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**GLI IDROIDI DI MONTECRISTO E DEL GIGLIO,  
DUE ISOLE A DIVERSI LIVELLI DI PROTEZIONE**

**HYDROID ASSEMBLAGES OF MONTECRISTO  
AND GIGLIO, TWO ISLANDS AT DIFFERENT  
LEVELS OF PROTECTION**

Candidato  
**Teo Marrocco**

Relatore  
Prof.ssa  
**Stefania Puce**

Correlatore  
Dr.  
**Camilla Roveta**

**Sessione Straordinaria**

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## RIASSUNTO

Le comunità bentoniche vengono considerate, fin dagli anni '50, ottimi bioindicatori e, ad oggi, il loro importante ruolo di monitor ambientali non è raccomandato solo in letteratura, ma anche da varie legislazioni internazionali, quali la Marine Strategy (2008/56/EC) e la Water Framework Directive (2000/60/EC). Grazie alle loro caratteristiche morfologiche ed ecologiche, gli idrozoi, soprattutto per quanto riguarda la loro fase polipoide bentonica, possono essere considerati ottimi bioindicatori dello status ambientale. A tale riguardo, il presente lavoro di tesi ha come scopo, non solo di ampliare le conoscenze tassonomiche ed ecologiche di questo gruppo in un'area poco studiata, come si è rilevata essere l'Arcipelago Toscano, ma soprattutto di confermare il potenziale ruolo degli assemblage bentonici di idrozoi come bioindicatori di differenti livelli di impatto antropico, anche su scala locale.

L'analisi è stata svolta su campioni provenienti dall'Isola del Giglio e dall'Isola di Montecristo, incluse nel Parco Nazionale dell'Arcipelago Toscano (TANP). La scelta di queste due isole è dovuta ad alcune loro caratteristiche: (1) simile composizione mineralogica, (2) differente distanza dalla costa toscana, (3) diverso livello di protezione e, quindi, di impatto antropico. I campionamenti sono stati svolti a Giugno 2019 e Giugno 2020, in immersione subacquea, per un totale di 16 siti campionati (8 per isola). In ogni sito, i campioni sono stati raccolti utilizzando la tecnica del "visually oriented sampling method" a cinque diverse profondità: 0, 10, 20, 30 e 40 metri. I campioni, conservati in alcol 95%, sono stati successivamente identificati al minor livello tassonomico possibile.

Poiché dalla letteratura è noto che molte specie di idroidi siano epifiti di macroalghe, durante il primo campionamento sono state inoltre effettuate foto, a 34 cm di distanza dal fondale con quadrati di 28x21 cm<sup>2</sup>, con lo scopo di valutarne la copertura algale. Utilizzando il software ImageJ è stata analizzata la copertura algale e, da essa, si è ricavato l'indice di valutazione della qualità ambientale (EEI-CEQR). Quest'ultimo è

stato messo poi in correlazione, tramite la correlazione di Spearman, con un indice di antropizzazione, calcolato per ogni sito, che tiene conto dei differenti impatti antropici ai quali le due isole sono soggette (come presenza di reflui cittadini, turismo, presenza di porti, pesca, ecc.). Ad ogni impatto è stato assegnato un valore tra 0 (nessun impatto) e 3 (impatto elevato).

Lo studio tassonomico dei campioni raccolti ha condotto all'identificazione di 16 famiglie di idrozoi (12 Leptothecata e 4 Anthoathecata) e 89 morfotipi (48 per l'Isola del Giglio e 75 per l'Isola di Montecristo). Delle 4 famiglie di Anthoathecata, a cui appartengono 14 morfotipi, 10 appartengono al genere *Eudendrium*. Dei 75 morfotipi di Leptothecata, 22 appartengono alla famiglia Campanulariidae, 13 a quella Aglaopheniidae e 12 a quella Haleciidae.

Tramite una nMDS (non-Metric Dimensional Scaling) è stata evidenziata una differenza tra le comunità bentoniche raccolte nelle due isole, con una maggiore biodiversità all'Isola di Montecristo, evidenziata anche da un valore dell'indice di Shannon maggiore rispetto a quello dell'Isola del Giglio. L'analisi di eventuali differenze tra le profondità è stata effettuata tramite la costruzione di GLMs (Generalized Linear Models), che però non hanno evidenziato particolari patterns.

Molti morfotipi identificati tra i campioni raccolti all'Isola di Montecristo sono stati rinvenuti su substrati secondari, in particolare macroalghe erette come *Cystoseira* spp., *Sargassum* spp. e alghe calcaree, che corrispondono ad un valore EEI-CEQR più elevato, mentre tra quelli raccolti all'Isola del Giglio la maggior parte dei si trovava su substrati primari, in quanto la copertura algale era costituita principalmente turf algale e specie opportuniste, con elevati tassi di crescita e cicli vitali brevi (con bassi valori di EEI-CEQR). Questo pattern può essere relazionato con le differenti pressioni antropiche a cui le isole sono soggette, ipotesi supportata anche dalla forte correlazione negativa (Spearman's  $\rho = -0.91$ ) trovata tra la presenza di macroalghe e il livello di antropizzazione calcolato.

In generale, nell'Isola di Montecristo sono state identificate molte specie e famiglie di idrozoi considerate in letteratura k-strateghe, mentre all'Isola del Giglio quasi tutti i morfotipi identificati possono essere considerati r-strateghi o opportunisti, in quanto caratterizzati da piccole dimensioni, elevata stagionalità e cicli vitali brevi.

Anche se condotto su scala locale, lo studio evidenzia un assemblage di idrozoi completamente diverso tra le due isole, non solo in struttura, ma anche in composizione e ricchezza in specie, suggerendo che la presenza di impatti diversi, anche se lievi, possa modificare profondamente le comunità bentoniche, sia direttamente (a livello di comunità algale) che indirettamente (a livello di assemblage di idrozoi).

In conclusione, i nostri dati forniscono una conoscenza più completa della biodiversità e dell'ecologia degli idrozoi dell'Arcipelago Toscano, in particolare per quanto concerne la Riserva Integrale dell'Isola di Montecristo, registrando 40 nuovi ritrovamenti rispetto alle specie elencate per l'area dell'Arcipelago Toscano, e pongono basi a supporto dei policy-makers per l'implementazione del TANP come futura Area Marina Protetta, in accordo con le direttive della Strategia Europea per la Biodiversità per il 2030. Ulteriori studi sono comunque necessari per estendere il campionamento anche ad altre isole dell'Arcipelago Toscano e in altre stagioni, in quanto molte specie di idroidi sono caratterizzati da cicli stagionali e quindi presenti solo in determinati periodi dell'anno.

## Chapter 1

### INTRODUCTION

#### *1.1 Hydrozoa*

The class of Hydrozoa Owen, 1843 is one of the 5 classes of the Phylum Cnidaria Hatschek, 1888, together with Cubozoa Werner, 1973, Staurozoa Marques and Collins, 2004, Scyphozoa Goette, 1887 and Anthozoa Ehrenberg, 1834. Hydrozoan classification and nomenclature have been infamous, posing difficulties for ecologists, taxonomists, biogeographers, as well as phylogeneticists who work with hydrozoans Collins, 2000. The present-day situation is that Hydrozoa comprises two reciprocally monophyletic clades, Trachylina Haeckel, 1879 and Hydroidolina Collins, 2000, which together contain approximately 3500 valid species (Collins, 2002; Daly et al., 2004). Despite wide consensus regarding the monophyly and composition of Hydrozoa, it has few unambiguous characteristics which identify sets of putative synapomorphies for Hydrozoa (Marques and Collins, 2004; Daly et al., 2007).



### **1.1.1. *Biology***

Hydrozoan's biology is characterized by a radial symmetry, with two or four axes, and are characterized by only two germ layers, the ectoderm and the endoderm. From the endoderm derives the gastrodermis (that surrounds the coelenteron), while from the ectoderm derives the epidermis. Between the two layers is present the mesoglea, mainly composed by water and fibrous protein, works as a hydrostatic skeleton. Moreover, Hydrozoa lacks a brain, replaced by a nerve net, which allow them to spread the nerve impulse in many directions. It is possible to divide the speed transmission of the nerve impulse in two types: mouth closing and tentacles retreat have a more rapid transmission, while the slower ones guide the body to become shorten. To the nerve net belongs cells distributed along the body and cells organized in a radial nerve ring. However, the organization of cells in the nerve net can vary among hydrozoans (Collins, 2002).

Belonging to the Cnidaria, Hydrozoa can present two different life stages, polyp and medusa, which share a general body structure of mouth and tentacles, but with a reversed oro-aboral axis: the polyp present tentacles and mouth on the upper side of the body, while the jellyfish have mouth and tentacles on the lower part. The two stages are alternated during the life cycle, even though

some species can present exclusively the medusae stage (e.g. Trachylinae clade) or the polyp stage (e.g. *Hydra* spp.) (Collins, 2002).

Hydrozoan's medusae are mainly characterized by:

- the bell, also called umbrella which generally recall a mushroom or a bell form, reaching various different sizes;
- the velum, a muscular horizontal marginal diaphragm, under the subumbrella cavity, with a fundamental role in swimming;
- bulbs and tentacles, usually on the marginal free rim of medusae, various in number and shape are armed with cnidocysts, attached to the bulbs;
- sense organs, including ocelli (light-sensitive organs linked to the bulbs), and statocysts (orientation and equilibrium organs);
- the manubrium, which contain the gastric cavity and often the gonads, can be attached directly to the subumbrellar roof or to a thickened layer of mesoglea cone-shaped (Boullion et al., 2006).

