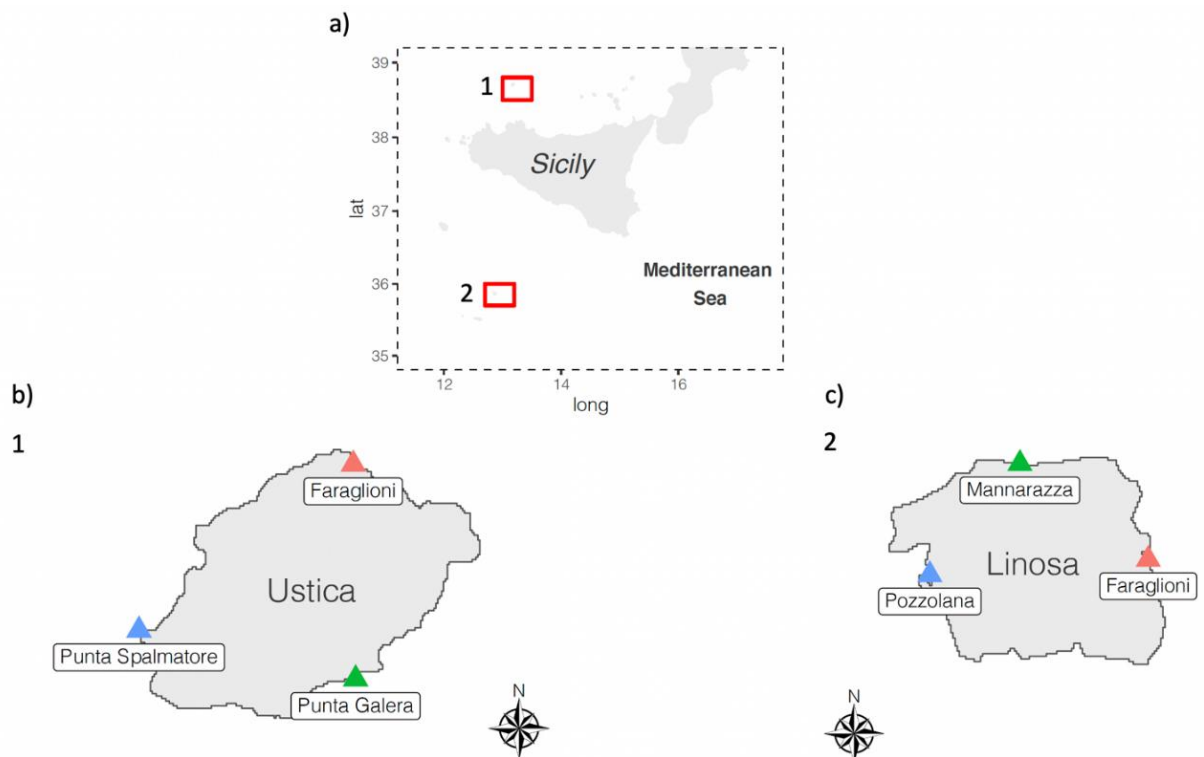
Figure 6- Study area: a) map of Sicily with highlights on 1-Ustica and 2-Linosa b) Ustica island with permanent monitoring locations (Faraglioni, Punta Galera, Punta Spalmatore) surveyed in the present study c) Linosa island with permanent monitoring locations (Faraglioni, Mannarazz,a Pozzolana) selected by Aversa (2020).

2.2 Data collection

Data were collected from June to October 2020 through the implementation of a standard method of underwater visual census (UVC) (Garrabou *et al.*, 2019), which was conceived to evaluate the abundance of target thermophilic species in order to monitor the effects of Climate Change in Marine Protected Areas (Azzurro *et al.*, 2019).

Sampling unit

The sample unit was represented by a 50m long x 5m wide transects (Fig.4). According to the simplified protocol provided by Garrabou *et al.*(Garrabou *et al.*, 2019), this distance was estimated by the observer, which was trained to swim at the constant speed of 10m/min for exactly 5 minutes. According to this protocol, individuals smaller than 2 cm were excluded from the counts.

Materials used to carry out these transects were: a pre-printed PVC board (Fig. 7) to collect data underwater, an underwater computer to count the timing and check water temperature (Fig. 8) and finally a paper sheet or logbook where to copy the data collected.

Figure 7- PVC board for underwater sampling
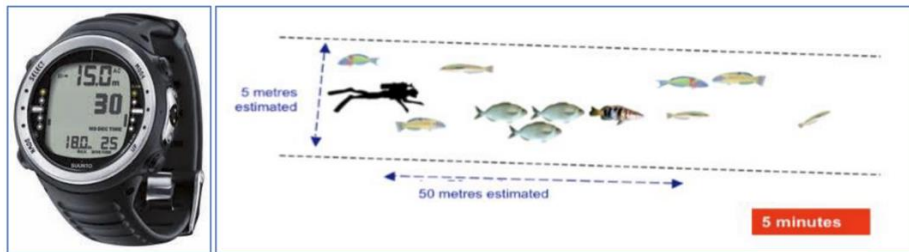
Figure 8- Underwater watch and transect scheme (From Garrabou et al, 2019)

The three selected locations for the island of Ustica were (Fig. 6b):

1. Punta Galera: Zone C; South of the Island

2. Faraglioni: Zone B; North of the Island

3. Punta Spalmatore: Zone B; East of the Island

In each monitoring location, data were collected at four different depth ranges: 1-3 m, 5-10 m, 11-20 m, 21-30 m. According to the experimental design, 4 consecutive replicates were carried out by each observer at each depth and surveyed location.

At the dept range of 1-3 m, all the transect were carried out by snorkeling (Fig. 9b), whilst and scuba diving was used to the other depth ranges (5-10/11-20/21-30m) (Fig. 9a). According to the basic safety rules, all the transect were performed in pairs (Fig. 9).
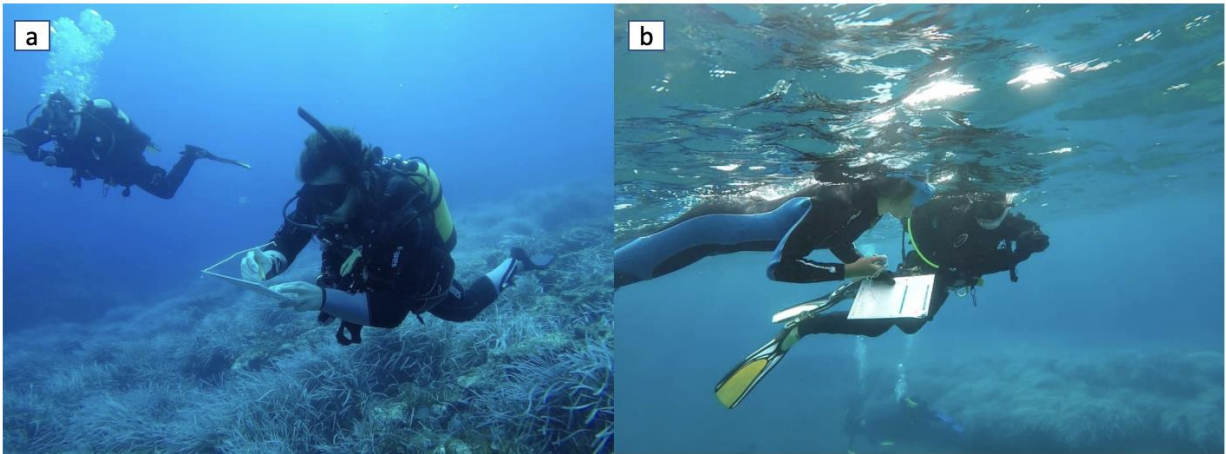
Figure 9- a) Diving operators working in pairs to carry out standard UVC transects of 5 min; b) Same technique carried out from the surface by snorkeling.

Target species

According to the protocol of Garrabou *et al.*(Garrabou *et al.*, 2019), nine different fish species were selected as target (Fig. 10): *Sparisoma cretense, Epinephelus marginatus, Thalassoma pavo, Sarpa salpa, Serranus scriba, Coris julis, Serranus cabrilla, Siganus* spp*., Fistularia commersonii.*

Further description of the selected species is present in the Appendix at the end of this study.

Figure 10- Pictures of the nine target species (credits Giovanni Ombrello)

2.2.1. Citizen Science and Visual Census

UVC Data were also collected with the help of trained volunteers engaged through the help of PADI-Professional Association Diving Instructor and DAN- Divers Alert Network (Fig. 9).

There are already other specific projects and training courses for PADI professionals, such as the "PADI Fish Identification", which is dedicated to Citizen Science. In this specific course the recreational diver has the possibility to audit different lectures on land and learn both a scientific sampling method and how to recognize the species of the project; after there are also lessons underwater with an instructor in order to apply the theory lessons.

Beside recreational divers, also the diving team must be trained: courses are held right before the summer season begin, allowing instructors and dive masters to teach these sampling methods to all divers and trainees during the entire diving period.

For this reason, the involvement of a diving center, such as Mare Nostrum in Ustica, gave the possibility not only to train professionals but also to engage many customers about the project and catch their attention trough the possibility to learn something during the dive.

Training of volunteers

Overall, a total of 111 volunteers were trained at the diving center to implement the UVC protocol. Training session were delivered during the recreational diving to all the interested customers. At each sampling location the dive master or the instructor usually introduced the project and divers were engaged in the Citizen Science activity (Fig. 11, 12). The briefing consisted in the explanation of each aspect of the PVC board, a short lesson on the identification and finally specific aspects on the transect technique.

Figure 11- Instructors and dive masters while doing a de-briefing with clients

Figure 12- PADI/DAN Training course for instructors in Ustica, 2020

Data input

After the underwater sampling, data registered in the PVC boards were checked by the diving instructors and manually uploaded to the interactive system provided by *Seawatchers* (https://www.observadoresdelmar.es/). Data uploaded to the system were finally downloaded as *csv files and submitted to a further data check.

Seawatchers is an interactive system in which not only scientists but also citizens can register and take action in different monitoring activities (Fig.13a,b). *Seawatchers* involves a number of recreational divers in different monitoring activities under the principles of sharing knowledge and mutual learning. Thanks to the system, volunteers have the possibility to follow each other, to do networking and to check other data on an interactive map.

In this study, we trained recreational divers, engaging them into the data collection.
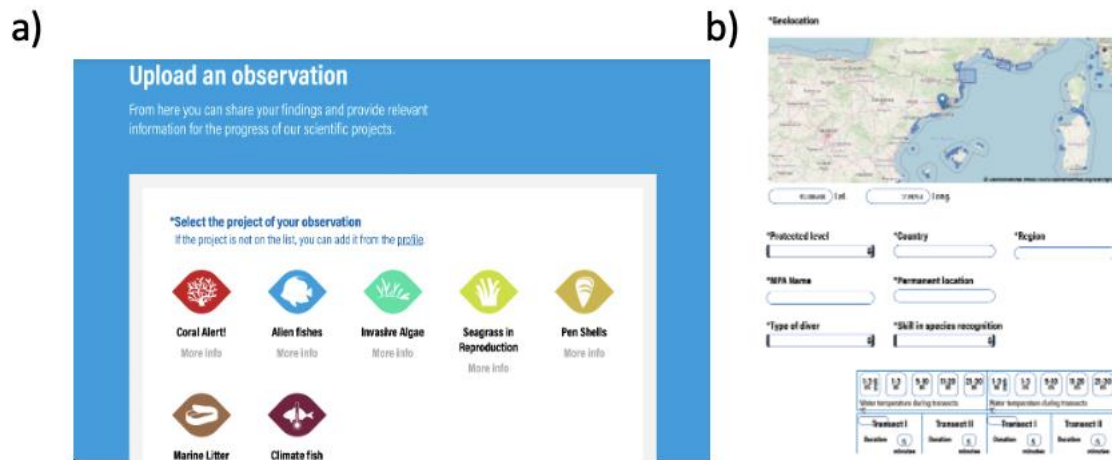
Figure 13– Example of uploading observation on Seawatchers web page: a) choosing the project ("Climate fish" in this case) and b) uploading the information through an interactive page, which mirrors the structure of the PVC board

2.3 Data analysis

Underwater visual census data collected in Ustica were assembled with the data of Aversa (2020) collected in Linosa and employed for univariate and multivariate analyses.

Both, the Principal Component Analysis (PCA) and the Correspondence Analysis (CA) were performed following the method of Hill and Smith (1976) and using both qualitative and quantitative variables. This method was crucial to show differences related to the different factors under study: skills in species recognitions, with 5 levels (Assisted, Low, Good, Very Good, Expert), MPA with two levels corresponding to the two different islands/MPAs, location, with three levels, nested in MPAs, and depth, with 4 levels (0-3m, 5-10m,11-20m, 21-30m). First of all, the goodness of data taken by recreational divers were tested for consistency with data acquired by experts, in order to avoid bias caused by non-expert divers in recognition of the target species, defining a final dataset.

To smooth sample variability, the total number of individuals of each species observed at a given depth was square root-transformed . The transformed dataset was used to create a graphic visualization of the distribution pattern of fish assemblages.

Based on the different thermic affinity of the target species (https://www.fishbase.se/search.php) and on their recent response to warming conditions (Moschella, 2008; Azzurro *et al.*, 2019) a simple index, here referred as *Tropicalization index (i^T)* was conceived and calculated on a presence/absence transformed matrix. The *Tropicalization index*, specifically applies to the Mediterranean coastal communities and it ranges from 0 (temperate) to 7 (highly tropicalized) fish assemblages, respectively (Fig. 14a). Conceptually, the index take into consideration three different indicators (Fig. 14b):

1) <u>Indicator 1</u>: *C. julis* and *T. pavo*;

2) <u>Indicator 2</u>: the herbivores *S. salpa* and *S. cretense*;

3) <u>Indicator 3</u>: invasive species of tropical origin.
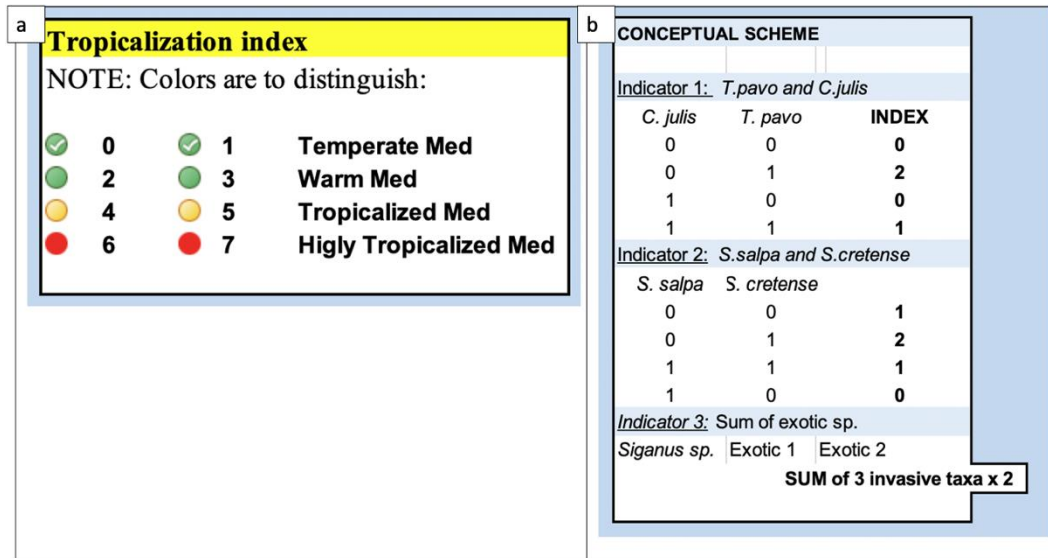
Figure 14-a) Conceptual score visualization of the i^T b) scheme to the indicators and presence/absence matrix

Each indicator is represented into a presence/absence matrix and the comparison between the two species results into a score index: Hence, the *tropicalization index* is calculated as the sum of the three indicators. We then explored the variation of the *Tropicalization index* in relation to the factors 'depth', 'location' and 'MPA': univariate analyses were performed through ANOVA after we properly verified through a Bartlett test, if variances were homogenous. We used Tukey's HSD (honestly significant difference) test to evaluate the trend of *tropicalization index* according to depth ranges and which range is the most significantly different. Then, the Hill-Smith (Hill and Smith, 1976) was used to analyze differences related to the factors 'site' and 'depth' in both MPAs.

