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Growth dynamics of the Demosponge *Chondrosia reniformis*: Current knowledge and its future potential in mariculture for collagen production

Dinamiche di crescita della Demospongia *Chondrosia reniformis*: Conoscenze attuali e potenziale futuro nella maricoltura per la produzione di collagene

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Index

Abstract

Chondrosia reniformis (Nardo, 1847) is a thick encrusting, smooth, and lobate sponge belonging to the class Demospongiae, with an Atlanto-Mediterranean distribution usually thriving in shallow coastal rocky bottoms (0–50 m). The main characteristic of the species is that it is almost entirely made of collagen, which makes this organism of great interest for the pharmaceutical and nutraceutical industries. Nevertheless, like many other sponges, its survival has been challenged in the last decades, given the increase frequency of marine heatwaves linked to climate change and the resulting mass mortality events of the shallower populations. Additionally, the potential exploitation of the sponge for biotechnological applications calls for a proper evaluation of its distribution, conservation status, growth and regeneration rates. In this context, a systematic review was conducted to summarize the current knowledge about *C. reniformis*, taking in special consideration studies on its *in situ* cultivation. Moreover, the use of 'Structure from Motion' (SfM) photogrammetric technique was evaluated as a non-invasive, long-term and cost-effective approach to assess this sponge's growth habit, volume and regeneration rates within an *in situ* mariculture system set up along the coast of Alassio (Liguria, Italy) in May 2021, over pre-existing artificial reefs. Biomass and collagen production have been also estimated from the volume obtained with SfM-

photogrammetry. Our review showed that the current knowledge on *C. reniformis* spans various fields (i.e., ecology, biochemistry, biotechnology, physiology, molecular biology, and microbiology), with certain fields having received more attention, such as biotechnology and biochemistry. Until now, few attempts have been made to develop suitable in situ mariculture methods for the sponge *C. reniformis,* highlighting that further studies are needed to optimize cultivation methodologies.

The average annual increases in volume, biomass and collagen production obtained in our study were of 108 ± 1 % year $^{-1}$; 129 ± 116 g DW dm^{-3} year^{-1}; 39 ± 35 g DW of collagen dm^{-3} year $^{-1}$, respectively. Overall, *C. reniformis* showed a significant growth in volume and biomass during the four years of monitoring period, with the largest increases between the initial times and intermediate time periods, with a stabilization of growth in the final months (January and July 2024).

This is the first study applying SfM-photogrammetry to assess the growth rates and the biomass production of *C. reniformis*, proving to be an effective approach for the long-term sponge growth's monitoring. Although presenting some limitations linked to water turbidity and sponges structural complexity, SfM-photogrammetry resulted as a suitable non-invasive compromise for *in situ* volume and biomass estimations and represented a strong candidate for the establishment of a one size fits all monitoring tool to assess different sponge morphologies. The outcomes of this study indicate that *C. reniformis* holds promise for sustainable mariculture aimed at collagen production, thanks to its high growth rates and phenotypic plasticity. However, further long-term monitoring studies are needed to ensure the feasibility of a sustainable exploitation of the species. It is, in fact, essential to develop sustainable management strategies to avoid negative ecological side effects and to promote local blue economy, while contributing to the conservation of natural populations.