On the other hand, the polyp stage is often colonial, characterized by the presence of numerous polyps, presenting many different morphology elements whose shape is used for taxonomical identification. Colonies are sometimes polymorphic, that is polyps are morphologically and physiologically different, and it is possible to distinguish their role through their form. Polyps called

gonozooids have the reproductive role, dactylozooids have the defensive role, while gastrozooids feed the colony.

The hydranth is the structure bearing the hypostome with a terminal mouth, which can be arranged in different ways. For example, Eudendriidae L. Agassiz, 1862 and Campanulariidae Johnston, 1836 have a peduncled hypostome. The mouth is usually conical or dome shaped, and surrounded of tentacles, which are the most characteristic elements of the hydranths, varying in type and structure (e.g. capitate, filiform, etc.). The hypostome is connected to the gastric column by the hydranth, characterized by an absorbing function. Some families, like Haleciidae Hincks, 1868 and Plumulariidae McCrady, 1859, have a differentiated gastroderm divided into two zones: the first one with glandular cells and digestive vacuoles, called oral digestive, and the second one called aboral non-digestive. The aboral limit of the hydranths is represented by the sphincter, characterized by muscular elements but without tentacles and digestive inclusions (Bouillon et al, 2004). Muscles also drive different movements, especially in the planula stage, like the modulating swimming direction in *Clava multicornis* Forsskål, 1775, allowing bending of the larva for an efficient phototaxis. At the polyp stage, muscles allow protection by retraction and peristaltic movement facilitating digestion and fluid circulation within the body Leclere et al, 2017.

The hydrorhiza and the hydrocaulus represent the stolonal system, allowing colony to be attached to the substrate. The horizontal growth is given by the coenosarc tube contained in a perisarc sheath, called hydrorhiza. This structure can survive under unfavourable conditions resorbing the colony and regrow in a new colony under proper conditions. Arising from the hydrorhiza there is the main stem of the colony which bears more than one hydranth, called hydrocaulus. The hydrocaulus can be branched with various shapes from arborescent to flabellate or flexuose, or it can be unbranched, like in some Halopterididae Millard, 1962.

The hydrocaulus may be monosiphonic or polysiphonic, thus with a single coenosarc tube or with two or more, each one with its perisarc, usually divided into segments or internodes. The perisarc completely surrounds the stolonal system of all benthic hydroids, with the exception of some “naked” species which are usually parasitic, pelagic or epizoic, or perisarc can also be calcified (under the calcite or aragonite form, or both), as in the families of Stylasteridae Gray, 1847 and Milleporidae Fleming, 1828.

The perisarc can form the hydrotheca, a solid theca around the polyps in which they can retract. The perisarc can have many forms and shapes supporting the taxonomic differentiation between thecate and athecate forms. In fact, in

Anthoathecates, the perisarc do not form a firm perisarc around polyps or it is less developed, and it is called pseudohydrotheca.

Nematothecae are the protective nematophores, variable in numbers and forms around the hydrotheca and containing the nematocysts (or cnidoblast). Cnidocytes are cells with a secretory organelle called cnidocyst, mostly contained in the tentacles. Cnidaria uses these cells to attach themselves to the substrate, capture preys or for defending purposes.

Some hydrozoan species, as *Physalia physalis* Linnaeus, 1758, have poisonous and dangerous cnidocysts even for humans, but most hydrozoan jellyfishes can cause only irritations. The cnidocysts can even be sticky (and so are called ptychocysts), which help to create mucous substances to protect the organisms, or can be lasso-like, called spirocysts, and can be wrap around the preys.

Almost all hydrozoan species are dioecious bearing gonads of one sex, but events of sex change and hermaphroditism are also reported. Hydroid sexual dimorphism is shown only on the colour and form of gonozooids or of its contents, as in the families of Plumulariidae, Haleciidae and Campanulariidae. Hydroids can also reproduce by asexual reproduction, that means the production of a separate soma with the same genotype as the parent (either medusae or hydroid). The hydroid clone can arise from natural fission or from a damaged colony which produce separate modular fragments. The budding

events usually took place in stolon with rearrangement of the cells population in terms of tissue folding and tissue accommodation for growth. The production of the stolonal clone is the most widespread mechanism of asexual reproduction among the benthic hydroids and it is particularly evident in the hydroids that are epiphytes of seagrasses and algae (Gili & Hughes 1995).

### *1.1.2. Ecology and covered ecological role*

Due to their modular body organization and high plasticity, hydroids can colonize different habitats and adapt to a variety of environmental conditions (Gili and Huges, 1995; Bouillon et al., 2004). In fact, hydroid assemblages can be found from cold water habitats, as the North-Atlantic Ocean, to tropical environments, as the Indo-Pacific area; moreover, they can reach a high depth range from 0 to almost 3000 m depth (Di Camillo et al, 2008; Voronkov et al, 2010; Cairns, 2011)

The Mediterranean hydrozoan fauna (Siphonophora excluded) comprises 400 species, which 20% of it are endemic, most of the species are Atlantic (68%) and the 8% are of Indo-Pacific origin. There are 69 nonindigenous (NIS) species in the basin: 44% of these are casual (recorded just one or very few times), 28% established (widely recorded in the basin), 6% invasive (established NIS that are able rapidly or largely to disseminate away from the

area of initial introduction, having a noticeable impact on the recipient community), and 22% questionable (of doubtful taxonomic status) (Gravili et al, 2013). Bouillon et al. (2004) suggested that Mediterranean hydroids can be divided in different categories: (a) “evident”, which comprises common species (e.g. *Eudendrium racemosum*, *E. glomeratum*, *Clytia hemisphaerica*, *Sertularella crassicaulis*, *S. gaudichaudi*, etc.); (b) “cryptic”, species usually difficult to sample but now collected more frequently due to the increase of sampling effort and efficiency (e.g. *Halocoryne epizoica*, strictly symbiotic with the bryozoan *Schizoporella sanguinea*); (c) species which are alternately rare and common.

The ecological role of Hydrozoa is related to their ability to increase the habitat spatial complexity, which they shape with their structures also enhancing interspecific interactions, therefore being considered habitat formers and ecosystem engineers. Hydroids have a large scale of forms, ranges and sizes. Their ability to create forests allow them to establish different relationships with many organisms. In fact, hydroid colonies are important benthic filter-feeders and represent a suitable substrate for the settlement of other sessile organisms, as gorgonians or algae (Di Camillo et al, 2017). For example, *Lytocarpia myriophyllum* Linnaeus, 1758 is largest leptothebate hydroid of the Mediterranean Sea, which presents colonies up to 1 meter in height. This

species creates wide forests on soft bottoms, stabilizing the sediment and providing refuge and food for several associated eukaryotic and prokaryotic organisms.

Hydroids can host many organisms on their colonies, also other hydrozoans, as *Plumularia setacea* observed on the hydrocladia, *Campanularia hincksii* on the hydrocaulus and the hydrorhizae, or some species of the genus *Bougainvillia* which have been found on the most superficial hydrorhizae. However, associated species are not only of the same phylum, in fact also vagile fauna was found, as some nudibranch species like *Dondice Banyulensis* and *Eubranchus exiguus* which lay eggs on hydrozoan branches and can feed on them (Bouillon et al, 2004).

The colony morphology can be affected by both abiotic (as temperature and salinity) and biotic factors (as food rates). In fact when food is scarce the polyps in the centre of the colony may undergo regression and new polyps are generated in peripheral locations, increasing the prey capture chances (Gili & Hughes 1995). In some experiments on *Laomedea flexuosa* Alder, 1857 is shown that there is a strict relation between the minor grow rate and shortage food and have different consequences on the hydrorhiza and on the hydrocauli: the first one continues to grow, while the second ones are shorter and more widely spaced. Another factor influencing colony morphology is current. Hydroids



size is inversely correlated with the water movement: large specimens are found in calm water while smaller ones are usually present where the water movement is higher. *Aglaophenia pluma* Linnaeus, 1758, for example, have unbranched hydrocauli in shallow turbulent water, with the size of 1.5 cm in height and, whereas the water is deeper and with a bidirectional current, the hydrocauli can be branched reaching 50 cm in height (Gili & Hughes 1995).

Hydrozoa have a key role in the energy transfer from the plankton to the benthos, due to their abundance, the high captures rates and the fact that they are consumed by many benthic invertebrates. This ability was highlighted by different studies, for example Gili and Coma (1998) estimated that hydroids can capture approximately the 10% of the annual algal production contrasting the only 0.5% of the hydrozoan community biomass. More, on the role of *Obelia* spp. as a regulator of the production of a local population of copepods, and on *Orthopyxis everta* Clark, 1876 (cited as *Campanularia everta*) which presents mean annual captured prey rates of 45,196 zooplanktonic prey m<sup>-2</sup> day<sup>-1</sup>, an equivalent of a mean biomass of 2260 µgC m<sup>-2</sup> day<sup>-1</sup>. The role of the hydrozoan on energy transfer is much more important than what expected basis on their size (Gili and Coma 1998). On the other hand, there are many invertebrates feeding on hydroids, like turbellarians, aplousobranchs, gastropods, polychaetes and pycnogonids. These are only some of hydroids'

predators, most of them presenting different adaptations to avoid the stings of cnidocysts or to be immune to them (Gili & Hughes 1995).