We also run a PERMANOVA test. The purpose of the test was to verify if there are differences in the distributions of the species assemblages in the two sites, taking into account the depth. The model we considered was:

$$Y = Si + PL + Depth + Si{:}PL + Si{:}Depth + PL{:}Depth$$

where "Si" was the Site of the sampling (Linosa or Ustica) and was considered as fixed, "PL" was the Permanent Location considered as fixed while "Depth" factor was considered as random.

The symbol ":" represents the interaction between the two factors.

Differences in the structure of fish assemblages were graphically represented in a two-dimensional ordination plot by non-metric multidimensional scaling (nMDS) using the Bray-Curtis measure of similarity.

The similarity percentage (SIMPER) procedure was employed to identify the major fish taxa contributing to dissimilarities between Permanent Location and also at different depth.

An inferential analysis was carried out using a negative binomial GAM-Generalized Additive Model to consider the abundances compared to other variables as temperature, skill in recognition, depth, permanent location, month, year, species. This modeling is made to comprehend if observed differences between groups are dependable or might have happened by chance in this study.

The negative binomial distribution was used because after appropriate overdispersion tests (package "qcc", p-value<0.001) we noticed over-dispersed data, hence Poisson distribution was not possible. The function used for the GAM is the following:

$$Ab = \beta o + f1(Yr) + f2(Mnt) + f3(WT) + [PL] + [Skill] + [Sp] + [Depth]$$

In which βo is the intercept, "Ab" is the abundance of the species, "f1,f2,f3" are smooth functions estimated by the model by maximum likelihood, "Yr" is the Year, "Mnt" the month, "WT" the water temperature, "PL" permanent location, "Skill" the skill in species recognition, "Sp" species. Last 4 represent linear functions.

Univariate and multivariate analyses were performed with the software PRIMER v7 and PERMANOVA+ software package (PRIMER-E Ltd, Plymouth, UK) and the R 4.1.0 software with following packages: "*ggplot2*", "*mgvc*" Mixed GAM Computation Vehicle with Automatic Smoothness, "*ade4*" Analysis of Ecological Data: Exploratory and Euclidean Methods in Environmental Sciences.

3. Results

Overall a total of 519 transects were carried out in Ustica (286 from researcher and 233 from recreational divers) and additional data were obtained from Linosa, consisting in 905 transects carried out from 2012 to 2018 (674 from researchers and 231 from recreation divers).

Discriminant analysis with the Hill & Smith function

The Hill-Smith analyses was carried out according to three different axes (Tab 1), keeping 46% of information from the entire dataset.

Table 1- Table showing the Hill and Smith analysis with 3 axis (CS1, CS2, CS3) and highlights on resulting main drivers for each axes (Year, Thalassoma pavo, Sparisoma cretense *for the first axis and* Serranus scriba, Serranus scriba *and Water temperature for the second axis)*

	CS1	CS2	CS3
Year	0.45	0.19	-0.15
Month	0.50	-0.33	0.57
Water. Temperature	-0.27	0.39	0.11
Sarpa.salpa	-0.34	-0.30	-0.38
Coris.julis	0.28	-0.35	0.17
Thalassoma.pavo	-0.47	-0.21	-0.25
Sprisoma.cretense	-0.38	-0.38	0.2
Epinephelus.marginatus	0.29	-0.34	-0.36
Serranus.scriba	0.22	-0.41	-0.16
Serranus.cabrilla	0.15	-0.14	0.26
Siganus.sp	-0.11	-0.04	0.38
Fistularia.commersonii	0.00	0.00	0

Main analyses were plotted with the first two of this axes (CS1, CS2 in the table) taking into account the most significant data (the three per column with the higher percentage) as the main driver of the plot.

Discriminant analysis with variable Skill in recognition

Discriminant analysis was firstly applied to evaluate the goodness of our data and to evaluate their quality according to the different self-assessed skill in recognition of divers (low, medium, good, very good, expert and assisted). Transects carried out by observers with "low, medium or good" skills were significantly different from our reference dataset (Very good skills, i.e. professional observers) (Fig. 15).
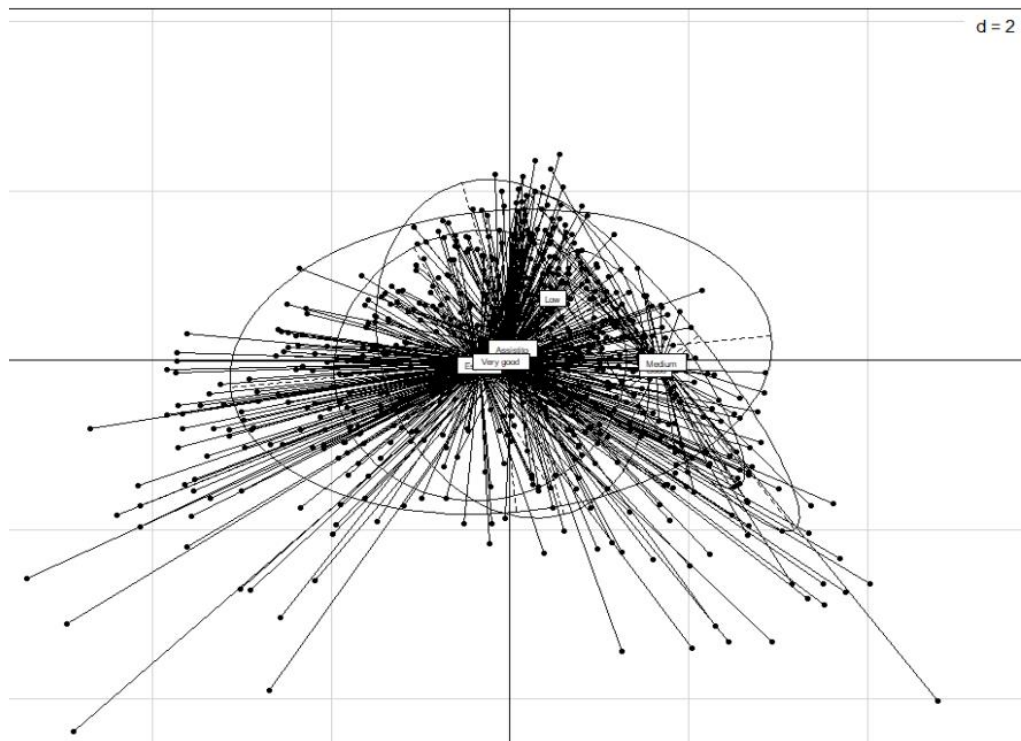
Figure 15- Hill and Smith graphic visualization of skills in species recognition used as discriminant variable

Based on this analysis, we decided to discard the data collected from observers evaluated as "low, medium or good" and use the remaining data for the following analyses, which resulted in a total of 393 validated transects for Ustica and 905 for Linosa.

General description of fish assemblages

A first exploration of the differences in target species abundances between sites (Linosa and Ustica) and among permanent locations (Punta Galera, Punta Spalmatore, Faraglioni-Ustica, Faraglioni-Linosa, Mannarazza, Pozzolana) is illustrated in Fig.16.

As showed by boxplots (Fig. 16a), the investigated fish assemblages were numerically dominated by *Sarpa salpa* and *Thalassoma pavo* both in Ustica and Linosa in which there is high presence also of S*parisoma cretense*

Fish abundances and species richness were not influenced by the permanent location nested in Ustica and Linosa (Fig. 16b).

Abundance ratio seemed to be maintained with three species (*Sarpa salpa, Sparisoma cretense* and *Thalassoma pavo*) numerically dominant in all sites (Figure 16); while the less abundant *Siganus* sp. (Lessepsian species) was only observed in Linosa.
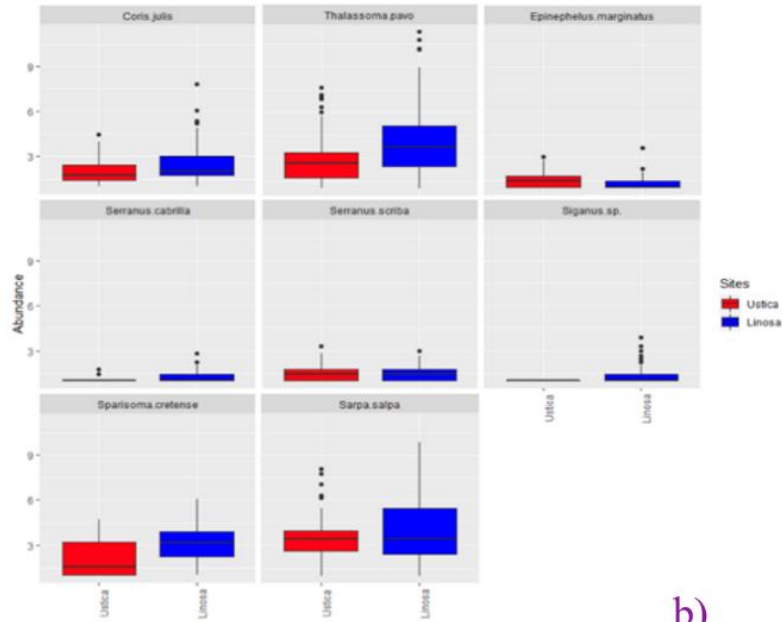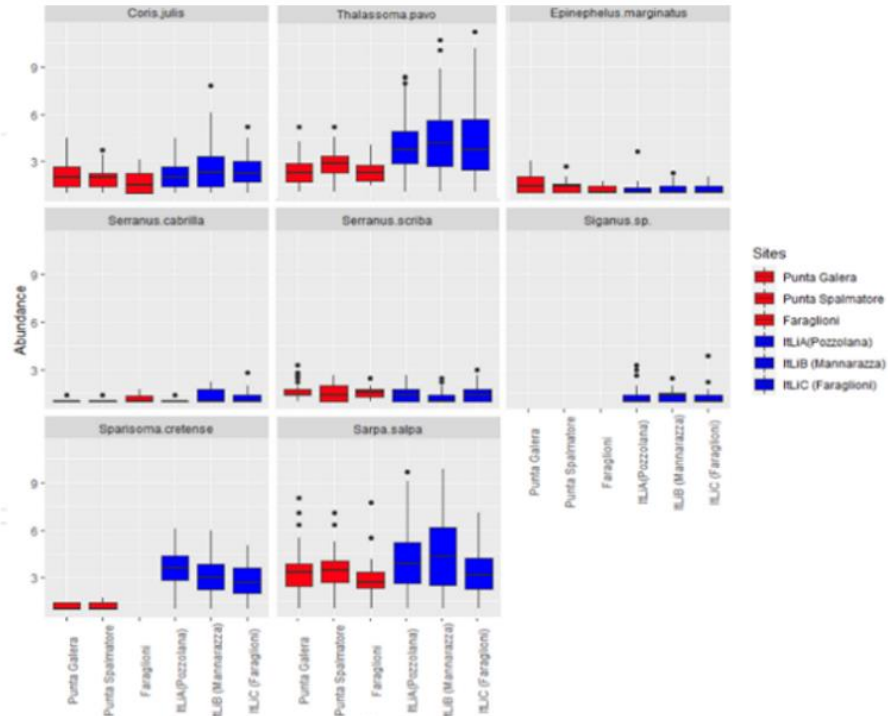
Figure 16- Abundance of target fish species, Boxplots*:* a) Ustica(red boxes) and Linosa(blu boxes) MPAs b) permanent locations (Punta Galera, Punta Spalmatore, Faraglioni-Ustica, Faraglioni-Linosa, Mannarazza, Pozzolana). *Abundance is expressed as the total number of individuals of each species observed in 50x5m stript transect transformed under square root*

The boxplots run at species level (Fig. 17) showed the higher abundances of *Sarpa salpa* and *Thalassoma pavo* in the first depth layer (1-3m).

On the contrary, the species *Coris julis, Serranus scriba*, and *Epinephelus marginatus* and *Siganus* spp. seemed to increase at deeper layers, 11-20m and 21-30m.
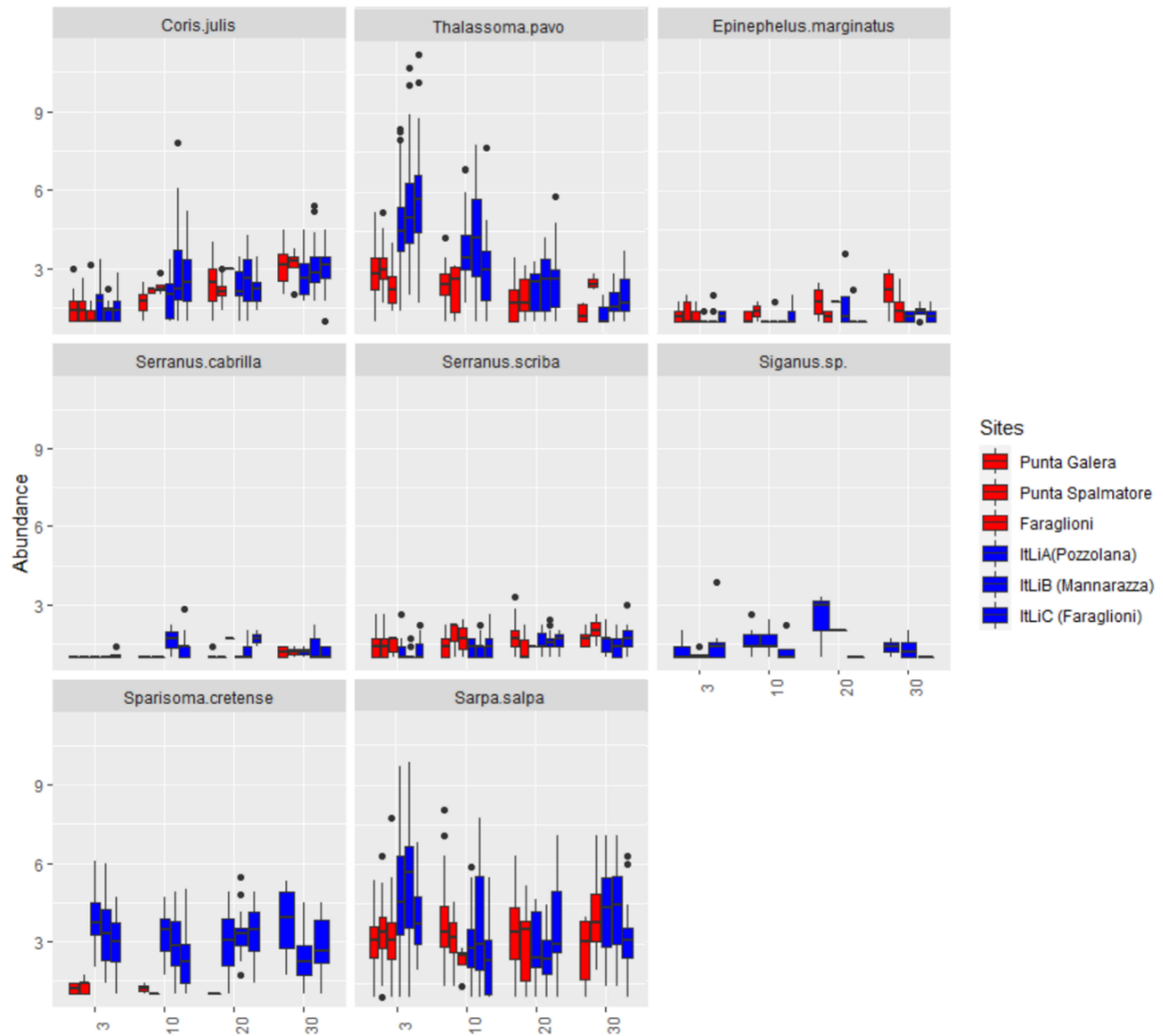
Figure 17-Abundance of target fish species per depth, Boxplots*: MPAs permanent Locations Punta Galera, Punta Spalmatore, Faraglioni-Ustica (red boxes), Faraglioni-Linosa, Mannarazza, Pozzolana (blu boxes). Abundance is expressed as the total number of individuals of each species observed in 50x5m stript transect transformed under square root*

Spatial variation in Tropicalization index

The *Tropicalization index*, calculated for each transect carried out in Ustica and Linosa, was here used to explore spatial variations at the scale of locations, MPAs, and also among the different depth surveyed.