Riassunto

Chondrosia reniformis (Nardo, 1847) è una spugna incrostante, spessa, liscia e lobata appartenente alla classe Demospongiae, con una distribuzione Atlanto-Mediterranea, che occupa solitamente i fondali rocciosi costieri a basse e medie profondità (0-50 m). La principale caratteristica della specie è di essere costituita quasi interamente da collagene, rendendola di grande interesse per l'industria farmaceutica e nutraceutica. Tuttavia, come molte altre spugne, la sua sopravvivenza è stata messa a dura prova negli ultimi decenni a causa dell'aumento della frequenza delle ondate di calore legate ai cambiamenti climatici, che hanno portato a eventi di mortalità di massa delle popolazioni più superficiali. Inoltre, il potenziale sfruttamento della spugna per applicazioni biotecnologiche richiede una valutazione adeguata della sua distribuzione, stato di conservazione, tassi di crescita e rigenerazione. In questo contesto, in questo elaborato di tesi è stata condotta una revisione sistematica per riassumere le conoscenze attuali su *C. reniformis*, con particolare attenzione agli studi sulla sua coltivazione in situ. Inoltre, è stato valutato l'uso della tecnica fotogrammetrica 'Structure from Motion' (SfM) come approccio non invasivo, e a basso costo per monitorare l'abitudine di crescita, il volume e i tassi di rigenerazione di questa spugna su un lungo periodo di tempo all'interno di un sistema di maricoltura *in situ*, allestito lungo la costa di Alassio (Liguria, Italia)

nel maggio 2021, sopra barriere artificiali preesistenti. La biomassa e la produzione di collagene sono state altresì stimate a partire dal volume ottenuto con la fotogrammetria SfM.

La revisione ha mostrato che le conoscenze attuali su *C. reniformis* coprono diversi campi, tra cui ecologia, biochimica, biotecnologia, fisiologia, biologia molecolare e microbiologia. Tuttavia, alcuni campi hanno ricevuto maggiore attenzione, come biotecnologia e biochimica, rispetto ad altri. Fino ad oggi, sono stati fatti solo alcuni tentativi per sviluppare metodi adeguati di maricoltura in situ per la spugna *C. reniformis*, evidenziando la necessità di ulteriori studi per ottimizzare le metodologie di coltivazione.

Gli aumenti medi annui di volume, biomassa e produzione di collagene ottenuti invece nel nostro studio sono stati rispettivamente di 108 ± 1 % anno^{-1}; 129 ± 116 g di peso secco dm^{-3} anno^{-1}; 39 ± 35 g di peso secco di collagene dm^{-3} anno^{-1}. Nel complesso, *C. reniformis* ha mostrato una crescita significativa in volume e biomassa durante i quattro anni di monitoraggio, con i maggiori aumenti tra i tempi iniziali e i periodi intermedi, con una stabilizzazione della crescita negli ultimi mesi (gennaio e luglio 2024). Questo è il primo studio che applica la fotogrammetria SfM per valutare i tassi di crescita e la produzione di biomassa di *C. reniformis*, dimostrando di essere un approccio efficace per il monitoraggio a lungo termine della crescita delle spugne. Sebbene presenti alcune limitazioni legate alla torbidità dell'acqua e alla complessità strutturale delle spugne, la fotogrammetria SfM si è rivelata un compromesso non invasivo adatto per le stime *in situ* di volume e biomassa e rappresenta un buon candidato per l'istituzione di uno strumento di monitoraggio standardizzato per valutare diverse morfologie di spugne. Inoltre, i risultati di questo studio indicano che *C. reniformis* ha un potenziale per la maricoltura sostenibile finalizzata alla produzione di collagene grazie al suo elevato tasso di crescita e alla sua plasticità fenotipica. Tuttavia, sono necessari ulteriori studi di monitoraggio a lungo termine per garantire la fattibilità di uno sfruttamento sostenibile della specie. È quindi essenziale sviluppare strategie di gestione sostenibile per evitare effetti ecologici negativi e promuovere un'economia blu locale, contribuendo allo stesso tempo alla conservazione delle popolazioni naturali.