Hydroids are generally assumed to be carnivores capturing their prey using cnidocysts. Usually, cnidocysts sizes are related to the size of the preys. Studies on the enteron contents of the hydroids using the scanning electron microscope (SEM) showed a variety of prey captured by hydroids: most of them are small zooplanktonic organisms like nauplii, small and adult copepods, and other small crustaceans. Moreover, diatoms were found in the enteron of *Silicularia rosea* Meyen, 1834 (cited as *Silicularia bilabiate*) and particulate organic matter as the mainly diet of *Campanularia everta* (Gili & Hughes 1995).

Many hydroids communities undergo sharp seasonal cycles, both in community structure and reproductive period cycles, which can occur through the year but generally each species have a limited period of fertility (Bavestrello et al 2007). Differences in hydroids communities is evident in the Mediterranean Sea where summer and winter have strictly different ecological requirements and zoogeographical affinities, and in tropical coral reef where the seasonality differences in terms of irradiance condition and temperature are small, but where the rainfall abundance on which depend the food availability and so the hydroids growth rate, lead the hydrozoan seasonal diversity (Di Camillo et al. 2008). In fact, many water characteristics, mainly temperature, and different

biotic interactions lead the resting life phase of the hydroids through the hydrorhiza as the resting form. This capability allows the colony survival during adverse seasons and to conserve a constant pattern in the season dynamics, as demonstrated with the study on *Eudendrium glomeratum* Picard, 1952 (Puce et al, 2009). Other examples are *Paracoryne huvei*, present as a resting form from June to October, and *Hydractinia (Podocoryna) exigua*, an epizoic species who live on hermit crab shell of *Diogenes pugilator*, usually abundant during the winter and rare during the summer (Bavestrello et al 2007). In addition, being important habitat-former, hydroids can affect water movements and light penetration, alter the substrate providing settling space and shelter, and be a food sources for other species. Hydrozoans also play an important role in the bento-pelagic coupling processes due to the releasing of planulae, medusoids and medusae in the water column, enhancing the local biodiversity (Di Camillo et al, 2017).

### ***1.2. Tuscan Archipelago National Park***

The Tuscan Archipelago National Park (TANP) is composed of 7 main islands (Capraia, Elba, Giannutri, Giglio, Gorgona, Montecristo and Pianosa) and other small islands and rocks between Tuscany and Corsica (www.islepark.it; Carrus et al., 2009). It occupies a total area of about 300 km<sup>2</sup> and 250 km of coastline,

and for this reason it is considered the largest marine park of the Mediterranean basin. Most of the area of the Archipelago is occupied by Elba, which is the bigger island with 147 km of coastline, while Giglio is second in size and only 14 km far from the mainland ([www.islepark.it](http://www.islepark.it)). On the other hand, Montecristo is the farthest island and considered one of the most pristine spots of the Mediterranean basin (Turicchia et al., 2018), being located 40 km from Elba and 63 km from Monte Argentario ([www.islepark.it](http://www.islepark.it)). More than 30,000 residents live permanently within the Park, while during the Summer season this number increases to over 200,000, with a high turnover ([www.islepark.it](http://www.islepark.it)).

The islands are spread within 2 distinct basins, the Ligurian Sea, washed by a cold west current, and the Tyrrhenian Sea, with the homonymous current (Fratini et al, 2013).The entire Archipelago presents heterogeneous characteristics among islands, from the steep coast of Montecristo to the tubular shape of Pianosa; the wide geological representations, sedimentary, metamorphic and igneous rocks are in part explained by its formation during the Triassic geological period and make the Archipelago a unique spot of heterogenous geology inside the Mediterranean ([www.islepark.it](http://www.islepark.it)).

These characteristics contributed to the high marine and terrestrial biodiversity rates of the Archipelago and to its uniqueness landscape and natural spots. In order to protect them from the increasing human pressures, The Tuscan

Archipelago National Park (TANP) (Italy) was established as a protected area on 22<sup>nd</sup> July 1996, after a long period of disputes and mediations between the central government and local stakeholders (Carrus et al, 2009).

The 7 islands are currently undergone to different levels of protection both in terrestrial and marine environments. Regarding the latter:

- in the Elba Island, the Ministerial Decree of 10<sup>th</sup> august 1971 established a one of biological protection in the area within Punta Falcone and Capo Bianco. In the area all fishery activities are prohibited whit the exception of some of some non-invasive and non-intensive fishery technics ([www.islepark.it](http://www.islepark.it)).
- Giglio is included only for 40% inside the protected area: adjacent waters and many terrestrial areas do not have any particular protection due to urbanization (DPR 22/07/1996; Casoli et al., 2016). Only the southern part of the island land is protected by the legislation of the TANP and it is divided into four zones ([www.islepark.it](http://www.islepark.it)).
- Most of coastline of Capraia is interested by a specific protection and legislation, in which activities (fishery, scientific research, bathing, etc.) are strictly regulated ([www.islepark.it](http://www.islepark.it)).

- The islands of Pianosa and Gorgona are characterized by a total protection, in fact the 100% of water, up to 1 nautical mile offshore, are considered zone 1( [www.islepark.it](http://www.islepark.it)).
- Montecristo was established as an Integral Nature Reserve by Ministerial Decree of 4<sup>th</sup> March 1971 and declared a biogenetic nature reserve by the Council of Europe since 1988. In addition, the island has the status of Special Protection Area (SPA, Directive 79/409/EEC). The land and the adjacent waters, up to 3 nautical miles offshore, are subjected to protection by the Carabinieri Corps and the Coast Guard (Angeletti et al., 2010; Bo et al., 2014). Fishing, bathing, diving, mooring and circumnavigating are forbidden, while only berthing, landing and scientific activities are allowed, under specific conditions, with a permission of the Territorial Office for Biodiversity of the Carabinieri Corps of Follonica (Angeletti et al., 2010).
- The sea around the island of Giannutri is divid in zone 1 and 2, whit the exception of two corridors which allow the access to boats ([www.islepark.it](http://www.islepark.it)).

### ***1.3 Aim of the work***

Although the taxonomy and ecology of Mediterranean hydroids is well documented, the literature available on the species (e.g. Sarà et al., 1993;

Balduzzi et al., 1996; De Ranieri et al., 2006; Leclere et al., 2009; Piazzini et al., 2014) of the Tuscan Archipelago is still scant, fragmentary and sparse, and many of the observations derive from sampling not deliberately targeting hydrozoans in a systematic way. Their modular body and plasticity, their ability to adapt to various environmental conditions, and their wide geographical distribution and being common components of the benthic communities make these organisms suitable indicators for environmental monitoring and to get information on the health of habitats and ecosystem (Ballesteros et al., 2007; Puce et al., 2009; Yilmaz et al., 2020; Roveta et al., 2021), as also recommended by the Marine Strategy Framework Directive (MSFD 2008/56/EC) and the Water Framework Directive (WFD 2000/60/EC).

In the current study, we explored the potential role of benthic hydrozoan assemblages as bioindicators of different level of anthropization at local scale, comparing the assemblages collected on the hard bottoms of two islands, Montecristo and Giglio, belonging to the Tuscan Archipelago National Park (TANP). Moreover, we provided new taxonomic and ecological information on the benthic hydrozoans of the area and our results will help to fill the gap of information of the biology ecology and distribution of this group of metazoans in the considered area.

## Chapter 2

### MATERIALS AND METHODS

#### 2.1. Study area

The study was focused on two islands belonging to the Tuscan Archipelago National Park, Montecristo (42.3317° N, 10.3083° E) and Giglio (42.3603° N, 10.9229° E) islands (Figure 1).

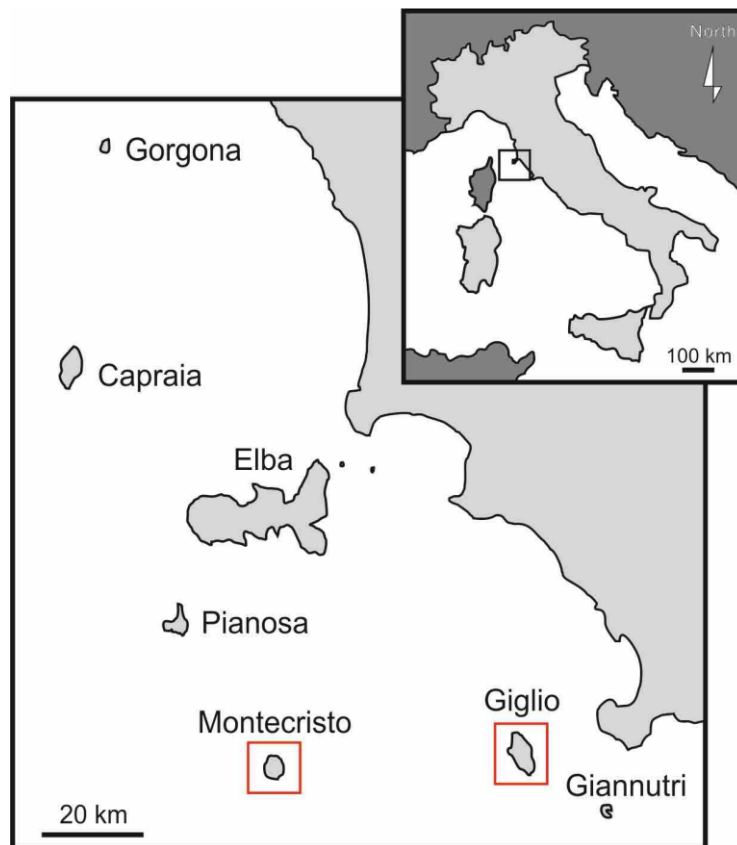


Figure 1 – Map of the Tuscan Archipelago National Park. The red square indicates the two islands considered for the study.