The three locations in Ustica (Faraglioni, Punta Galera and Punta Spalmatore) results in an average tropicalization index of 1.15±0.04, while the three one in Linosa (Pozzolana, Mannarazza and Faraglioni in Linosa) have an average i^t of 2.35±0.04 (Fig. 18).
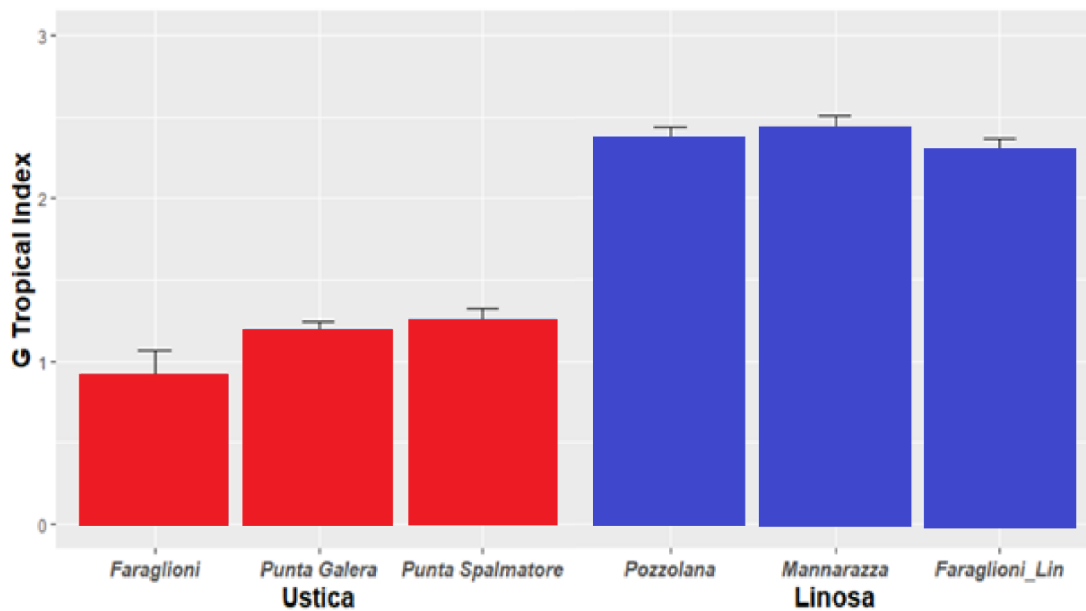
Figure 18- Barplot with error bars for the permanent location in both islands (red for Ustica and blue for Linosa). Tropicalization index is expressed as the average of i^t of each species observed per location in 50x5m strip transect.

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The influence of depth on the *Tropicalization index*, is tested with ANOVA, and the depth

resultes to significantly contribute to its variation (Tab 2.).

Table 2- Table a) showing Bartlett test and b) the ANOVA test with the tropicalization index as response variable and depth as independent variable.

a) **Bartlett test of homogeneity of variance**

Data:	Tropical index x depth
Bartlett's K-squared	2.414
P-values	0.4911

b)

ANOVA	Df	F value	Pr (>F)
Tropi(Depth)	1	43.62	$1.15\ e^{-10}$ ***
Residual	440		

After the ANOVA test, a Tukey's HSD test is performed to evaluate the most influencing depth

range affecting the *Tropicalization index*, giving us the 0-3m as the most significant.

In figure 19 data collected in the six permanent Locations and at different depths are showed

according to the *Tropicalization index*. In Ustica (Fig. 19 a-b-c) the higher *Tropicalization index*

was found in Punta Spalmatore in the range 0-3m (i^T=1.53±0.1)(Fig. 19b), while Faraglioni (Fig.

19c) appears to be the least tropicalized at the same depth (i^T=1.36±0.1). Tropicalization index

was generally higher in Linosa than Ustica (Fig.19 d-e-f), and concerning the different

permanent locations, Mannarazza (Fig. 19d) results to be the most tropicalized Location

(i^T=2.72±0.1) at the 0-3 depth layer.
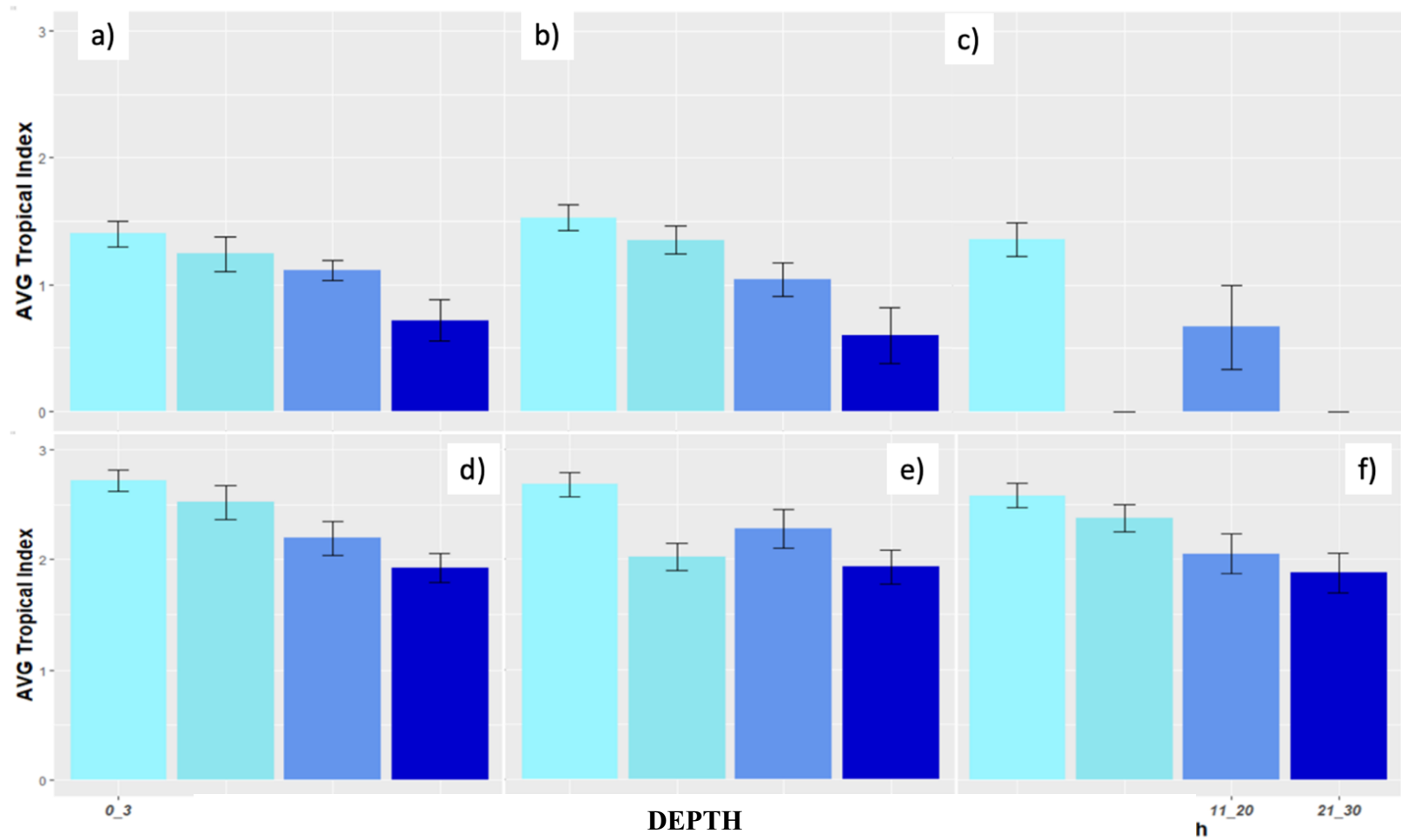
Figure 19- Barplot with error bars for the permanent location in both islands of the average tropicalization index per depth (with color grading darker for deeper ranges): a)P.ta Galera; b)P.ta Spalmatore; c) Faraglioni-Ustica; d) Mannarazza; e) Faraglioni-Linosa; f) Pozzolana.

Discriminant analysis with variable Sites

The two MPAs, Ustica and Linosa, resulted well separated in two cartesian quadrants (Fig. 20a). According to Figure 20b, the species contributing the most to typify the two clusters were *E. marginatus*, *Serranus* sp. and *Coris julis* and *Sarpa salpa*, *Thalassoma pavo*, *Sparisoma cretense*, *Siganus sp* and *Fistularia commensonii*, respectively.
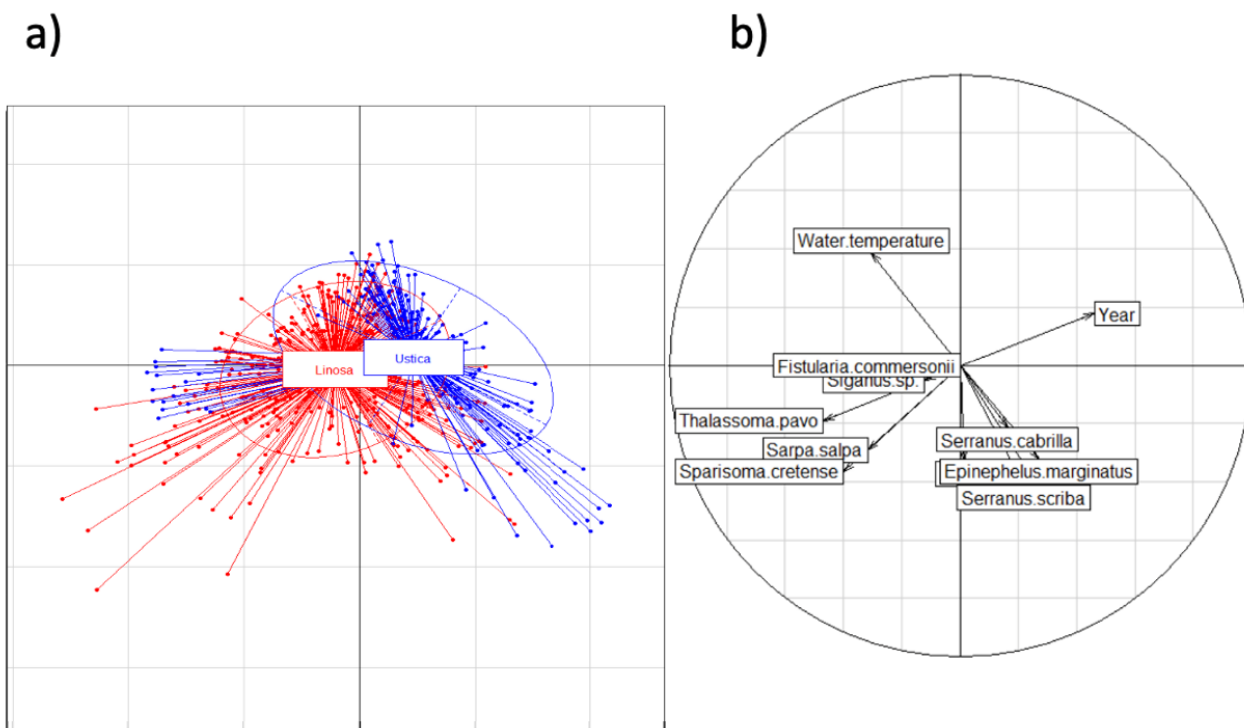
Figure 20- Hill and Smith graphic visualization using sites as discriminant variables: a) Linosa (blu) and Ustica (red) are separated in different quadrants; b) main drivers of the separation of the two main islands.

The analysis of permanent location rather than sites provided similar outputs (Fig. 21).
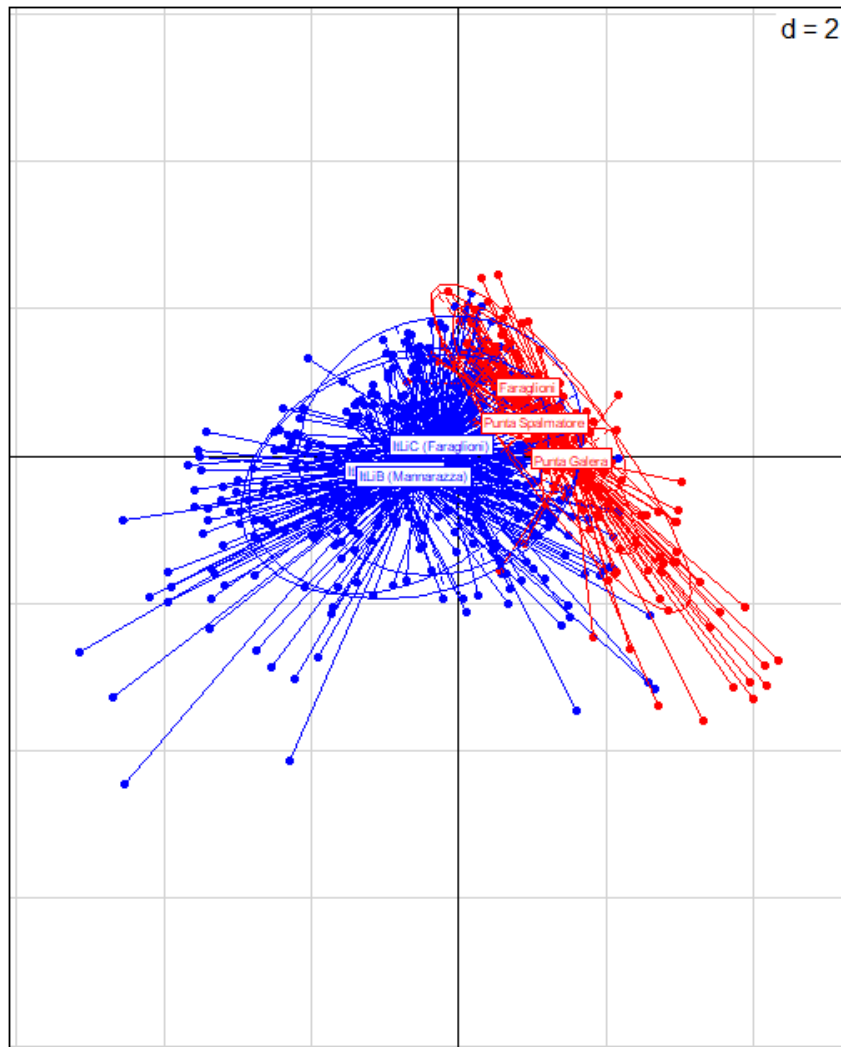
Figure 23- Hill and Smith graphic visualization using Permanent Location as discriminant variables: the ones of Linosa (blu) and the ones of Ustica (red) are separated in different quadrants.

The combination of the two factors (Depth & Sites) allowed to explore site related differences across the different depths (Fig. 22). Observations followed the bisector between IV an II quadrant, exactly the same direction of the vector Water Temperature in figure 22b.
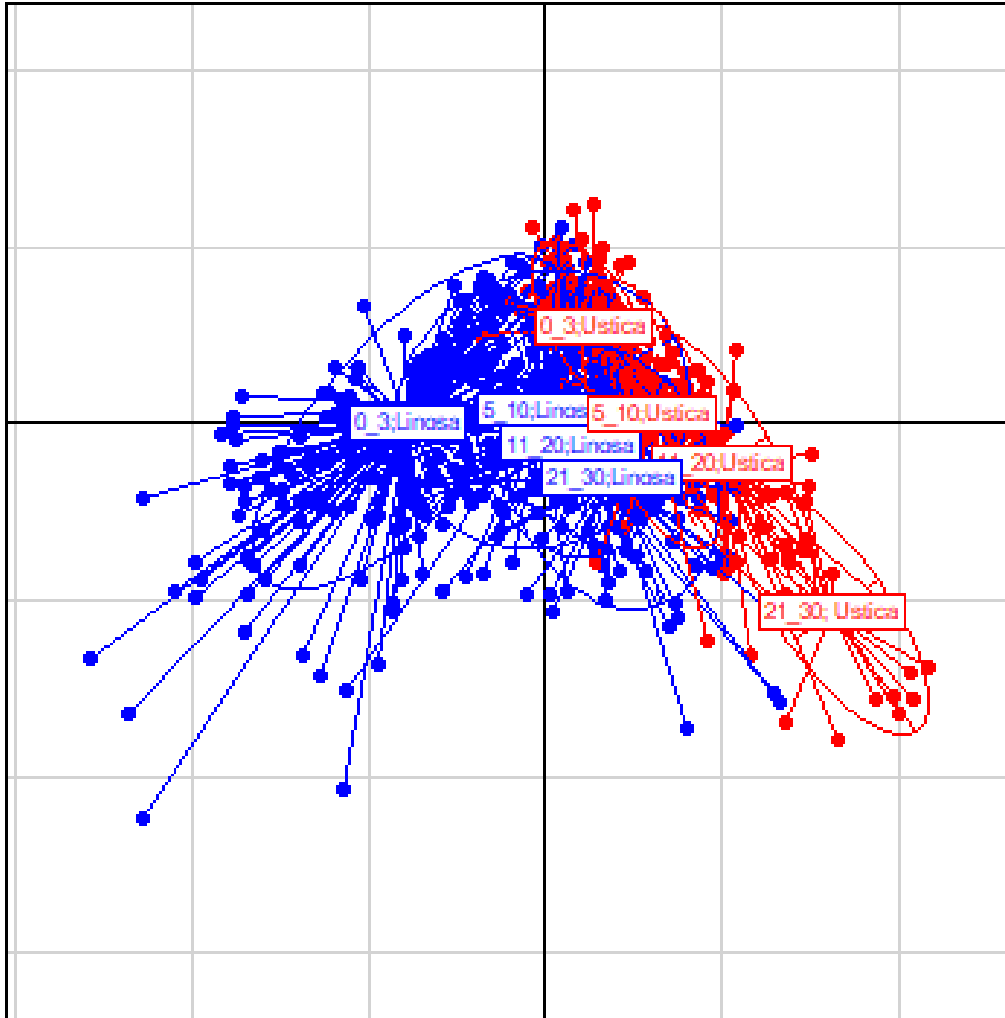
Figure 22– Hill and Smith graphic visualization using depth ranges as discriminant variables in both Islands (blu=Linosa, red=Ustica)

NMDS analysis

Differences between the two MPAs

The nMDS ordination plot shows a clear-cut separation between Ustica and Linosa MPAs (Fig. 23a).

The 2d-bubble plot (fig. 23b) illustrate two superimposed species, *S. salpa* and *S. cretense*, with *S. salpa* dominating in Ustica and *S. cretense* in Linosa.

a)

b)
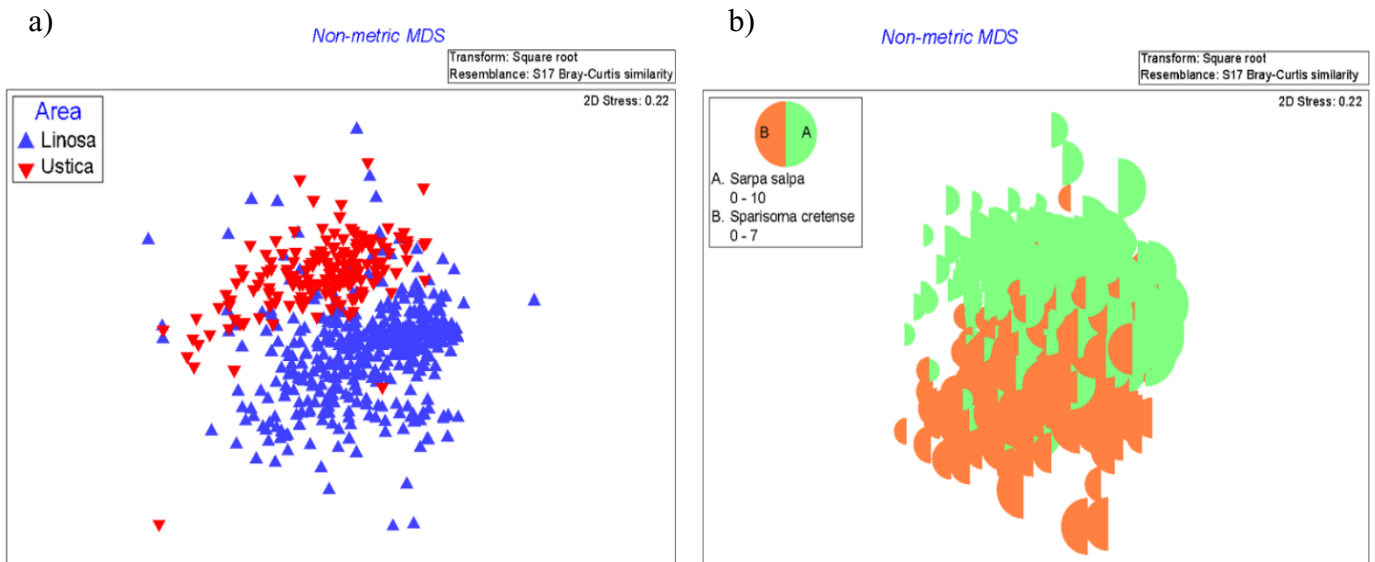
Figure 23- a)Non-metric multi dimensional scaling (nMDS) ordination plot of individual replicates comparing Linosa (blu triangles)and Ustica (red triangles) fish assemblages. b) Same plot with superimposed abundances of Sparisoma cretense *(orange semicircles) and* Sarpa salpa *(green semicircles).s.*

Depth-related differences

In the graph shown in Figure 24 we noticed a clear separation on the nMDS ordination plot
across different depths.

On the right (fig. 24b) we have the 2d-bubble version with two species examined *T. pavo-C.
julis*, in which *T. pavo* dominated in the surface layers and *C. julis* in the deeper layers.
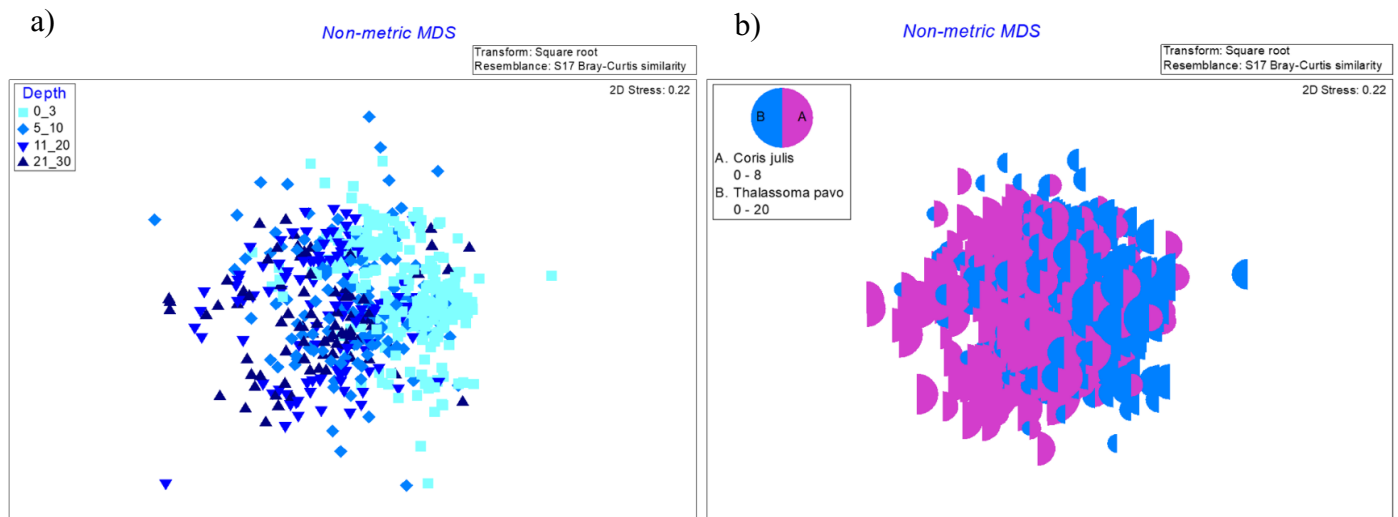
Figure 24- a)Non-metric multi dimensional scaling (nMDS) ordination plot of individual replicates comparing different depth (with color grading darker for deeper ranges) fish assemblages. b) (nMDS) ordination plot of individual replicates comparing Thalassoma pavo (blu semicircles) and Coris julis (violet semicircles) assemblages.

Analysis of similarity

According to SIMPER analysis, three fish species mostly contributed to typify the Ustica Island
(Tab. 3a) (*Sarpa salpa*, *Thalassoma pavo* and *Coris julis)*, while in Linosa (Tab. 3b) the
assemblage was dominated by *Sparisoma cretense*. Dissimilarity between Ustica and Linosa (c)
resulted 51.43% with *S. cretense* giving the highest contribution (12.65%).

Table 3 - Results of SIMPER analysis showing fish taxa contributing the most to similarity in Ustica (a) and in Linosa (b). c) Pairwise between Ustica and Linosa showing dissimilarity

a)

Linosa

Species	Average abundance	Average similarity	Contrib%	Cum.%
Sparisoma cretense	2.89	16.52	28.94	28.94
Thalassoma pavo	3.48	16.31	28.58	57.52
Sarpa salpa	2.99	11.55	20.23	77.76
Average similarity	57.06			

b)

Ustica

Species	Average abundance	Average similarity	Contrib%	Cum.%
Sarpa salpa	3.01	21.77	34.85	34.85
Thalassoma pavo	2.15	15.87	25.4	60.25
Coris julis	1.68	11.68	18.07	78.95
Average similarity	62.46			

c)

Ustica VS Linosa

Species	Average abundance	Average similarity	Av.Diss	Contrib%
Sparisoma cretense	2.89	0.14	12.65	24.6
Sarpa salpa	2.99	3.01	11.14	21.67
Thalassoma pavo	3.48	2.15	10.59	20.59
Coris julis	1.66	1.68	6.62	12.87
Average dissimilarity	51.43			

SIMPER analysis in Ustica correlating all consecutive depths 0_3m-5_10m (Tab. 4a); 5_10m-11_20m (Tab 4b); 11_20m-21_30m (Tab 4c) showed how Average Abundances of *C. julis* increased according to the depth and the opposite behavior of *T. pavo* and *S. scriba*, the most representative in upper layer. In deeper habitats also *E. marginatus* appeared dominant. In all depth ranges, the driving species was *S. salpa*.

Table 3- Results of SIMPER pairwise analysis showing the trend of fish taxa contributing most at different depth layer in Ustica MPA..

a) **Simper pairing per depth 0-3m; 5-10m Ustica**

Species	Average abundance (0-3m)	Average abundance (5-10m)	Av.Diss	Contrib%
Sarpa salpa	3.19	3.41	9.44	27.06
Thalassoma pavo	2.87	1.88	8.19	23.47
Serranus scriba	1.19	1.21	5.98	17.14
Coris julis	1.03	1.69	4.79	13.72
Average dissimilarity	**34.90**			

b) **Simper pairing per depth 5-10m;11-20 Ustica**

Species	Average abundance (5-10m)	Average abundance (11-20m)	Av.Diss	Contrib%
Sarpa salpa	3.41	2.65	12.1	31.52
Thalassoma pavo	1.88	1.47	7.73	20.13
Coris julis	1.69	2.31	5.61	14.6
Epinephelus marginatus	0.5	1.06	5.2	13.53
Average dissimilarity	**38.40**			

c) **Simper pairing per depth 11-20; 21-30m Ustica**

Species	Average abundance (11-20m)	Average abundance (21-30m)	Av.Diss	Contrib%
Sarpa salpa	2.65	2.42	11.25	30.07
Thalassoma pavo	1.47	0.96	6.28	16.78
Epinephelus marginatus	1.06	1.71	6.17	16.49
Coris julis	2.31	3.11	5.93	15.84
Average dissimilarity	**37.42**			

SIMPER analysis in Linosa correlating all consecutive depths 0_3m-5_10m (Tab. 5a); 5_10m-11_20m (Tab 5b); 11_20m-21_30m (Ta b 5c) showds how the species assemblages varied according to depth. The abundance of *C. julis* increased with depth, while *T. pavo* and *S. cretense* had the opposite trend.

Table 4- Results of SIMPER pairwise analysis showing the trend of fish taxa contributing most at different depth layer in Linosa MPA. Showing Average abundances of the driven species and the composition of the assemblage.

a)

Simper pairing per depth 0-3m;5-10m Linosa

Species	Average abundance (0-3m)	Average abundance (5-10m)	Av.Diss	Contrib%
Sarpa salpa	3.98	2.18	11.44	26.3
Thalassoma pavo	5.17	3.12	10.53	24.21
Sparisoma cretense	3.21	2.35	6.86	15.77
Coris julis	0.78	2.07	6.74	15.48
Average dissimilarity	51.43			

b)

Simper pairing per depth 5-10m; 11-20m Linosa

Species	Average abundance (5-10m)	Average abundance (11-20m)	Av.Diss	Contrib%
Sarpa salpa	1.83	2.18	9.78	23.02
Thalassoma pavo	1.78	3.12	9.3	21.89
Sparisoma cretense	3.11	2.35	7.4	17.42
Coris julis	2.2	2.07	6.73	15.83
Average dissimilarity	42.48			

c)

Simper pairing per depth 11-20m; 21-30m Linosa

Species	Average abundance (11-20m)	Average abundance (21-30m)	Av.Diss	Contrib%
Sarpa salpa	2.63	1.83	10.59	25.52
Thalassoma pavo	1.07	1.78	7.24	17.44
Sparisoma cretense	2.63	3.11	6.97	16.78
Coris julis	2.87	2.2	5.9	14.22
Average dissimilarity	41.51			

Permanova analysis

According to the PERMANOVA, abundance varied significantly with Sites (Ustica and Linosa), although a non significant effect is found for the interaction between 'Site' and 'Permanent Location'. A significant effect was found crossing 'depths' with 'P.Location' and 'Sites' (Table 6).