The choice of the two islands is related to some characteristics of the islands themselves:

1. They have a **similar mineralogical composition**, since they are mainly composed by granitic rocks (Alvisi et al., 1994; Innocenti, 1997), except for Promontorio del Franco in the Giglio Island which is mainly limestone (Alvisi et al., 1994). Different studies (e.g. Canessa et al., 2019; Canessa et al., 2020) showed that the substrate lithology, together with chemical-physical factors and biological interactions, can deeply affect the structure and diversity of sessile zoobenthic assemblages. Moreover, Bavestrello et al. (2000) conducting different laboratory experiments demonstrated on *Eudendrium glomeratum* larvae, demonstrated that their larvae prefer to settle on marble (carbonatic) than quartz (granitic).
2. Even though both Montecristo and Giglio are included in the TANP, they are subjected to **different levels protection**. In fact, the Montecristo Island is uninhabited and isolated (Innocenti et al., 1997), and it was established as an Integral Nature Reserve in 1971. Nowadays it also present the status of Special Protection Area (SPA, Directive 79/409/EEC). The land and the adjacent waters, up to 3 nautical miles offshore, are subjected to protection by the Italian Carabinieri Corps and

the Coast Guard (Angeletti et al., 2010; Bo et al., 2014). Almost all the activities are forbidden (e.g. fishing, bathing, diving, mooring and circumnavigating), while berthing, landing and scientific activities are allowed, under specific conditions, with a permission of the Territorial Office for Biodiversity of the Carabinieri Corps of Follonica (Angeletti et al., 2010). Due to all these reasons, Montecristo is considered one of the best-preserved sites of the Mediterranean, previously identified as a reference site for Western Mediterranean benthic assemblages (Cecchi et al., 2014; Turicchia et al., 2018). On the contrary, Giglio hosts 1,500 residents, living in three small villages: Giglio Castello, Giglio Porto and Campese (Bavestrello et al., 2000; [www.islepark.it](http://www.islepark.it)). The island is included only for 40% under the protection of the TANP: in fact, the adjacent waters and many terrestrial areas do not have any protection due to urbanization (DPR 22/07/1996). In addition, the high flow of tourists during the summer period, undergone the island to high anthropogenic pressures (Casoli et al., 2016).

3. The two islands have a **different distance from the mainland**. Giglio is only at 14 km from the mainland, thus, it can be more exposed to possible anthropic impacts deriving from the Italian coast (Casoli et al., 2016) compared to Montecristo, which is 63 km far ([www.islepark.it](http://www.islepark.it))

## 2.2. Field activities

Samplings were carried out in SCUBA diving during June 2019 and 2020 in both Montecristo (TANP permission #00068010) and Giglio, and a total of sixteen sites were sampled (Table 1; Figure 2).

Table 1 – List of the sites surveyed in the current study in the study area.

Island	Site	Code	Latitude (N)	Longitude (E)
Montecristo	Punta Rossa	M-HB01	42°18'48.12"	10°19'01.62"
	Punta Forata	M-HB02	42°19'09.60"	10°19'35.16"
	Punta del Diavolo N-O	M-HB03	42°21'02.46"	10°17'55.86"
	Punta di Cala Maestra	M-HB04	42°20'07.56"	10°17'18.96"
	Punta delle Grotte	M-HB05	42°18'57.0"	10°17'48.0"
	Punta Fortezza	M-HB06	42°20'46.3"	10°19'17.6"
	Punta di Cala St. Maria	M-HB07	42°19'41.21"	10°17'20.53"
	Punta del Diavolo N-E	M-HB08	42°21'3.94"	10°18'50.63"
Giglio	Le Scole	G-HB01	42°21'17.04"	10°55'50.94"
	Punta Capel Rosso	G-HB02	42°18'58.08"	10°55'16.74"
	Punta Fenaio	G-HB03	42°23'21.54"	10°52'48.18"
	Scoglio del Corvo	G-HB04	42°20'17.76"	10°53'21.36"
	Secca di Campese	G-HB05	42°22'23.85"	10°52'47.95"
	Punta di Cala Cupa	G-HB06	42°22'8.01"	10°55'12.01"
	Punta Corbaia	G-HB07	42°19'30.5"	10°55'45.3"
	Scoglio di Pietra Bona	G-HB08	42°19'47.97"	10°53'57.99"

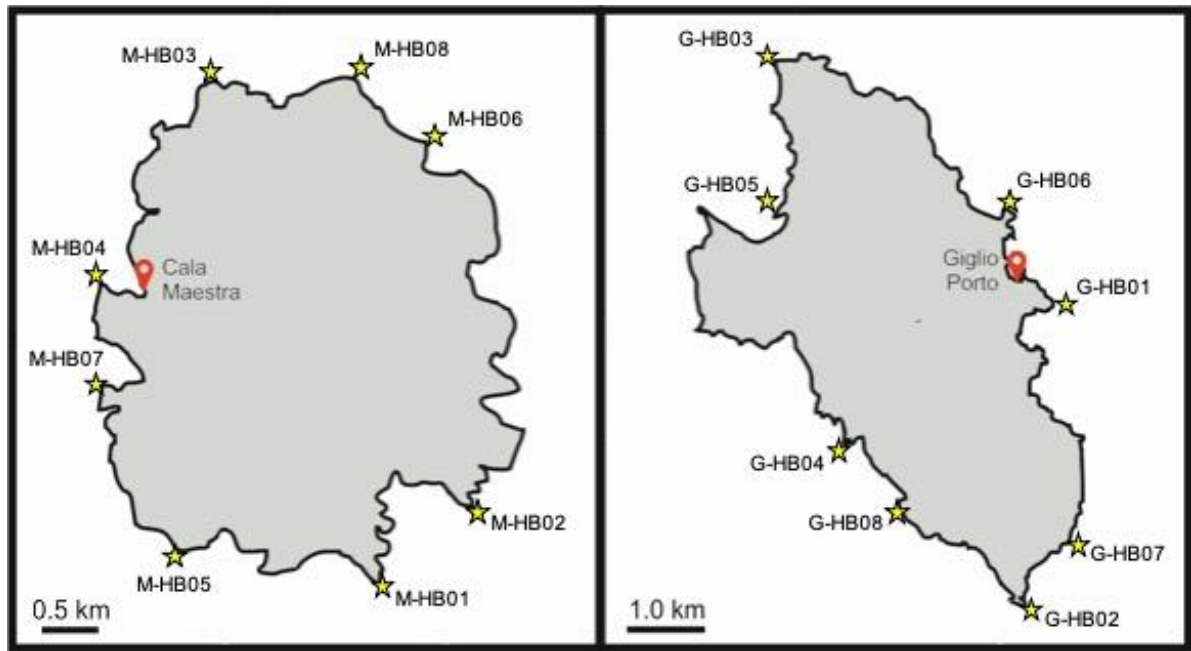


Figure 2 – Map of the Montecristo Island (left) and of the Giglio Island (right), with the location of the sampling sites.

The visual oriented sampling method was chosen for the collection of samples, since this type of sampling is considered as one of the most effective methods in the detection of highest numbers of hydrozoan species of an area (as stated by Piraino et al., 2013). Samples were collected on rocky bottom assemblages at five depths: 0, 10, 20, 30, 40 meters, with a mean of 6 minutes at every depth. Hydroid samples were stocked in alcohol 95% and, once in the laboratory, specimens were identified at the lowest taxonomic level, when possible. The status of taxa was validated using the website World Register of Marine Species (WoRMS).

### ***2.3. Anthropic index***

Since the considered islands are subject to different levels of protection and, thus, to different anthropic pressures, in order to evaluate the impact affecting each study site, we defined an Anthropic index following Gobert et al. (2009) and Piazzzi et al. (2015; 2021). The index was calculated considering a total of six impacts, that are urbanization and urban wastes, port, tourism, artisanal fishing, sailing and anchoring, and mainland distance, and each one was classified from 0 (no impact) to 3 (high impact), taking into account the presence and distance from the source. Data were obtained using ARPAT technical reports on the areas (Cicero and Serena, 2016; Verniani, 2018; Verniani et al., 2018; Verniani, 2019), consulting different websites, and direct observations of our underwater operator (Table 2).

Table 2 – Anthropogenic index values calculated for each study site.

	Urbanization & urban waste	Port	Turism	Fishing	Sailing & anchoring	Mainland distance	<b>TOTAL</b>
M-HB01	0	0	0	0	0	0	<b>0</b>
M-HB02	0	0	0	0	0	0	<b>0</b>
M-HB03	0	0	0	0	0	0	<b>0</b>
M-HB04	1	1	1	0	1	0	<b>4</b>
M-HB05	0	0	0	0	0	0	<b>0</b>
M-HB06	0	0	0	0	0	0	<b>0</b>
M-HB07	0	0	0	0	0	0	<b>0</b>
M-HB08	0	0	0	0	0	0	<b>0</b>
G-HB01	1	1	3	3	3	3	<b>14</b>
G-HB02	0	0	1	3	3	3	<b>10</b>
G-HB03	1	0	1	2	3	3	<b>10</b>
G-HB04	0	0	2	1	3	3	<b>9</b>
G-HB05	3	0	3	3	3	3	<b>15</b>
G-HB06	2	3	2	1	3	3	<b>14</b>
G-HB07	0	0	1	3	3	3	<b>10</b>
G-HB08	0	0	2	3	3	3	<b>11</b>

#### ***2.4. Photo survey and analysis: Ecological Evaluation Index and Ecological Quality Ratio (EEI-cEQR)***

Since many Hydrozoa species have been observed to live as epiphytes on macroalgae, during the first sampling, together with the collection of organisms, a photographic survey was conducted, in order to analyse the macroflora of the two islands.