*Table 5-Results of univariate permutational multivariate analysis of variance (PERMANOVA) performed on the bray-Curtis distance of square root-transformed abundance data of Sites, Permanent Location X Sites and Depths X Permanent Location X Sites; *: p < 0.05; **: p < 0.001; ***: p < 0.0001; ns: not significative.*

Source	df	MS	Pseudo-F	P(perm)
Area	1	45596	24.253	0.0002
Location(Area)	4	3360.4	0.4413	0.8258
Depth(Loc.(Area))	17	9766.5	13.78	0.0001
Residuals	563	708.73		

GAMs analysis

Species abundances for each region are obtained by fitting a Generalized Additive Model (GAM) on the total abundance of species (Wood, 2011).

The GAM validity of the assumption was verified by the residuals analyses: the QQ-plot (Fig. 25a) showed the trend of quantiles and as our points remained on the bisector in the first quadrant, we had a normal distribution of our data. Supporting the QQ-plot, also the Histogram of Residuals demonstrated the normal distribution, while the Residuals vs Linear predictors (Fig. 25b) verified the homoscedasticy.

GAM is a model that linearly depends on smooth functions that works on the average of the values providing us the potential response for better fits to data.
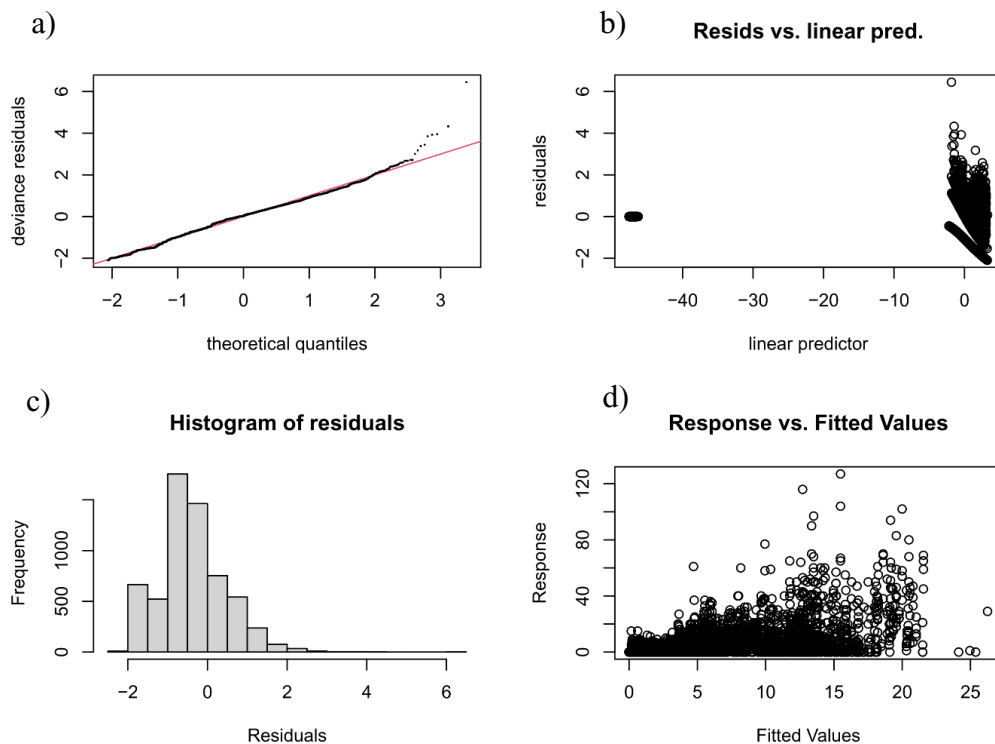
Figure 25- GAM check plots: a)qq-plot: we have the theoretical quantiles (x axes) and the residuals (y axes), as our residuals remains almost on the bisector of this quadrant, we have a normal distribution; b) Linear predictors (x axes) and residuals (y axes) plot, supporting the homoscedasticy; c) histogram of residuals, showing the normal distribution as in a); d) Fitted values (x axes) and Response (y axes)

4. Discussion

Here we applied a simplified monitoring strategy (Garrabou *et al.,* 2019) in two Sicilian MPAs, to explore the abundance pattern of a series of fish species considered as climate change indicators (Moschella, 2008). Ustica and Linosa are both volcanic island characterized by similar habitats (Romagnoli *et al.*, 2020; Sulli *et al.*, 2021) but located at different latitudes and submitted to different climate regimes (Gianguzza *et al.*, 2009). They therefore provide an ideal field of study where to test for climate related differences in the abundances of target species. Our basic expectations were that thermophilic fishes, including tropical invaders, were more abundant at lower (Linosa) than a higer (Ustica) latitudes and more common at shallower depths, as also observed in other Mediterranean locations (Milazzo *et al.*, 2011, 2013). A further aim of this study was to test the possible contribution of *Citizen science* for data collection, being *Citizen science* an increasingly valued strategy to engage volunteers in the process of data collection, such as in biodiversity monitoring (Wiersma, 2010; Alipanah *et al.*, 2020); image classification (Smith *et al.*, 2011; Williams *et al.*, 2015); transcribing old records and annotating images from past biodiversity collections (Elizabeth R., 2015).

According to our analyses on the *Tropicalization index*, Linosa MPA appears to be significantly more tropicalized (average of i^T=2.36) with respect to Ustica (i^T=1.16) and the two islands are clearly separated by different pattern of species abundance, as illustrated by nMDS plots. This result was somehow expected, because the Tropicalization phenomenon occur along with a south-north and east-west gradient. Moreover, the Strait of Sicily acts as a biogeographical boundary, separating western and eastern Mediterranean sub-regions (Di Lorenzo *et al.*,2018) and thus, significant changes are expected when comparing biotic assemblages sampled in and out of this sector. The Strait of Sicily is crossed by the February 15°C , which is is recognized as a sort of

''physiological'' barrier to the westward expansion of warm-affinity species occurring in the Eastern Mediterranean basin (Bianchi, 2007), with a clear change in species composition between the west and east sectors (Bianchi *et al.*, 2018). In the last twenty years, an increasing number of tropical species has been reported from the Sicily straits, particularly in the Pelagie Island MPA (Galil, 2008; Moschella, 2008) and Malta (Azzurro *et al.,*2013) as a consequence of the progressive and westward expansion of tropical NIS from the Levantine sectors (Di Lorenzo *et al.,* 2018). Therefore, the observed differences in terms of target species abundances and *Tropicalization index*, in Linosa and Ustica, well illustrate the current tropicalization level of two MPAs characterized by a similar geological structure but different biogeographical sectors and climate regimes.

Our set of target species includes some native thermophilic species, such as *Thalassoma pavo, Epinephelus margìnatus, Sparisoma cretense, Sphyraena viridensis,* which are moving northwards eith respect to theyr current distribution and especially along the North-western sectors of the Mediterranean (Moschella, 2008). This phenomenon, indicated under the term of "meridionalization" (Bianchi, 2007) (Feliatra and Bianchi, 1993) is generally explained as one of the most visible consequences of Climate change in the Mediterranean area. Warming climates are also contributing to the increasing abundance and success of thermophilic Non Indigenous Species (NIS). This phenomenon, often indicated as "tropicalization" of the Mediterranean Sea (Bianchi and Morri, 2000; Bianchi, 2007), is associated to a variety of ecological and socio-economic impacts.   Tropical NIS are mostly represented by Red Sea species entered in the Mediterranean through the Suez Channel (lessepsian migrants).

Data on the abundance of our climate change indicators have been also employed to explore the influence of depth, being this axis the main gradient along which faunal changes occurs in

marine ecosystems. Similar to what happen in terrestrial systems, where under warmer conditions and greater forest losses (e.g., in the tropics or lowland areas), species tend to shift more rapidly towards higher elevations (Guo, Lenoir and Bonebrake, 2018), warming temperatures are pushing marine species to deeper waters in search of thermal refugia. Our results clearly highlight vertical pattern in warm affinity species such as *T. pavo* and *S. cretense* further changes are expected in locations where the density of *T. pavo* has recently increased (Milazzo *et al.*, 2011) according to the ongoing trends of meridionalization (Bianchi, 2007). Therefore, interannual observations at permanent monitoring locations may be useful to periodically track these changes. This would also increase our understanding about the ecological consequences of climate change as, even if species composition is not altered, the strength of interspecific interactions might change (Harley and Rogers-Bennett, 2004).

Increasing water temperatures are expected to influence patterns of species richness, composition, densities and distribution of aquatic organisms (Eady *et al.*, 2013). Here, the *Tropical index* i^T revealed a vertical gradient in the abundance and distribution of climate change indiocators, with decreasing i^T at higher depths. The discriminant analysis combining depth and sites (Hill Smith) graphically demonstrates that depths were moving in the same direction as the water temperature vector, empathizing the strict correlation between these two explanatory variables. PERMANOVA analyses also confirmed the significant influence of depth (Fitzpatrick *et al.*, 2012) in some of our targets.

One limit of this study is related to the fact that the effect of the MPA protection were not taken into account in our analyses. As a matter of facts, the experimental design was not appropriate to consider the factor 'protection level' in both Islands. Nevertheless, we might consider that fishing

activities are all banned in all the surveyed locations, therefore potential differences due to a different fishing pressure can be considered as negligible.

In the next chapters we discuss aspects related to the single species, considering their thermal affinity and recent knowledge of their pattern of distribution and abundance.

The Mediterranean parrotfish - *Sparisoma cretense*

The Mediterranean parrotfish *Sparisoma cretense* is widely distributed along the eastern Atlantic coasts (Portugal, Azores, Madeira, Canary Islands and Senegal), as well as throughout southern Mediterranean coasts; in Italy, the species historically occurs in Sicily and southern islands present in all western seas, from the Ligurian to the Ionian Sea, for some years also present in the southern and central Adriatic with sub-tropical affinity (Froese, 2021).

Results from this study showed that *S. cretense* is a dominant species in Linosa (average sightings 9.52 ind/250m^2) but also occurs in Ustica (0.13 ind/250m^2), especially in the locations of P.ta Spalmatore and P.ta Galera. In Ustica, the presence of *S. cretense* resulted mostly restricted to the first bathymetric range (0-3m), while in Linosa we already have a widespread presence also at 30m depth. Recently in Italy, the Mediterranean parrotfish has been observed northward with respect to its known distribution limits. Indeed, in the last two decades, it has been reported from Giglio Island (Tuscan Archipelago, Italy;(Bianchi C.N., Morri C., 1998)), were it is currently well established (Ventura *et al.*, 2019). The establishment of a new population of this warm-water species in the Giglio Island confirms its northward expansion, as also suggested after the occasional observations of this species in the Northwestern sectors of the basin (Morri *et al.*, 2017). In fact, the expansion of the Mediterranean parrotfish was also documented by records of the species in other sectors of the North Mediterranean, such as the North Aegean Sea (Yapıcı, 2016),

North Ligurian Sea (Astruch *et al.*, 2016), Eastern Ionian Sea (Nicolaidou *et al.*, 2012) and Eastern Adriatic (Dulčić and Pallaoro, 2004).

The Mediterranean parrotfish is taking advantage of warmer waters, and hence improved thermal habitat conditions supporting key life processes, such as growth, spawning and recruitment (Marras *et al.*, 2015). Therefore, in a warming scenario, we expected *S. cretense* to become more abundant in Ustica and increasing its presence along deeper layer, as already occur in Linosa MPA.

Ornate wrasse *-Thalassoma pavo*

Ornate Ornate wrasse -*Thalassoma pavo* is largely distribuited in Mediterranean, especially in the southern part, rare in the Ligurian Sea and in the northern Adriatic, common in the central and southern Tyrrhenian Sea Atlantic ocean (Froese, 2021).

The ornate wrasse has its optimal distribution in the Canary Islands (Falcón *et al.*, 1996) and also occur with high abundances in the Azorean and Madeira islands (Ribeiro *et al.*, 2005) and south to the Canaries; it is also abundant in the Cape Verde islands (J. M. Falcon unpublished data)

In this study *T. pavo* resulted abundant in both islands (Ustica 5.8 ind/250m^2, Linosa 16.2 ind/250m^2) and almost equally distributed in each permanent locations. As expected, we found greater abundances of *T. pavo* in Linosa. Considering the depth related differences, the Ornate wrasse dominates shallower waters in both Islands and gradually decreases at greater depths. Also the nMDS showed the depth variability of the assemblages of *T. pavo* and *C. julis*, two competing wrasses species which occupy two different bathymetric ranges.

In Italian waters, *T. pavo* is more common along the southern coasts of the basin, where SST varies between 14°C in winter and 29 °C in summer. However, *T. pavo* has progressively expanded in the northernmost sectors (Milazzo *et al.*, 2011). In the recent years, it has progressively

expanded northwards, an event which was related to the ongoing water warming of the NW Mediterranean (Guidetti and Ambrosio, 2004). According to these recent evidences, *T. pavo* received an increasing attention due to its potential role as a climate change indicator, and so it was included in the protocol. Our analysis of the vertical distribution patterns confirms an intrinsic between-region variability in the overall density, likely reflecting the subtropical affinity of *T. pavo* as illustrated by Milazzo (Milazzo *et al.*, 2011).

These potential between-species differences in temperature preference are confirmed partially by the northward expansion of the southern species *T. pavo*, as a consequence of water warming in the Mediterranean Sea (Astraldi *et al.*, 1995).

The rainbow wrasse -***Coris julis***

The rainbow wrasse is a species with boreo-Atlantic origin, its geographic distribution in the Mediterranean Sea is reported as fairly evenly distributed in the basin, without any evident latitudinal gradient (Guidetti and Ambrosio, 2004).

This species is ecologically similar to the 'warm-adapted *Thalassoma pavo* and the fact that these two species have the same size, habitat preferences and feeding strategy (Marion and Uhl-Bien, 2007) means that they may potentially overlap in habitat and trophic requirements suggesting possible competition for resources (e.g., space, food). These two native wrasses give us a unique opportunity to explore distribution patterns of two competing and ecologically similar species. The current abundance and distribution of *C. julis* in Ustica and Linosa along the different depth layers, is expected to be influenced by habitat shifts due to the increasing relative dominance of the competing *T. pavo*. The degree of trophic specialization of *C. julis* is therefore an important adaptation to reduce habitat overlaps, related ecological costs and probably increases the

possibilities of coexistence of these two wrasses under a warming conditions (Milazzo *et al.*, 2013). Our experiments demonstrate the importance of latitudinal and depth related factors, supporting the hypothesis of a rapid change in the abundance patterns of *C. julis* in relation to the increasing abundances of *T. pavo*, as highlighted by (Milazzo *et al.*, 2013);

We documented the occurrence of *C. julis* in both islands (average at 0-3m= Ustica 3.9 ind/250m^2, Linosa 4.3 ind/250m^2). These values of abundance linearly increase as increasing s depths. These results are supported by studies by (Milazzo *et al.*, 2016), in which they present the shifts in distribution of these species suggesting that in a warming scenario the W-Mediterranean will become more suitable for *T. pavo* whilst large sectors of the E-Mediterranean will be unsuitable for *C. julis*, progressively restricting its distribution range. *Posidonia oceanica* meadows might be regarded as a suboptimal habitat, underused by *T. pavo* and representing a 'refuge' for *C. julis* where this species can avoid potential detrimental inter-specific effects when its normally preferred habitat is occupied by the dominant antagonist *T. pavo*. Similarly, a marked vertical shift in sloping algal seabeds was recently observed, with *C. julis* expanding to deeper and cooler environments when the relative dominance of *T. pavo* was high in the warmer and shallow water algal habitat, and the two species coexisting in slowly sloping algal seabeds (Milazzo *et al.*, 2011). These shifts might result in fish communities' re-arrangement and novel functional responses throughout the entire coastal food-web.