Pictures were taken at the same depths of the collection of samples, using a Canon G16 camera, located at 34 cm distance from the substrate with the support of a 28x21 cm frame (0.0588 m<sup>2</sup>). The photographic samples followed a regular disposition, placed at every 2 m. Five pictures per depth were randomly chosen for the analysis, for a total of 200 photos. Using ImageJ

(Rasband, 2012), the cover percentage of macroalgae was extrapolated from each photo, and macroalgal species were identified at the best possible level of taxonomic resolution and classified into different Ecological Status Groups (ESG-IA, IB, IC and ESG-IIA, IIB). Their Ecological Evaluation Index and Ecological Quality Ratio (EEI-cEQR) values were estimated according to Orfanidis et al. (2011) and are presented in the supplementary material S1. For the analysis, the median value of EEI-cEQR of every site was considered.

### ***2.5. Statistical analysis of data***

The visual oriented sampling method allowed us to express data as the presence/absence of the various hydrozoan species at block of observations, which included different sampling sites and 5 different levels of depth each site (0, 10, 20, 30, 40 m). A non-metric dimensional scaling (nMDS), based on a similarity matrix and a Jaccard similarity index for presence-absence data, was used to compare the assemblages found in the different sites of the two islands. Depths were not considered since they would have produced missing values.

We also compared how islands and depths influenced the diversity of hydrozoans. Therefore, we calculated the Shannon's Entropy index (Guevara et al. 2016) for each depth level of each site, considering hydrozoans divided in families. Then, we adopted a Hurdle Gamma Generalized Linear Model to

explore the influence of depth and island considering the Shannon's Entropy index (Zuur et al., 2020). The GLM was fitted with STAN, using a Bayesian MCMC approach (Carpenter, 2017). We also considered an interaction between depth levels and islands, to disentangle any potential difference in the effect of depth between Montecristo and Giglio. Moreover, as different depths from the same site could have been autocorrelated, we included a random intercept term to look for differences between sites. We also modelled the zero-part of the model by comparing four models accounting for the effect of island, site and depth, assuming that these covaries affected sampling with no observations. Models were compared with leave-one-out cross validation (Vehtari et al, 2017).

Finally, to see if a relation between the  $EEI-C_{EQR}$  values and the Anthropic index calculated existed, the Spearman correlation coefficient was calculated.

All statistical analysis and figures were performed and produced using the free software R version 4.0.3 (R Core Team).



## Chapter 3

### RESULTS

Among the samples collected, 16 families (12 Leptothecata and 4 Anthoathecata) and 89 morphotypes (48 in Giglio and 75 in Montecristo) were identified. Considering the 4 families of Anthoathecata, 14 morphotypes were found, 10 belonging to the genus *Eudendrium*, and among the 12 Leptothecata families, 75 morphotypes were identified, most of them belonging to Campanulariidae (22), Aglaopheniidae (13) and Haleciidae (12) (Table 3).

Only the family Zygophylacidae and 15 morphotypes were exclusive of the Giglio Island, while the 4 families of Cladocorynidae, Corynidae, Bouganvilliidae and Lovellenidae together with 38 morphotypes were exclusively found in Montecristo. The site with the highest number of morphotypes identified was M-HB08 (41), followed by M-HB05 (29), G-HB08 and G-HB06 (24), while the sites G-HB03 and G-HB01 were the ones with the lowest number species richness, with 7 and 10 morphotypes recorded respectively (Table 3).

Considering the Hydrozoa species listed for area 2 (Relini, 2008: VII, Figure 1; Gravili et al., 2008), 32 morphotypes were in common and 40 were new records for the area (Table 3).

Following Gravili et al. (2013), six of the identified morphotypes (*Clytia hummelincki*, *C. linearis*, *Filellum serratum*, *Plumularia pulchella*, *Eudendrium carneum*, *E. merulum*) are considered as non-indigenous species (NIS), in particular *C. hummelincki* and *C. linearis* were classified as “invasive” and the other four morphotypes as “established” (Table 3).

Table 3 – List of the identified morphotypes collected. + = new records for area 2 (see Relini,2008; Gravili et al.,2008).

Hydrozoa	Montecristo Island								Giglio Island							
	M- HB01	M- HB02	M- HB03	M- HB04	M- HB05	M- HB06	M- HB07	M- HB08	G- HB01	G- HB02	G- HB03	G- HB04	G- HB05	G- HB06	G- HB07	G- HB08
<b>Leptothecata</b>																
<b>Aglaopheniidae</b>																
<i>Aglaopenia acacia</i> Allman, 1883				X			X	X								
<i>Aglaopenia elongata</i> Meneghini, 1845		X	X		X	X										
<i>Aglaopenia harpago</i> Schenck, 1965		X					X							X		
<i>Aglaopenia kirchenpaueri</i> (Heller, 1868)												X			X	
<i>Aglaopenia lophocarpa</i> Allman, 1877		X	X					X								
<i>Aglaopenia octodonta</i> Heller, 1868	X	X		X	X	X		X		X		X				X
<i>Aglaopenia picardi</i> Svoboda, 1979						X		X								
<i>Aglaopenia pluma</i> (Linnaeus, 1758)	X							X								
<i>Aglaopenia</i> sp.1	X															
<i>Aglaopenia</i> sp.2			X													
<i>Aglaopenia</i> sp.3				X												
<i>Aglaopenia tubiformis</i> Marktanner-Turneretscher, 1890	X				X	X	X	X						X	X	
<i>Aglaopenia tubulifera</i> (Hincks, 1861) +			X	X				X								X
<b>Campanulariidae</b>																
<i>Campanularia hincksii</i> Alder, 1856 +	X	X			X											

<i>Campanularia</i> sp.1									X										
<i>Campanularia</i> sp.2																			X
<i>Campanularia</i> sp.3																			X
<i>Campanularia volubilis</i> (Linnaeus, 1758) +		X																	X
Campanulariidae sp.																			X
<i>Clytia gracilis</i> (Sars, 1850) +																			X
<i>Clytia hemisphaerica</i> (Linnaeus, 1767)	X		X		X	X	X	X	X	X									X
<i>Clytia hummelincki</i> (Leloup, 1935)		X																	X
<i>Clytia linearis</i> (Thorneley, 1900)		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Clytia noliformis</i> (McCrary, 1859) sensu Calder, 1991+*					X	X		X						X	X	X			X
<i>Clytia paulensis</i> (Vanhöffen, 1910)																			X
<i>Clytia</i> sp.1																			X
<i>Clytia</i> sp.2																			X
<i>Gonothyrea loveni</i> (Allman, 1859) +														X					X
<i>Laomedea angulata</i> Hincks, 1861														X					X
<i>Laomedea calceolifera</i> (Hincks, 1871)														X					X
<i>Laomedea flexuosa</i> Alder, 1857 +														X					X
<i>Obelia bidentata</i> Clark, 1875														X					X
<i>Obelia dichotoma</i> (Linnaeus, 1758)														X					X
<i>Orthopyxis crenata</i> (Hartlaub, 1901)	X	X	X	X	X	X	X	X						X					X
<i>Orthopyxis integra</i> (MacGillivray, 1842)		X												X					X
<b>Lafoeidae</b>																			
<i>Acryptolaria conferta</i> (Allman, 1877) +																			X
<i>Filellum disaggregatum</i> Peña Cantero, García-Carrascosa & Vervoort, 1998 +																			X
<i>Filellum serpens</i> (Hassall, 1848) +																			X
<i>Filellum serratum</i> (Clarke, 1879) +														X	X	X	X	X	X



**Kirchenpaueriidae***Kirchenpaueria pinnata* (Linnaeus, 1758)

X X X X X

**Lovenellidae***Mitrocomium cirratum* Haeckel, 1879 +

X

**Plumulariidae***Plumularia obliqua* (Johnston, 1847)

X X X

*Plumularia pulchella* Bale, 1882 +<sup>b</sup>

X

*Plumularia setacea* (Linnaeus, 1758)

X

X

**Hebellidae***Scandia gigas* (Pieper, 1884) +

X X X

*Scandia* spp.

X

X X X X

X X

**Sertulariidae***Dynamena disticha* (Bosc, 1802)

X X X X X X X X X X X X X X X

*Salacia desmoides* (Torrey, 1902) +

X

X

X

X

*Sertularella crassicaulis* (Heller, 1868) +

X

X

X

*Sertularella cubica* García Aguirre & Gonzalez, 1980 +

X

*Sertularella distans* (Lamouroux, 1816) +

X

X

X

*Sertularella ellisii* (Deshayes & Milne Edwards, 1836)

X

X

X

X

X

X

*Sertularella polyzonias* (Linnaeus, 1758)

X

X

**Zygophylacidae***Zygophylax biarmata* Billard, 1905 +

X

**Anthoathecata**

**Bougainvilliidae**

Bougainvilliidae sp.