Salema - *Sarpa salpa*

The Salema, *S. salpa* is a strictly coastal species, widely distributed throughout the Mediterranean basin and in the eastern Atlantic Ocean from the Bay of Biscay to Sierra Leone and the Canary Islands, Madeira and Cape Verde and from Congo to South Africa (Froese, 2021).

This species is widespread along the Mediterranean coasts (Verlaque, 1990), but in the easternmost sectors of the Mediterranean, is currently rare or absent, due to the combination of unfavorable climate conditions (Marras *et al.*, 2015) and competition with invasive rabbitfishes (Coll *et al.*, 2010). A competitive superiority of invasive rabbitfishes (*Siganus luridus* and *S. rivulatus*) vs. salema has been hypothesized by different authors (Barichea, 2004), especially in the warmest sector of the Mediterranean, which are suitable for tropical rabbitfishes but already unsuitable for the temperate *S. salpa* (Marras *et al.*, 2015). In Ustica, the pattern of distribution of *S. salpa* showed its abundance (11.8 ind/250m^2) at almost every bathymetric range, same for Linosa (13.7 ind/250m^2). Nonetheless, in a future perspective, this native species is expected to follow a declining trend according to climate change scenarios and to the rapid expansion of invasive rabbitfishes, their tropical antagonists (Marras *et al.*, 2015). It is important to recall that invasive rabbitfishes (*S. luridus* and *S. rivulatus* are capable of grazing the same algal resources consumed by *S. salpa*, and cause large-scale impact on natural habitats and local food chains (Vergés *et al.*, 2014).

The dusky spinefoot - *Siganus luridus*

The dusky spinefoot *Siganus luridus*, is an invasive herbivorous species, naturally distributed along the western Indian Ocean coasts, including the Red Sea and the Persian Gulf. Following the Lessepsian migration, the species colonized the eastern part of the Mediterranean Sea with high abundances. It is currently very common along the coasts of Crete and continental from Cyrenaica to the island of Rhodes. Rarer on the coasts of Tunisia and mainland Greece. The native *salema* is very common and widespread along the Mediterranean coasts (Verlaque, 1990), but in the easternmost sectors of the Mediterranean, this species has probably been outcompeted by the

marbled spinefoot (Barichea, 2004; Coll *et al.*, 2010) and a competitive superiority of marbled spinefoot vs. salema has been hypothesized (Barichea, 2004). Furthermore, other siganid species (e.g. *Siganus luridus*) have recently extended their distribution, colonizing the islands of the Sicilian Channel (Castriota and Andaloro, 2008). In general, warming conditions are believed to be facilitating the arrival and spread of tropical invaders at an unprecedented rate (Hiddink *et al.*, 2012). Marras et al. (2015) demonstrated that a large difference in the optimal temperature for aerobic scope between the salema (21.8°C) and the marbled spinefoot (29.1°C), highlighting the importance of temperature in determining the energy availability and, potentially, the distribution patterns of the two species; he predict that by 2050, the thermal habitat suitability (THS, an index based on the relationship between MS and temperature) of the marbled spinefoot is predicted to increase throughout the whole Mediterranean Sea, causing its westward expansion.

Siganus sp. is in direct competition for algal resources with its native counterpart: the salema, and they are among the few herbivorous fishes occurring in the Mediterranean (Azzurro *et al.*, 2007). Expected changes in the relative dominance of these two herbivorous species might have important ecological consequences in the near future. However, from our data in Ustica, this species has not been detected yet, indicating still a low tropicalization for the area, while in Linosa we already have individuals (0.3 ind/250m^2).

In the Central sectors of the Mediterranean, the species is currently expanding its distribution and abundance. At the moment, the average abundance of the dusky spinefoot is still far lower than that of the coexisting native herbivore fish (*S. salpa* and *S. cretense*) and generally less than that in the invaded sectors of the Eastern Mediterranean (Bodilis *et al.*, 2014).

Nevertheless, although the population of *S. luridus* in Linosa has not yet assumed the character of a 'true' invasion, the recent increase in its abundance should be considered seriously and taken as a further step towards the tropicalization of the Mediterranean Sea (Azzurro *et al.*, 2017).

Last report in Capo Milazzo, Sicily (Facebook: Carmelo Isgrò, 2021) and further expansion of this species is expected in the near future according to the prediction of a general rise in average temperature up to 1°C with an aver- age increase of around 0.6°C was found between the pres ent-day projection and the future scenario(Marras *et al.*, 2015)

Brown grouper -*Epinephelus marginatus*

The brown grouper, is one of the seven species of grouper recorded in the Mediterranean Sea. The geographical range of the dusky grouper includes the Mediterranean, the Eastern Atlantic from the Southern coasts of the British Isles to South Africa, and part of the coasts of Brazil (Froese, 2021). This native species has extended its distribution margins, in both Mediterranean and extra-Mediterranean areas due to climate changes (Moschella, 2008). The presence of this rocky-associated grouper is very low in areas such as Linosa (0.4 ind/250m^2) while in Ustica we have individuals (1.3 ind/250m^2) at each bathymetric range, increasing with depth.

Painted comber - *Serranus scriba* & Comber - *Serranus cabrilla*

Serranus scriba known as Painted comber it is distributed in Eastern Atlantic from the Bay of Biscay to Mauritania including the Canaries and the Azores. Also from the Mediterranean and the Black Sea (Froese, 2021).

Serranus cabrilla (Comber) widespread along the coastal strip of the eastern Atlantic Ocean, from the English Channel to the Cape of Good Hope, including the Azores, Madeira and Canary Islands.

It is very common throughout the Mediterranean, especially in the Tyrrhenian Sea, it is also present in the Black Sea. It is not certain whether it is also present in the Red Sea due to immigration from the Mediterranean (Froese, 2021).

These two species are common and ecologically similar.

Painted comber is usually distributed in a higher bathymetry according to its sub-tropical affinity (Froese, 2021) while Comber is related to deeper and cooler layer.

Unlike *T.pavo* and *C.julis* , our analyses did not resulted in any clear pattern according to both spatial and depth related factors, but the future implementation of the UVC protocol is expected to reveal hidden pattern, as expected by the different thermal affinity of these two ecologically similar species (Froese, 2021).

Bluespotted cornetfish *- Fistularia commersonii*

The bluespotted cornetfish is a highly invasive species detected first time in 2000 in the Mediterranean Sea. Areas environmentally suitable for this species were identified along the Levantine Sea, Sicily Strait and Tyrrhenian Sea

Concerning the MPA-Engage project in Pelagie Archipelagos, we recorded a single observation (one individual) of *F. commersonii* in Linosa, at Faraglioni permanent location, in the bathymetric range of 11-20m. No individuals of this tropical invaders were recorded in Ustica, despite the species has been already reported allover from the Mediterranean sea (Azzurro *et al.*, 2013).

Based on the data collected in the present study, we could affirm that in both Linosa and Ustica, the species, if present with a permanent population, is still very rare and so at the early phases of the invasion process. Considering its carnivorous habit, a possible outbreak of this population

could deeply change fish communities if the ecological integration of invaders takes place (Azzurro *et al.*, 2013).

The contribution of trained volunteers

An important merit of this study was to engage a number of volunteers in the process of data collection. The engagement process was structured with the help of *Mare Nostrum Diving,* a local diving center operating in the island of Linosa and Ustica. The training process started in each sampling location, when the dive master or the instructor introduced the project and divers were engaged in the Citizen Science activity.

Citizen science is today recognized as in important contribution for ecological and conservation studies in both Terrestrial and aquatic systems (Mori *et al.*, 2017). In many places worldwide, resource managers have taken advantage of volunteer networks for ornithology studies, reef ecology, stream and water quality monitoring (Bray and Schramm, 2001; Fore *et al.*, 2001).

The scientific community, however, seems reluctant to accept citizen science due to a current lack of certified audits to assess the validity of using such data in academic research and resource management decisions. Quality assurance and validation of these initiatives, through compliance to rigorous scientific methodology, is necessary for the scientific community to accept and utilize the data collected by citizen scientists (Boudreau and Yan, 2004). Acceptance by the scientific community would also allow additional personnel to participate in bio-monitoring, and thereby increase our biological understanding of these species by creating large standardized spatial and temporal datasets (Balton *et al.*, 2006). Adequately describing measures taken to assure quality, such as thorough data verification or volunteer training, is therefore critical for addressing the credibility challenge (Freitag *et al.*, 2016) that citizen science studies face.

Validated citizen science data have not only contributed to reductions in costs associated with research endeavors, but have also been quite useful in previous environmental assessments (Snäll *et al.*, 2011).

For example, a statewide citizen science ornithological study provided more than 200,000 h of data collection that is valued at over a million dollars, even based on minimum wage (Bonney *et al.*, 2009).

It is clear that scuba divers have a positive inclination towards Marine Citizen Science (MCS). However, retention of current volunteers and engagement of future participants in MCS may be made difficult by a lack of new stimuli in the case of the former group, and an overall lack of knowledge of CS in the case of the latter group. Evidently, the scuba diving industry, particularly dive centers, play a critical role in assisting CS initiatives, by making MCS publicly known. However, a proper integration of MCS into the scuba diving industry would necessitate the effort of professional intermediaries and hired experts working alongside the scientific community and the diving community (Lucrezi *et al.*, 2018).

For our study, we trained volunteers with the MPA-Engage protocol through a PADI/DAN courses on fish identification and transect technique. Data taken by citizen science has been evaluated at different levels. First by a self-evaluation of the skills, then by a team of expert scientists, and finally by the hill-smith discriminant analyses. This latter analysis showed that information collected by observers with "Medium, Low, Good" auto-evaluated skills in fish recognition were significant skewed with respect to the reference data provided by expert observers. However, trained volunteers who self-evaluated themselves with "Very Good", skills were useful for our data collection: these levels were in the exact center of the axis, with no significant differences with the data recorded by "Expert" observers, such as professional researchers and skilled

University students. This also apply to the category "Assisted", in which an expert observer supervised the process of data collection made by another diver during the entire transect.

These results support Dickinson et al. (2010) whose work presented the fact that opportunely trained citizen can be pivotal in collecting huge amount of data. In our case in particular, the help of trained non-scientists people not only support the monitoring in the MPA, but also help raise awareness on important environmental problems such as climate change and shifts in species composition.

5. Final remarks

The Mediterranean marine biota is dramatically changing under the combined influence of anthropogenic drivers and warming climates (Bianchi and Morri, 2000). In this situation, Marine Protected areas are considered today as the best sites were to observe and monitor these changes (Garrabou *et al.*, 2019). Moreover these areas of high conservation value offer nature-based solutions for repairing environmental damages and sustaining the wide array of services provided by marine ecosystems (MedPAN and SPA/RAC, 2019). Well-managed MPAs effectively protect natural habitats and species from multiple local stressors (Sala *et al.*, 2018)

However, seen the rate of threats (overfishing, destructive forms of fishing, pollution, and coastal development) and now also the climate-change impacts that can adversely affect marine environments (McLeod *et al.*, 2009), there is a pressing need to provide resource managers with approaches and information that can help to conceive appropriate strategies for mitigating related impacts (Fulton *et al.*, 2015).

In this context "MPA-ENGAGE", led by the Institute of Marine Sciences of the Spanish National Research Council and funded by the Interreg MED program, is supporting Mediterranean MPAs to adapt to and mitigate the ongoing climate change effects in the Mediterranean Sea. The acquisition of abundance data through the method presented in this study and the continuous updating of the dataset provides a baseline where to test the expected information on the expansion of invasive species, contributing to the assessment of vulnerability of other species and providing useful baseline for the adaptive management of these phenomena (Cramer *et al.*, 2018).

Importance of the close connection between temperature and depth in the study of variations in the distribution of species (Milazzo *et al.*, 2016)lead our future studies to the use of materials and methods capable of storing such a quantity of data, over large temporal spaces and in different

areas at the same time, to allow a comparison of all Mediterranean MPAs in order to acquire a global map of thermophilic species and better understand Mediterranean Sea trend of changing.

Due to the short time laps, the results obtained from our visual census did not provide historical information, nor time series. Nevertheless, using a limited number of target species we were able to define clear spatial patterns of the distribution and abundance of climate change indicators, indicating expected trajectories of change in the MPA of Ustica.

Moreover, we had the chance to include participatory actions such as citizen science, contributing to defining a collaborative and site-specific adaptive management plans within the marine protected areas of the Mediterranean included in the MPA-Engage project. In this way it could be possible to create a capillary network that allows greater communication and participation between all the MPAs of the Mediterranean.

The method made possible to collect a substantial amount of data in a relatively short time and with significantly reduced costs, as well as having statistically valid data that supported the effectiveness of the study. This type of monitoring also allows to increase climate awareness and ocean literacy in a number recreational divers. A continuous and well-structured collaboration with the diving industry could in the near future represent a support to monitoring activities in Mediterranean MPAs with a 'win-win' situation.

Future developments of the project could lead to engage other Mediterranean MPAs in the process of data collection to closely track the change of coastal fish assemblages at the regional scale, offering a common tool to monitor the ongoing tropicalization of our Mediterranean Sea.

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7. Appendix

Target Species (Froese, 2021)

The target teleost species of the MPA-ENGAGE project were selected for monitoring in MPAs due to their sensitivity to water temperature changes. Due to their thermal preferences, it is expected that some of these species may be favored (eg Thalassoma pavo, Siganus luridus) and others disadvantaged (eg Coris julis, Serranus cabrilla) by the ongoing warming with consequent variations in abundance and distribution.

Brown grouper - Epinephelus marginatus (Lowe, 1834)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Serranidae

Genus: Epinephelus

Species: Epinephelus marginatus

Figure 26 -Epinephelus marginatus. Foto credit Bruno Pitruzzela

The brown grouper (Fig. 26) is a large sedentary fish, which shows a robust body, with a voluminous head and above all with a very wide mouth. It can reach large sizes and have a very long life, even reaching 50 years.

The most common sizes are between half a meter and one meter in length, with weights ranging between 30 and 50 kilograms. The color is brown on the back and yellow or orange on the belly, with typical light spots around the eye. On the back it has yellowish-green spots arranged variably on the sides and on the head which vary according to age and reproductive period, it tends to have a darker livery in the older specimens. The young groupers have a much more varied pint livery than the adult specimens and an extremely curious attitude.

Ecology: Solitary, territorial, rather shy, although it has been shown that in areas where it is not hunted, it does not show excessive distrust of humans and is easily approached by divers. It prefers rocky and sandy bottoms and Posidonia, rich in ravines and caves at a depth between 8 and 100

m. It is a slightly euryhaline subtropical species, but it can penetrate lagoon or lake environments with a strong marine influence. The younger specimens live near the coast and at shallower depths. It has sedentary habits, so much so that it has frequented the same area and the same den for many years.