X

*Calyptospadix cerulea* Clarke, 1882 +

X

**Cladocorynidae**

*Cladocoryne floccosa* Rotch, 1871 +

X

X

X

**Corynidae**

Corynidae sp.

X

**Eudendriidae**

*Eudendrium armatum* Tichomiroff, 1890

X

X

X

X

X

X

*Eudendrium capillare* Alder, 1856

X

X

*Eudendrium carneum* Clarke, 1882 +

X

X

*Eudendrium glomeratum* Picard, 1952

X

X

*Eudendrium merulum* Watson, 1985 +

X

X

X

*Eudendrium moulouyensis* Marques, Peña Cantero & Vervoort, 2000 +

X

*Eudendrium racemosum* (Cavolini, 1785)

X

X

*Eudendrium rameum* (Pallas, 1766) +

X

*Eudendrium ramosum* (Linnaeus, 1758)

X

*Eudendrium* spp.

X

X

X

X

X

X

X

X

With the nMDS analysis on the 16 sampling sites, it was possible to observe how all sites of an island are clustered together, suggesting the presence of two distinct hydrozoan assemblages in Montecristo and Giglio (Figure 3).

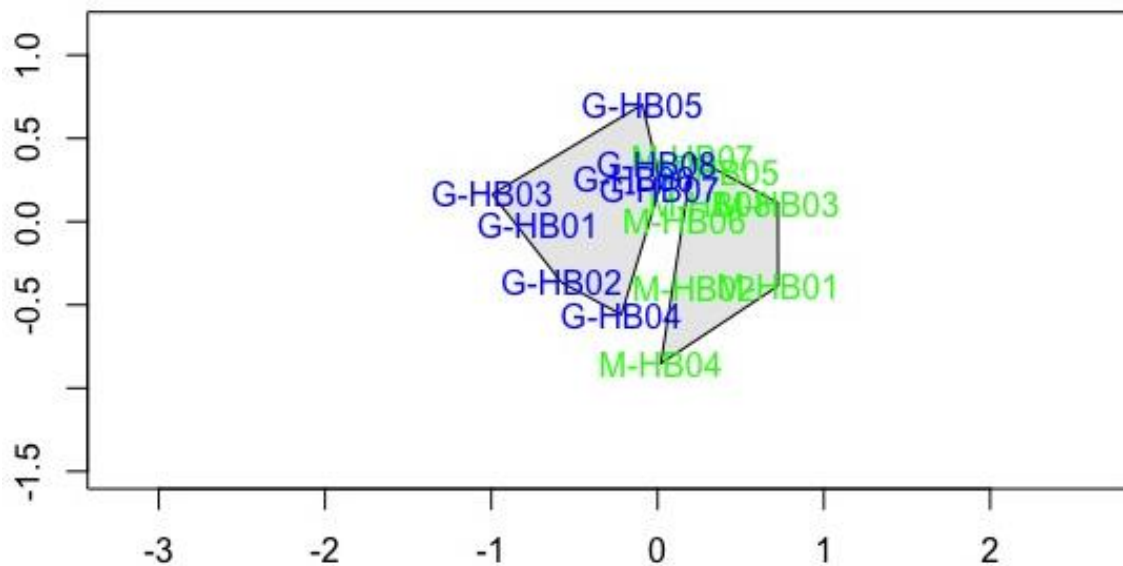
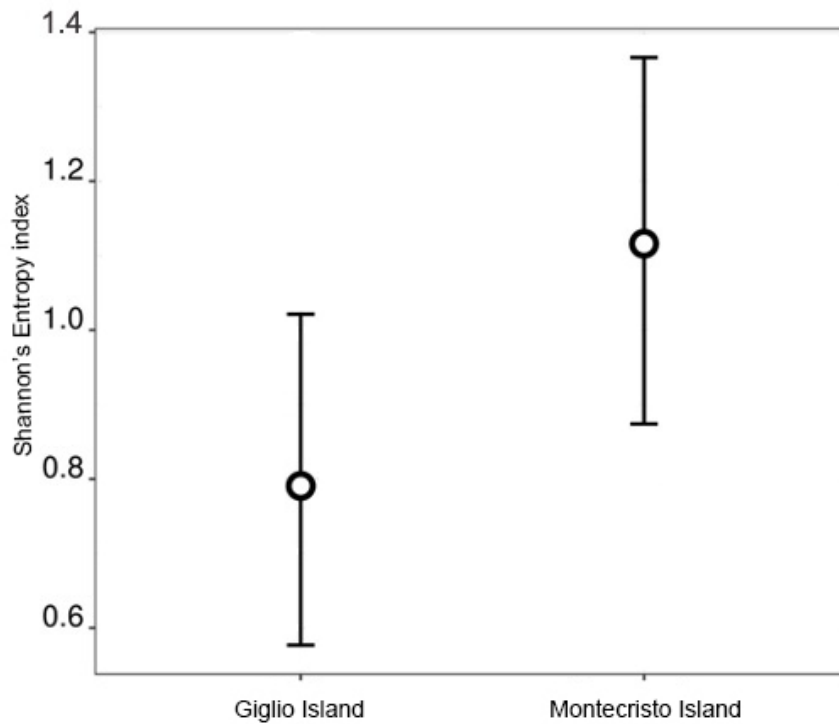


Figure 3 – Non-metric multi-dimensional scaling (nMDS) plot, comparing the hydrozoan benthic assemblages between sites.

In addition, the best candidate model ( $R^2 = 0.14$ ) showed that the only clear difference found was between islands, with higher values of the Shannon's Entropy index in Montecristo (Figure 4).





*Figure 4 – Marginal effect of the GLM: differences between Giglio and Montecristo islands in the Shannon's Entropy characterizing the hydrozoans benthic assemblages.*

The model, which predicted higher values of the index in the Montecristo Island, also indicated more balanced counts at four of the Montecristo sites, that are M-HB05, M-HB06, M-HB07 and M-HB08 (Figure 5).

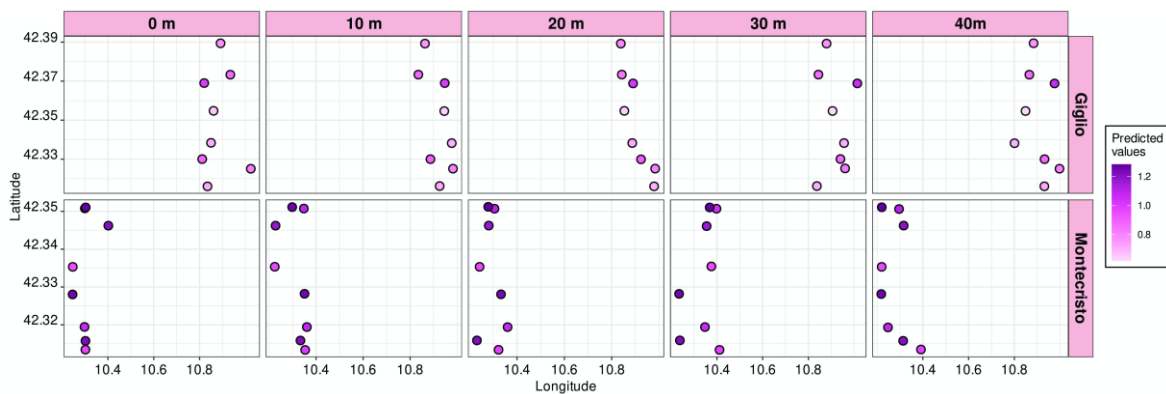


Figure 5 - Predicted values of the GLM, at the various sites and depths

Considering the morphotypes recorded at the different sampling depths, most of them were found at 0 m in Montecristo (Figure 6), with a decreasing trend with depth. On the contrary, in the Giglio Island the number of morphotypes was almost constant at all depths except for the 30 meters, where a lower number of morphotypes were identified (Figure 6). No morphotypes have been found in samples collected at 20 at G-HB03, G-HB05 and M-HB01, at 30 m at G-HB01, G-HB03, G-HB05 and M-HB04, and at 40 m at G-HB04.

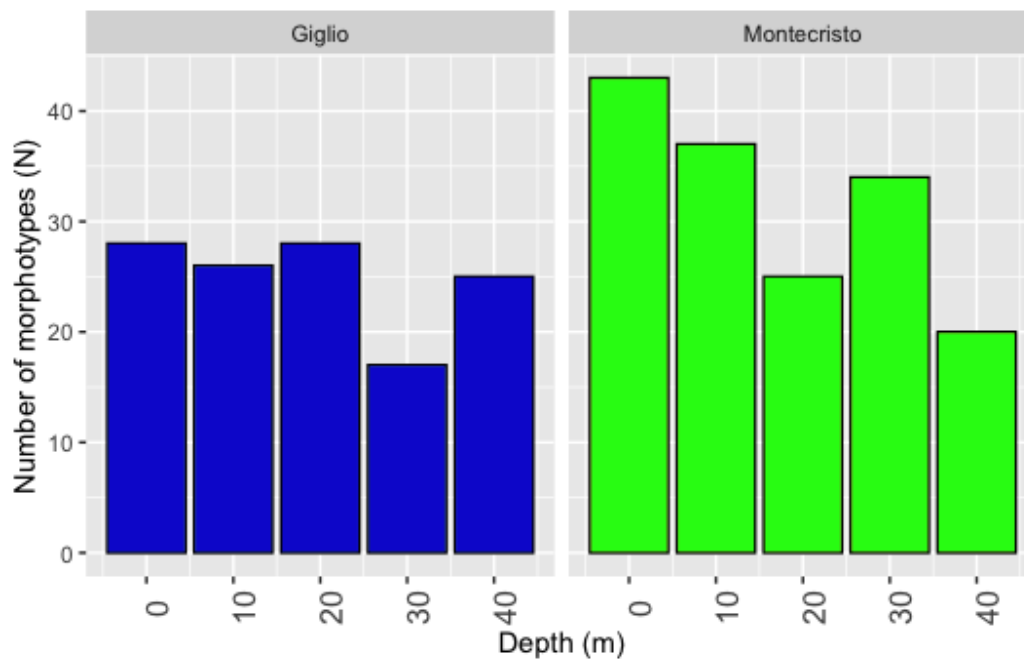
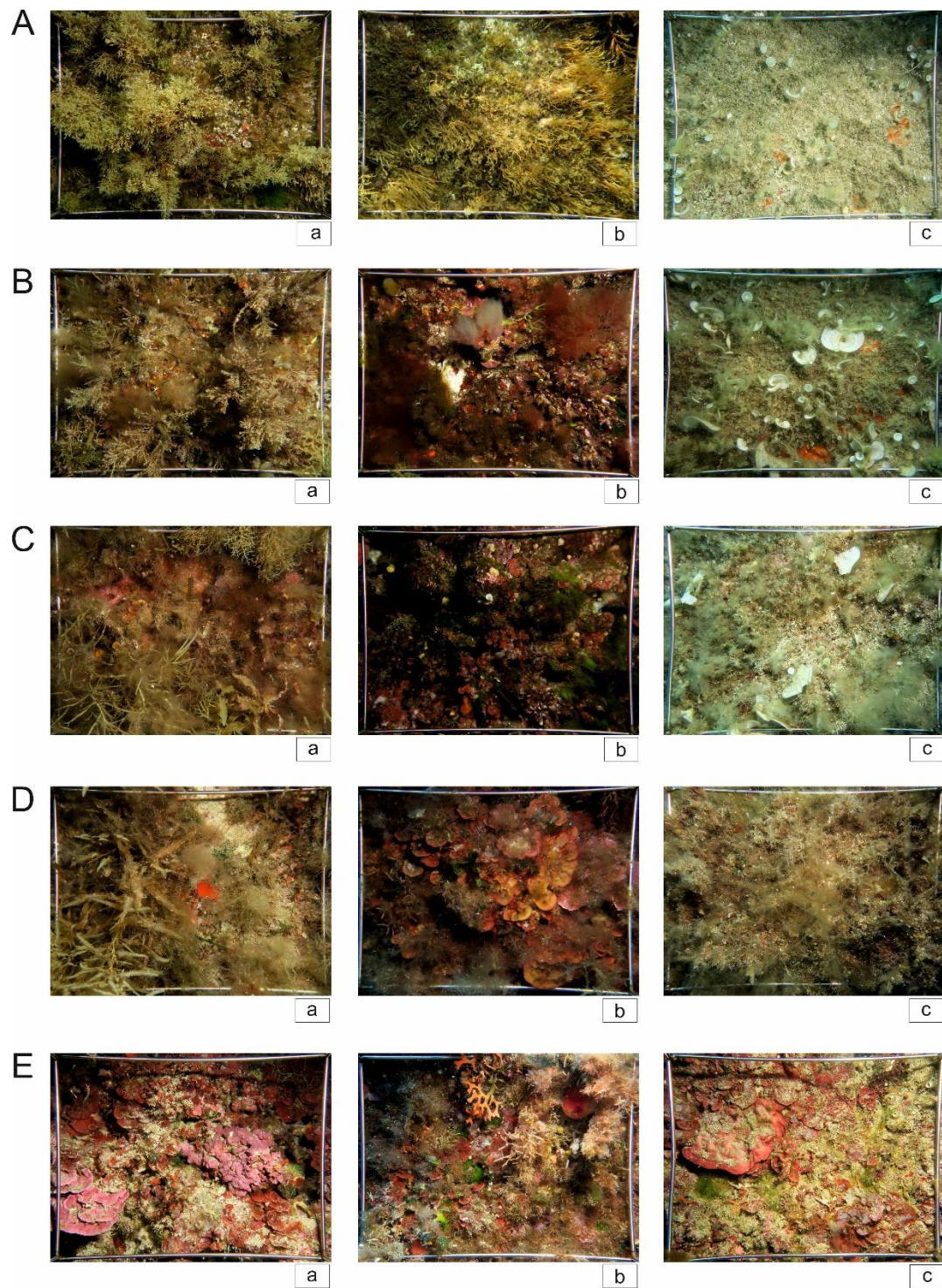


Figure 6 – Number of morphotypes per depth per island.

Most of morphotypes, except for some of the families Aglaopheniidae, Eudendriidae, Halopterididae and Plumulariidae, were mainly found on secondary substrates as epiphytic on erected macroalgae. From the photographic sampling was possible to differentiate three types of macroalgal assemblages for the two islands, two of them in the Montecristo Island (Figure 7). The I type was characterized by mainly fuclean brown algae (*Cystoseira*, *Ericaria*, *Gongolaria*) between 0 and 20 m depth (Figure 7Aa, Ba, Ca), *Sargassum* and calcareous algae (both articulated and encrusting corallines) at 30 m depth (Figure 7Da), and *Peyssonnelia* and crustose calcareous algae at 40 m (Figure 7Ea). The II type identified in Montecristo was composed by Dictyotales at 0 m (Figure 7Ab), and from 10 to 40 m by articulated and

crustose corallines, turf-forming filamentous rhodophytes, *Flabellia petiolata* and *Peyssonnelia* spp. (Figure 7Bb, Cb, Db, Eb). In the Giglio Island, a more homogeneous algal composition was observed among sites, with an abundance of algal turfs, *Padina pavonica* and *Acetabularia acetabulum* at 0 m depth (Figure 7Ac), and with also articulated calcareous algae at 10 m (Figure 7Bc). At 20 and 30 m, the same vegetation was heavily covered by a thick mucilaginous sheath (Figure 7Cc, Dc). At 40 m, crustose calcareous algae were the most abundant forms, together with a small amount of Dictyotales and filamentous algae (Figure 7Ec).



*Figure 7 – Field images of the macroalgal communities characterizing the hard bottoms of Montecristo and Giglio islands. A) 0 m, B) 10 m, C) 20 m, D) 30 m, E) 40 m depths at: a) Montecristo Island type I; b) Montecristo Island type II; c) Giglio Island*

Due to the different protection levels of the islands and, thus, the possible different anthropogenic impacts, in addition to the different algal substrates (Figure 7), we correlated the two aspects through the calculation of two indexes, an Anthropogenic index (Table 2) and the Ecological Evaluation Index coupled with the Ecological Quality Ratio index (EEI- $c_{EQR}$ ) (supplementary material S1). The Spearman correlation showed a strong negative correlation (Spearman's  $\rho = -0.91$ ) between the presence of three-dimensional erected macroalgae and anthropic pressures (Figure 8).

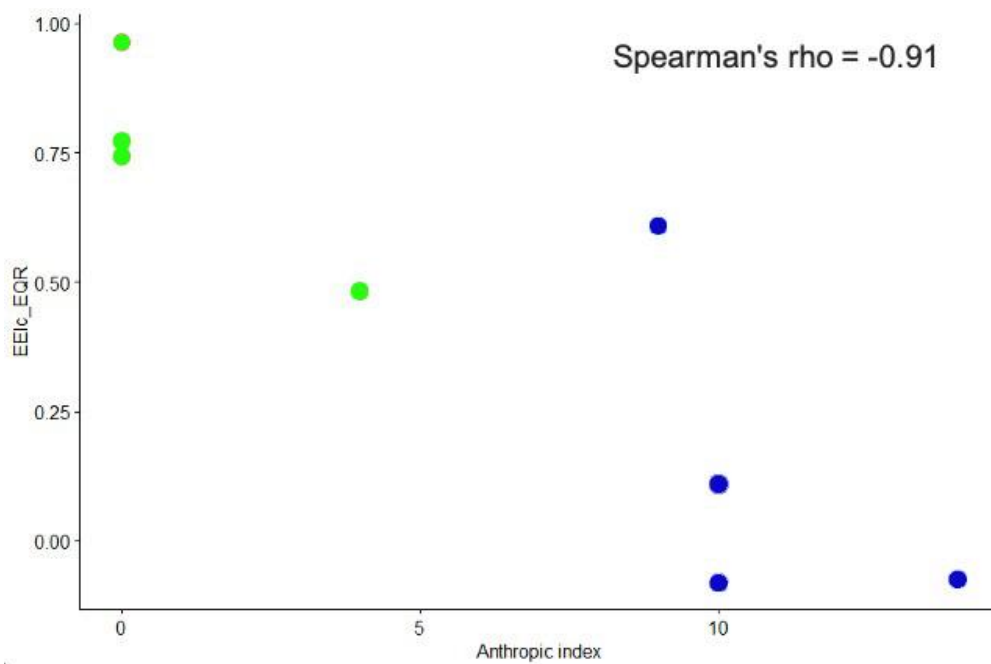


Figure 8 – Spearman correlation between the Ecological Evaluation Index (EEI- $c_{EQR}$ ) and the anthropic index calculated for sites sampled during June 2020.