Reproduction: It has a protogynous hermaphroditic sexual development; the specimens born as a female, reach sexual maturity at the 5-9th year (about 40 cm in length), and later a part of them becomes male at about 15 years (about 80 cm in length). Feeding: It feeds mainly on octopuses, mollusks, crustaceans, and other fish.

Distribution: It commonly lives in the Mediterranean Sea and is however also encountered in the eastern Atlantic Ocean and the western Indian Ocean, in the British Isles, and up to Mozambique and Madagascar. In the western Atlantic along the southern coasts of Brazil. In the Mediterranean, the Brown Grouper is found in optimal conditions only within the protected marine areas (Fig. 27).
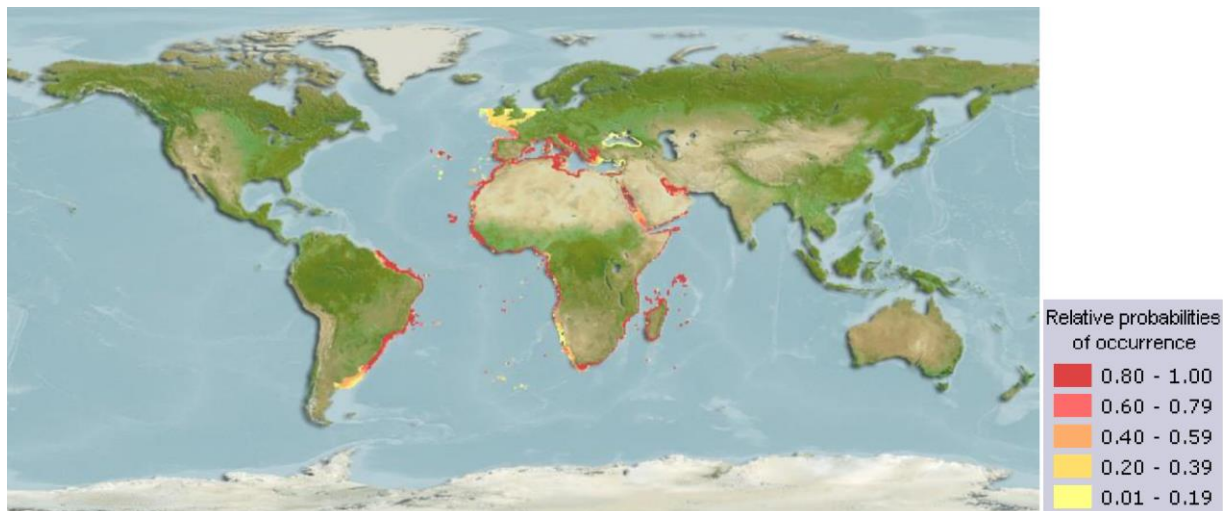

Figure 27- Current distribution of brown grouper - (Source: www.fishbase.org)

Mediterranean rainbow wrasse - Coris julis (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Labridae

Genus: Coris

Species: Coris julis

Figure 28- Ph. Giovan Ombrello - Mediterranean rainbow wrasse (on the right male, on the left female)

Mediterranean rainbow wrasse (Fig. 28) is easily recognizable by its tapered body shape, long pointed muzzle, and small mouth. The livery shows a marked sexual dimorphism. The belly is white and above, the female has a dark red color while the male has bands of green, gray, and orange with different reflections that make them very colorful fish. The dimensions commonly vary between 10 and 20 cm (max 30 cm) in adults.

Ecology: It inhabits rocky bottoms or Posidonia, it is a strictly coastal fish and its bathymetric diffusion ranges from a few centimeters to about 60 meters deep. It inhabits coastal waters near rocks and harbors even in very shallow waters. Usually solitary, sometimes in small groups.

Feeding: It feeds on algae, small molluscs, and crustaceans.

Reproduction: The damsel is a protogynous hermaphrodite species, with distinct sexual dichromatism: individuals (sexually active at 1 year) are born female and grow up to be male. Studies have shown that all individuals over 18 cm are male. The sex change takes about 5 months. The eggs are pelagic and transparent yellow, they are laid between April and August.

Distribution: The genus is represented in the Mediterranean with only one species. This species is widespread in the Mediterranean Sea and the eastern Atlantic Ocean from the English Channel to central Africa (Fig. 29)
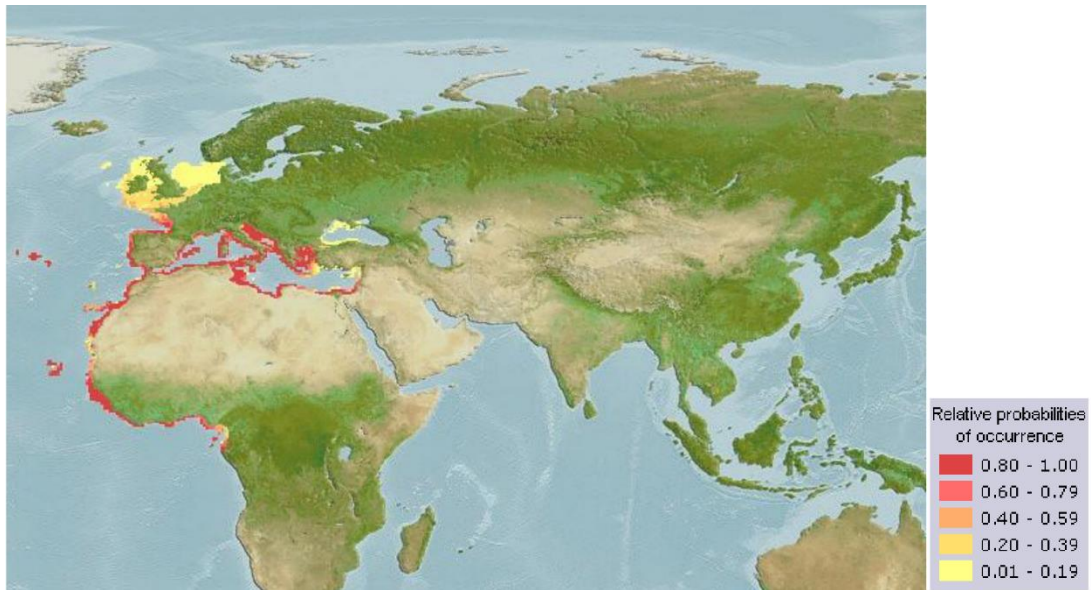

Figure 31- Current distribution of Coris julis - (Source: www.fishbase.org)

Ornate wrasse - Thalassoma pavo (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Labridae

 Genus: Thalassoma

Species: Thalassoma pavo

Figure 30- Ph. Giovan Ombrello - Ornate wrasse (on the left male specimen, on the right female specimen)

Ornate wrasse (Fig. 30) has a fusiform body, compressed laterally. Tapered body, golden yellow, tending to green or orange, with typical reticular designs on the blue head, for females 5 vertical stripes of the same color, a black spot between the second and third band. As an adult, it changes color until it completely loses the blue bands and black spot.

 Ecology: It is a coastal species that inhabits shallow and well-lit rocky bottoms, up to 20 meters, even if it goes up to 100. Curiously, it is easily attracted by moving sediment from the bottom. The female specimens often move in large groups.

Feeding: Carnivores like the other wrasses, feeds on small mollusks and crustaceans

Reproduction: Protogynous hermaphrodite, born female and after a few years becomes male. Breeding takes place from June to July. The eggs are pelagic.

Distribution: Present in the Mediterranean, especially in the southern part, rare in the Ligurian Sea and in the northern Adriatic, common in the central and southern Tyrrhenian Sea. In recent

years there has been an expansion towards the north of the range of this species, probably

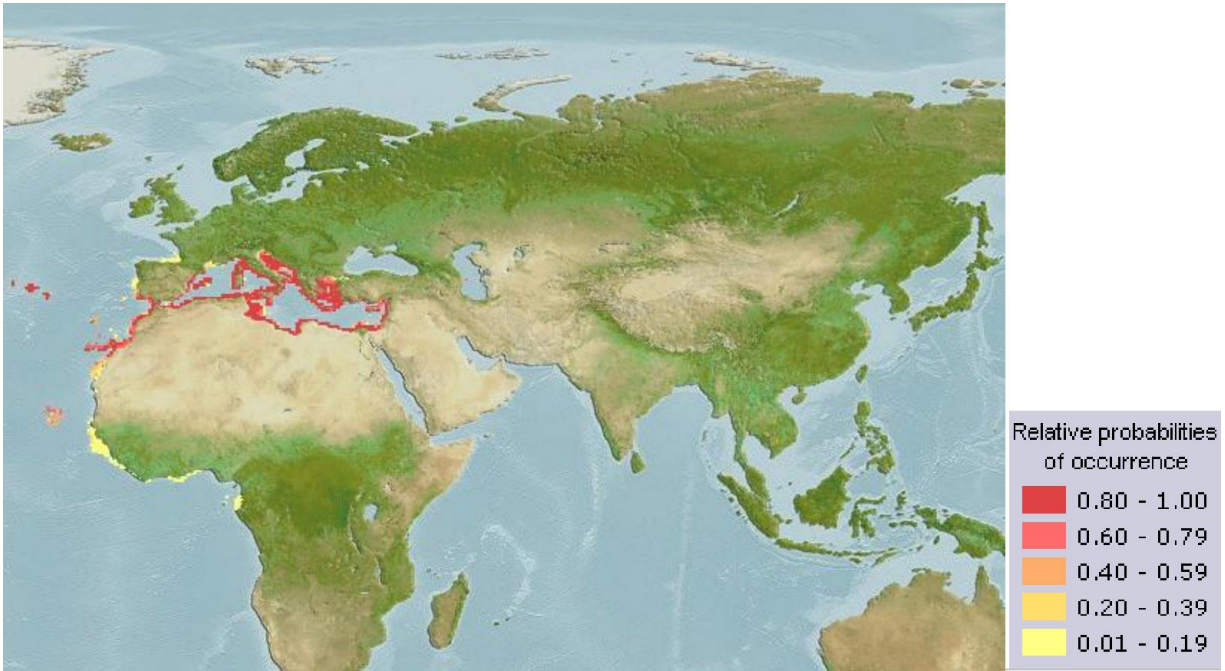following the southernization of the Mediterranean Sea (Fig. 31)

Figure 33- Current distribution of Coris julis - (Source: www.fishbase.org)

Salema - Sarpa salpa (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Sparidae

Genus: Sarpa

Species: Sarpa salpa

Figure 32- Ph Giovan Ombrello , Salema

Salema (Fig. 32), with an oval and oblong body, laterally compressed with a slightly less curved dorsal profile than the ventral one. Large head with a rounded snout. The livery tends to take on a silver-gray color on the back and sides, whitish on the belly. Presence of 10-12 golden stripes on the sides that run from the caudal peduncle to the head. Presence of a small black spot at the base of the pectoral fins. Maximum length: 50 cm - on average 30-35 cm. Maximum weight: 3 kg.

Ecology: gregarious marine species. It is a strictly coastal species living on rocky bottoms with the growth of aquatic plants and *Posidonia oceanica* meadows. Generally between 1 and 15 meters deep, however, being able to reach bathymetric depths close to 70 meters, it normally does not exceed 20 meters and is also found in very shallow waters. In lagoons and estuaries, it is present only in waters with a strong marine influence.

Feeding: It is a mainly herbivorous fish. When young it feeds mainly on annelids, crustaceans, and other invertebrates.

Reproduction: Protandric hermaphrodite, the salpa is born male and then becomes female during growth. Reproductive period September-October.

Distribution: It is a strictly coastal species, present throughout the Mediterranean basin and in the eastern Atlantic Ocean from the Bay of Biscay to Sierra Leone and the Canary Islands, Madeira and Cape Verde, and from Congo to South Africa (Fig. 33).
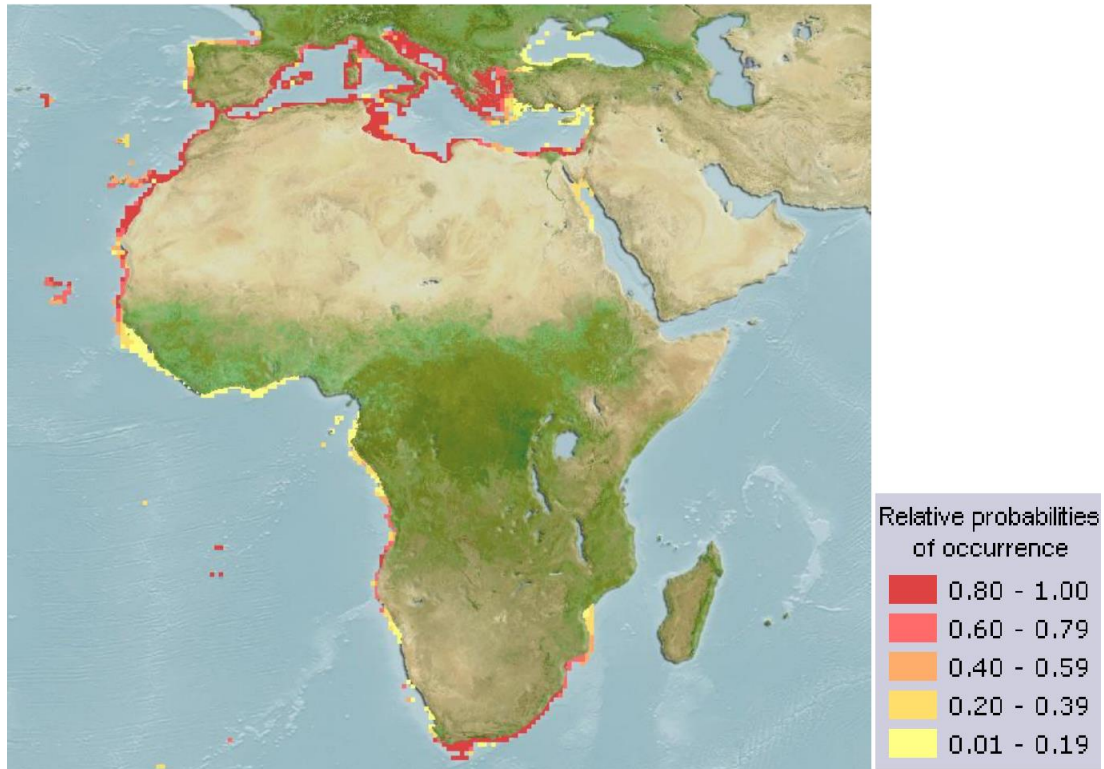

Figure 35- Current distribution of Sarpa salpa - (Source: www.fishbase.org)

Painted comber - Serranus scriba (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Serranidae

Genus: Serranus

Species: Serranus scriba

Figure 34- Ph Giovanni Ombrello, painted comber

Painted comber (Fig. 34) has a similar appearance to that of the Comber but with a more pointed muzzle, a straight forehead and a slightly larger mouth.

The livery is variable but generally presents a reddish or brownish background color that gradually becomes lighter on the sides and belly where there is a characteristic more or less dark blue spot. There are 5-7 brown vertical bands coupled two by two that do not reach the belly.

Ecology: It is a solitary species, distinctly territorial. It aggressively defends its territory from intrusions. Unlike the Comber, it is a rather diffident animal. When it is about to attack a prey it assumes a characteristic oblique position with the head turned upwards. It prefers rocky bottoms and *Posidonia oceanica* meadows. It is an almost exclusively coastal fish and, although it is known to catch 200 meters deep, it is normally rare to encounter it below 30m.