## Chapter 4

### DISCUSSION

All the species of hydrozoans collected in Montecristo and Giglio islands were already known for the Mediterranean Sea and typical of its rocky bottom assemblages (e.g. Bouillon et al., 2006; Morri et al., 2009; Puce et al., 2009; Yilmaz et al., 2020). Nonetheless, these new data fill an important distributional gap for the area 2 (see Relini, 2008 for the definition), adding 40 new records, most of them already present and well-known in the surrounding areas (see Gravili et al., 2008). In the Tyrrhenian Sea, Iacono et al. (2013) observed that water masses move from the southern part to its northern part. It is possible that the direction of the Tyrrhenian currents can promote larval dispersal and their settlement in the studied area (Cowen and Sponaugle, 2009). Although in hydrozoans and most cnidarians in general the planula is considered only partly suitable for long range dispersal (Boero and Bouillon, 1987), longer distances can be reached by the medusa stage, rafting objects, or free-living fragments present in the water column (Aliani and Meloni, 1999). Thus, the assemblages found in the two islands could be affected by the species present in the surrounding areas. A similar pattern was observed for the recorded NIS. Apart from *Plumularia pulchella*, for which no previous records were found in the surrounding areas, and *Clytia hummelincki* and *C. linearis*

which were already listed in the area (Gravili et al., 2008), the other species (*Filellum serratum*, *Eudendrium carneum* and *E. merulum*) could have reached the islands thanks to water movements. The two “invasive” NIS, *C. hummelincki* and *C. linearis*, originally described in the West Indies and New Britain respectively, are now spread in all the Mediterranean Sea (Gravili et al., 2013), and found in most of the Italian seas (Gravili et al., 2008).

In some cases (Table 3) the identification to species level was prevented for the small amount of material available, poor material or the lack of structures essential for identification (e.g. reproductive structures). Among the 89 identified morphotypes, *Acryptolaria conferta*, *Aglaophenia octodonta*, *Antennella secundaria*, *A. siliquosa*, *Clytia hemisphaerica*, *C. linearis*, *Dynamena disticha* and *Scandia* spp. were the most common among sites and depths (Table 3); moreover, these species have been recognized by many authors (e.g. Boero and Fresi, 1986; Svoboda and Cornelius, 1991; Bouillon et al., 2004; Boero et al., 2005; Moura et al., 2011; Gravili et al., 2013) as among the most frequent and abundant hydroid species characterizing the Atlanto-Mediterranean hard bottom assemblages. In addition, seven of the recorded species were zooxanthellate, as *A. harpago*, *A. octodonta*, *A. tubiformis*, *A. tubulifera*, *Eudendrium moulouyensis*, *Halecium nanum* and *Sertularella*



*polyzonias* (Bouillon et al., 2004, 2006; Gravier-Bonnet and Bourmaud, 2005; Di Camillo et al., 2008; Puce et al., 2009; Stabili et al., 2018).

Both species richness and the Shannon's Entropy index showed clear differences between the hydrozoan assemblages of the studied islands: the sites sampled in the more anthropic Giglio were characterized by a lower species richness, together with the dominance of few species and a low number of epiphytic hydrozoans. On the other hand, in the more pristine Montecristo, the recorded assemblages were characterized by a higher number of species, growing both on primary and secondary substrates. In particular, the different assemblages found in the two islands could be mainly related to the different substrate, in terms of algal coverage and community. In fact, most of morphotypes were found on macroalgae, mainly *Cystoseira*, *Ericaria*, *Gongolaria*, *Sargassum*, that are seaweeds with a thick or calcareous thallus, low growth rates and long-life cycles, and defined by Orfanidis et al. (2011) as ESG-I (late-successional). However, in sites were lower values of EEI- $C_{EQR}$ , also a lower alpha-diversity was recorded. These sites were mainly composed by Dictyotales, classified as ESG-IIA (filamentous sheet-like opportunistic) and coralline algae, which are considered as ESG-IC (shade-adapted plastic) (Orfanidis et al., 2011). Different hydrozoan families and species (e.g. Campanulariidae, Haleciidae, Lafoeidae, Bouganvillidae, and many others)

usually grow as epiphytes of various macroalgal species (Morri and Bianchi, 1999; Faucci and Boero, 2000; Calder, 1991), together with other groups of invertebrates, as sponges, bryozoans, polychaetes, molluscs, crustaceans, echinoderms and ascidians (Mazzella et al., 1989; Morri and Bianchi, 1999; Frascchetti et al., 2002; Ávila et al., 2007; Piazzini et al., 2018). Moreover, it has been observed that different portions of an algal thallus (basal, middle and distal) can host different hydrozoan species (Frascchetti et al., 2006). On the other hand, in all sites of the Giglio Island, the dominant algal group was the ESG-II, characterized by species with high growth rates and short life cycles (Orfanidis et al., 2011). The observed pattern can be related to the different anthropic pressures (e.g. fishing, sailing, anchoring, urban wastes, mainland inputs) to which the islands are subjected. These results and observations are also supported by the strong negative correlation found between the Anthropogenic index and the EEI-CEQR.

As observed by Megina et al. (2013), that analysed the hydrozoan assemblages found in harbours and natural rocky cliffs of the southern coast of Spain, the hydrozoan families Aglaopheniidae, Halopterididae and Sertulariidae, described as k-strategist (Cornelius, 1990; Leclère et al., 2009), were much more represented in the natural hard bottom of Montecristo. Other hydroids species, as the family Campanulariidae and some species belonging to the

genus *Halecium*, considered as opportunistic (i.e. small size, extensive hydrorhizal growth, seasonality) (Gili and Huges, 1995; Genzano and Rodriguez, 1998; Migotto et al., 2001), were found in both islands and were among the most representative morphotypes identified in Giglio. However, as observed by Castellanos-Iglesias et al. (2018), some species among the Campanulariidae showed a different sensitivity to pollution, being more abundant in pristine areas (e.g. *Clytia gracilis*). However, Castellanos-Iglesias et al. (2018) conducted their studies in a different biogeographical area, where the different environmental conditions and abiotic factors (temperature, salinity, pH, dissolved oxygen, ect.) can play an important role in the biological effects of contaminants and affect differently the biota (Sánchez et al., 2007; Schiedek et al., 2007; Edzwald and Haarhoff, 2011; Manciocco et al., 2014).

Megina et al. (2016) also reported that different species of *Eudendrium* can co-occur in pristine areas and more polluted environments or be exclusive of one of the two habitats. An example is *E. moulouyensis*, which they recorded only in non-impacted areas of southern Spain, while in the present study this species was found only in one site of Giglio. However, since some species of *Eudendrium*, as *E. racemosum*, are characterized by a high body plasticity, they can colonize different habitats and adapt to a variety of environmental conditions (Megina et al., 2016). Therefore, these characteristics may allow the

same species to occur both in natural, protected environments and in more anthropized areas.

This study, conducted at local scale, showed completely different hydrozoan assemblages between the pristine area of Montecristo (Turicchia et al., 2018) and the more anthropized Giglio (Casoli et al., 2016). These results suggested that even the effect of low and limited pressures (such as urban wastes of small villages, artisanal fishing, seasonal tourism, recreational diving and sailing, low concentration of contaminants in the seawater, ect.) can strongly affect the hydrozoan benthic biodiversity (Castellanos-Iglesias et al., 2018; Yilmaz et al., 2020) at different levels. In fact, we observed not only a different composition of the hydrozoan community, but also differences in the macroalgal communities between the two islands. Therefore, being mostly epiphytic, hydrozoans can suffer anthropization both directly and indirectly (Faucci and Boero, 2000; González-Díaz et al., 2003). Moreover, since macroalgae can host a high biodiversity, including not only hydroids but also other invertebrates, further studies on the whole epiphytic community are needed to expand the knowledge and to create successful management plans for macroalgal habitats. Moreover, being substrate generalists (Cornelius, 1992), several hydrozoan species settle on the surface of other animal organisms, as Porifera, Cnidaria, Mollusca, Annelida, Bryozoa, Crustacea, Echinodermata and Chordata

(Piraino et al., 1992; Puce et al., 2008). Therefore, the conservation and protection of these groups are necessary to maintain high rates of hydrozoan biodiversity.

## Chapter 5

### CONCLUSIONS

The differences observed, in terms of species composition and richness, in the hydroid assemblages of Montecristo and Giglio, allow us to suggest these organisms as suitable bioindicators for the good environmental status of an area, even at local scale, as previously stated by Yilmaz et al. (2020). Moreover, since many hydrozoan species are seasonal (Bavestrello et al., 2006; Bouillon et al., 2006), further studies are needed, increasing the sampling effort to other period in order to have a general picture of the species composition along a year and to create a baseline used to compare how these assemblages can change through time, also in respect to the water temperature increase of the Mediterranean Sea (Puce et al., 2009). In conclusion, this work is the first focussed exclusively on the hydrozoans of the TANP, being the other information scant, fragmentary and sparse in the literature, and many of these observations derive from samplings not deliberately targeting hydroids in a systematic way. Our data provide additional taxonomical and environmental insights on the Tuscan Archipelago, especially on the Integral Nature Reserve of Montecristo, and lay the groundwork to the policy-makers for the establishment of the TANP as a future Marine Protected Area (L.979/82 art.31 and 394/91 art 36).

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## **GREETINGS**

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