Feeding: It feeds mainly on small fish (particularly silversides) and crustaceans, occasionally also on polychaeta and mollusks. It also feeds on the remains of the octopus meal: its presence is therefore often an indication of the proximity of one of them.

Reproduction: It is a hermaphrodite species, in which eggs and sperm mature at the same time, even if self-fertilization never occurs. The eggs are laid among the rocks near the coasts in the spring and summer months.

Distribution: Eastern Atlantic from the Bay of Biscay to Mauritania including the Canaries and the Azores. Also from the Mediterranean and the Black Sea Fig. 35).
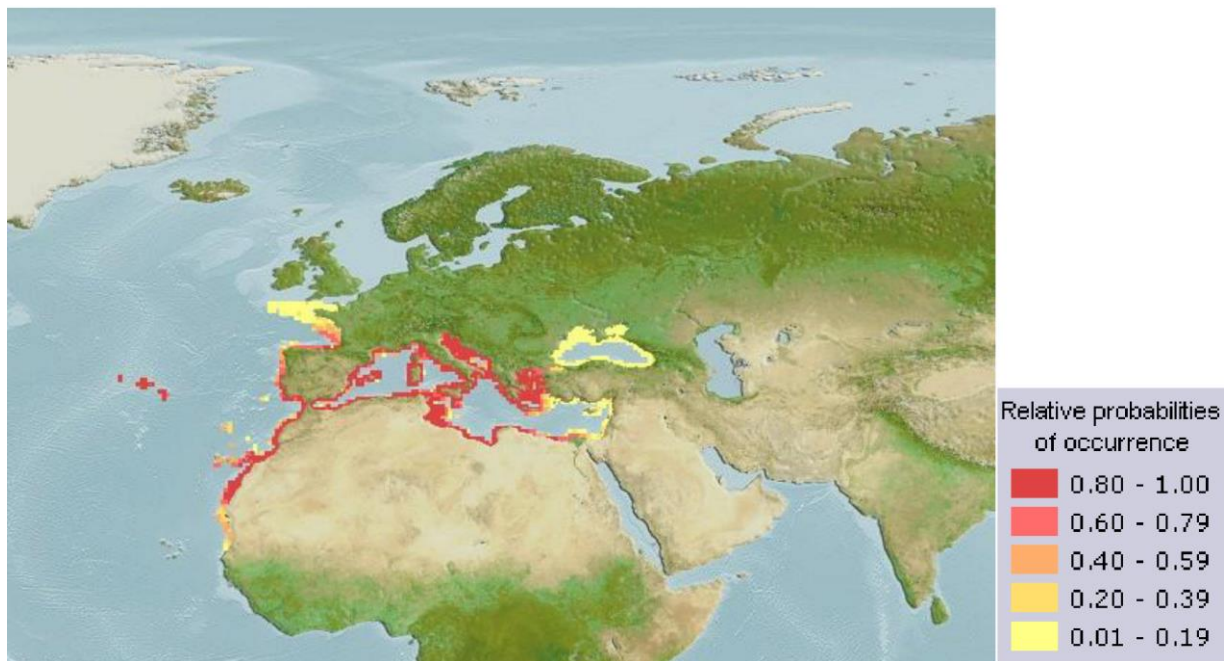

Figure 37- Current distribution of Serranus scriba - (Source: www.fishbase.org)

Comber - Serranus cabrilla (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Serranidae

Genus: Serranus

Species: Serranus cabrilla

Figure 36- Ph Giovanni Ombrello, Comber

Comber (Fig. 36) body is similar to those of the other serranids. The body is elongated, with a round section with a pointed muzzle. The mouth is very wide, with small teeth and full lips. The eyes are large. The livery is quite variable and consists of a light brown or reddish brown background, vertically streaked by 7-8 wide irregular reddish brown bands more or less dark and horizontally by 1-3 regular orange or golden lines which continue obliquely on the fish's head. In the middle of the body there is a clear longitudinal band.

The fins are yellowish. It exceptionally reaches a length of 40 cm, it is often found in sizes from 5 to 25 cm in adults.

Ecology: It is a territorial fish with solitary habits. It is strictly diurnal, at night it takes refuge on the seabed where it takes on a lighter livery. It lives on the rocky bottoms and on the *Posidonia oceanica* meadows. In deep water it can also populate sandy or muddy bottoms. It is usually a strictly coastal fish but can be encountered up to many meters deep. Feeding: It feeds on small

fish, molluscs and crustaceans. In spite of its small size, it is a very aggressive predator, which does not hesitate to attack even prey of a size slightly smaller than its own. Reproduction: The reproduction takes place between April and July in the Mediterranean. Hermaphrodite. Distribution: Widespread along the coastal strip of the eastern Atlantic Ocean, from the English Channel to the Cape of Good Hope, including the Azores, Madeira and Canary Islands. It is very common throughout the Mediterranean, especially in the Tyrrhenian Sea, it is also present in the Black Sea. It is not certain whether it is also present in the Red Sea due to immigration from the Mediterranean (Fig.37).
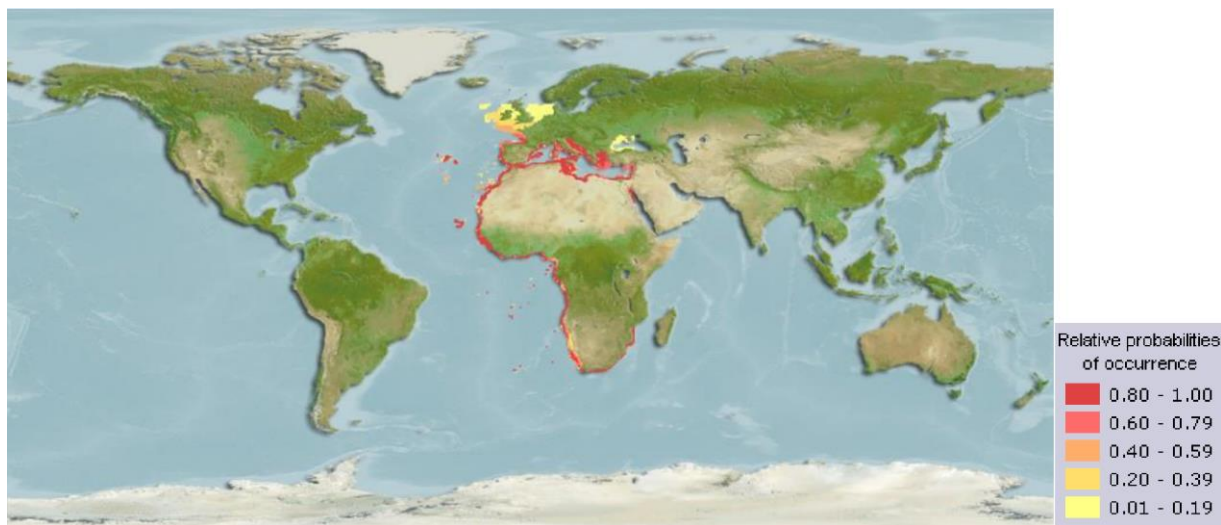

Figure 37- Current distribution of Serranus cabrilla - (Source: www.fishbase.org)

Parrot fish - Sparisoma cretense (Linnaeus, 1758)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Scaridae

Genus: Sparisoma

Species: Sparisoma cretense

Figure 38- Ph Giovanni Ombrello (female on the left, male on the right)

Parrot fish (Fig. 38) body is oval, slightly compressed at the hips, similar to a labride. Its most striking feature is the mouth, which, as in all scarides, is equipped with a bony beak formed by welded teeth. The female livery is very showy: bright red with a large greenish patch edged with yellow on the head and another patch, yellow and smaller, on the caudal peduncle. The male has a less showy, brown livery, with a lighter belly. It reaches a length of 50 cm. Ecology: It is a sedentary fish, of shallow rocky areas, but it also goes into the Posidonia meadows, up to twenty meters deep. Feeding: It feeds mainly on algae and small invertebrates that it scrapes off the rocks with its modified teeth.

Reproduction: Occurs in summer-autumn, the eggs are floating.

Distribution: Currently, parrotfish is widespread in the eastern Atlantic (Portugal, Azores, Madeira, Canary Islands and Senegal), as well as throughout the Mediterranean Sea except the coldest parts; in Italy it is present in all western seas, from the Ligurian to the Ionian Sea, for

some years also present in the southern and central Adriatic (sighted in the seas of the island of Lastovo, Croatia). The distribution in the Mediterranean has certainly had fluctuations over the course of history, linked both to intense fishing and to documented attempts to repopulate in Roman times. The most recent fluctuations are linked to the phenomenon of the southernization of the Mediterranean due to the warming of the waters (Fig. 39).
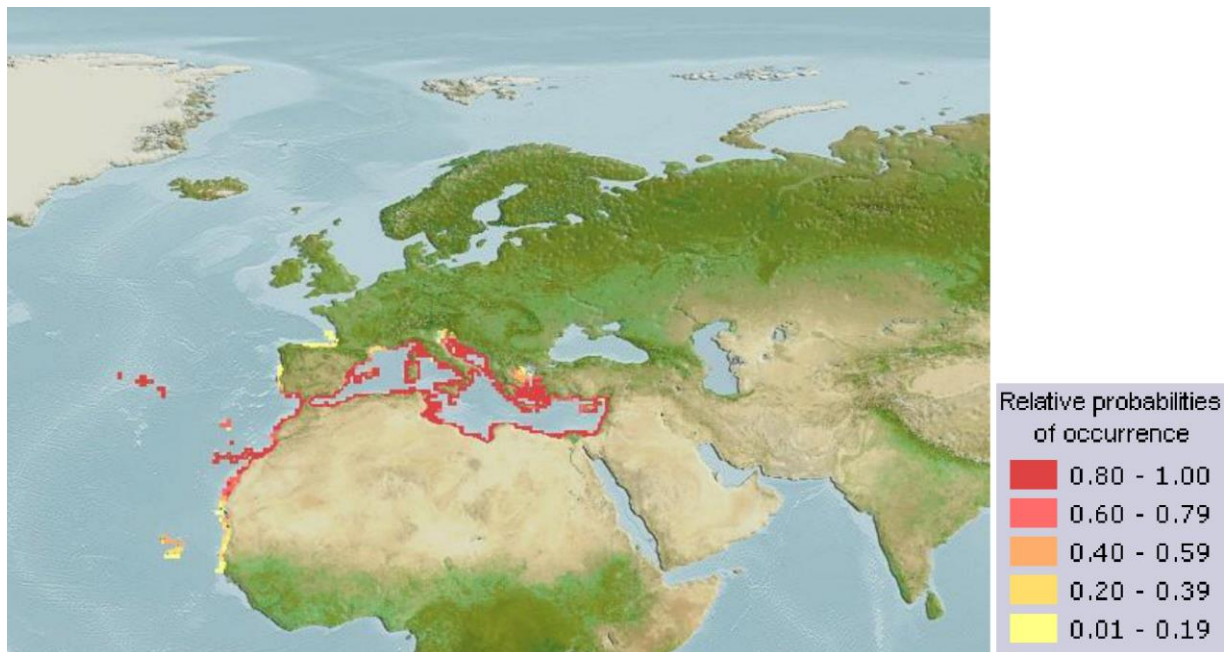

Figure 41- Current distribution of Sparisoma cretense - (Source: www.fishbase.org)

Rabbit fish – Siganus luridus (Rüppell, 1828)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Perciformes

Family: Siganidae

Genus: Siganus

Species: Siganus luridus

Figure 32- Ph Giovanni Ombrello, juveniles of rabbit fish

Rabbit fish (Fig. 40) has an oval body shape, laterally compressed. Small, sunken scales in the skin that has a smooth appearance. Small and terminal mouth, open downwards (typical of a herbivore), with plump and evident lips. Incisor teeth form. Dorsal fin with a long anterior part with sharp spiny rays and a shorter posterior part with soft rays. Variable color from beige to dark brown, the ventral part may be lighter, a blue punctuation, with blue lines on the operculum, is evident in young people. The nocturnal livery is marbled. Maximum length 25 cm.

Ecology: The juveniles are strongly gregarious and form dense schools, the adults are solitary or gather in small groups, often mixed with other species. When threatened it erects the toxic spiny rays of its fins. Coastal, it is not found more than 20 meters deep. It lives on hard coral bottoms with the presence of sediment and vegetation. In the Mediterranean it populates hard bottoms rich in algal cover or Posidonia meadows. Diet: it has a herbivorous diet and feeds (in the Mediterranean) mainly on brown algae of the genera Padina, Sargassum, Dictlyotales and Sphacelaria. The juveniles are planktophagous. Reproduction: Occurs in spring and summer. The

eggs and larvae are pelagic and are found near the surface. In the Mediterranean, it reproduces from April to August.

Distribution: This species is endemic to the western Indian Ocean including the Red Sea and the Persian Gulf. Following the Lessepsian migration, it colonized the eastern part of the Mediterranean Sea. It is currently very common along the coasts of Crete and continental from Cyrenaica to the island of Rhodes. Rarer on the coasts of Tunisia and mainland Greece. There are 103 reports from southern France (Provence), Albania, Croatia and the Strait of Sicily (Fig. 41)

First report in the Mediterranean: 1955, Israel

First report in Italian waters: 2003, Linosa

Figure 43- Current distribution of Siganus luridus - (Source: www.fishbase.org)

Bluespotted cornetfish – Fistularia commersonii (Rüppell, 1838)

Phylum: Chordata

Class: Actinopterygii

Subclass: Osteichthyes

Order: Syngnathiformes

Family: Fistulariidae

Genus: Fistularia LINNAEUS, 1758

Species: Fistularia commersonii

Figure 42- Ph. Salvatore Tuccio, Bluespotted cornetfish

Bluespotted cornetfish (Fig. 42) has a characteristic appearance typical of the members of the Fistulariidae family, very thin, depressed and with very elongated mandibles joined in the shape of a tube and a long and thin filament protruding from the center of the caudal fin. The dorsal and anal fins are placed far back, opposite and symmetrical, rather small. The flakes are absent. The color is olive or greenish on the back and sides to brown with two stripes or lines and thin blue dots on the back and sides. It may have dark marbling on the back, especially at night. It measures up to 160 cm, the average size is around one meter.

Ecology: It lives in coastal waters near the cliffs, more rarely on soft bottoms or on Posidonia oceanica meadows. It is often gregarious and is met in schools but it can also be solitary. It swims with a sinuous movement of the whole body and with the anal and dorsal fins well extended which give it greater hydrodynamics almost to make it look like an arrow.

Feeding: It feeds mainly on small fish that it catches in ambush or approaching slowly and sucks in with the buccal tube. It also catches shrimp and small cephalopods. Reproduction: It is oviparous, large eggs develop outside the body and then hatch. The larvae are 6-7mm in size. Distribution: It is widespread in the tropical belt of the Indian and Pacific Oceans including the Red Sea; from here it penetrated, through the Suez Canal, into the Mediterranean Sea (Lessepsian migration) where it was reported for the first time in 2000 in Israel; in the following years it rapidly expanded reaching Turkey, the island of Rhodes, Crete, the 105coasts of southern Italy, Sicily, Sardinia and the Tyrrhenian and Adriatic coasts, reaching as far as the coasts of the Iberian peninsula. Lessepsian species; reporting of catches in all Italian waters starting from the Sicilian seas at the beginning of the 2000s (Fig. 43).

First report in the Mediterranean: 2000, Israel

First report in Italian waters: 2004, Sicily

Figure 45- Current distribution of Fistularia commersonii - (Source: www.fishbase.org)