1. Introduction

1.1 Characteristics of the phylum Porifera

1.1.1 Biology and ecology of Porifera

Porifera are the phylogenetically oldest, extant metazoans. These invertebrates are important sessile filter-feeders distributed worldwide, in all oceans and in many freshwater habitats, from the tropics to the highest latitudes. Thanks to their phenotypic plasticity, they are able to adapt to different environmental conditions, occupying a wide bathymetric range, from the intertidal zone to hadal depths (up to 8000 m) (Hooper et al., 2002; Van Soest et al., 2012). Their simple body organization and relative plasticity of the cellular elements have led to a great diversity inside the phylum Porifera, with about 9650 species considered valid to date (de Voodg et al., 2024). Unlike the other animals, sponges are the simplest group, lacking true tissues and organs, and presenting a simple level of organization (Figure 1): all sponges have an epithelium of T-shaped or flattened cells (called pinacocytes) which covers the outside of the sponge, as well as its internal body chamber (called spongocoel), containing flagellated choanocytes (choanoderm), and an intermediate non-epithelial layer of varied thickness, called mesohyl, comprised between the pinacoderm and the choanoderm (Adamski, 2021). Choanocytes are flagella-bearing cells surrounded by a collar of microvilli, which, with their beating, maintain a unidirectional water current through the body and are specialized in the capture of food particles (Leys et al., 2012).

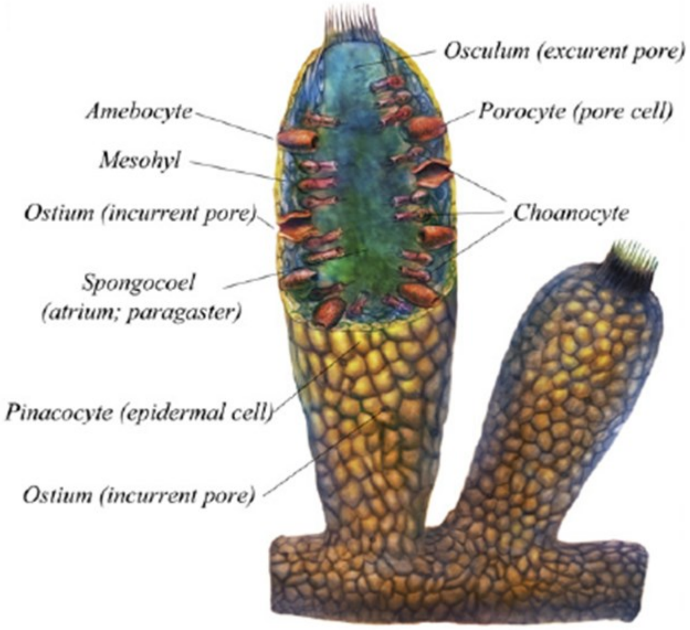


Figure 1. Schematic cross-section of a sponge anatomy showing the three layers of the body wall, including the external pinacocyte cells, the internal choanocyte cells, and the mesohyl separating them, incurrent pores in the body wall (ostia), and an excurrent opening (osculum) to the body chamber (spongocoel) (from Yin et al., 2015).

The water enters sponges' body through numerous small pores, called ostia, moves through inhalant canals to choanocytes and leaves through larger excurrent openings, the oscula. The presence of a differentiated inhalant and exhalant aquiferous system with external pores can be considered an autapomorphy of the phylum, and is used to pump the water efficiently, given the characteristic filtering activity of these organisms (Hooper et al., 2002). The pumping rate of sponges is considerable; for example, a massive sponge can pump about 1000 times its own volume of water over 24 h (Reiswig, 1971), and they can process up to 35 ml min^{-1} (cm sponge) $^{-3}$ (Weisz et al., 2008). The only exceptions are the so-called carnivorous sponges, highly adapted deep-sea forms, in which the aquiferous system is non-existent, but present a sticky outer surface with which small preys are captured (Van Soest et al., 2012). Depending on the organization of the aquiferous system in the internal region of a sponge and the presence of chambers in the body wall, sponges can be described as having an ascon, a sycon, or a leucon structure: in its simplest form – asconoid organization – the body chamber is constituted by a simple tube lined by choanocytes; in the syconoid structure, the body chamber is formed by a tube with fingers lined by choanocytes; then, in the leuconoid structure, the body chamber presents several canals leading to spherical or ovoid chambers lined with choanocytes (Figure 2) (Leys et al., 2012). A solenoid and sylleibid organization have also been described as characteristic in some species (Figure 2) (Adamska, 2021).

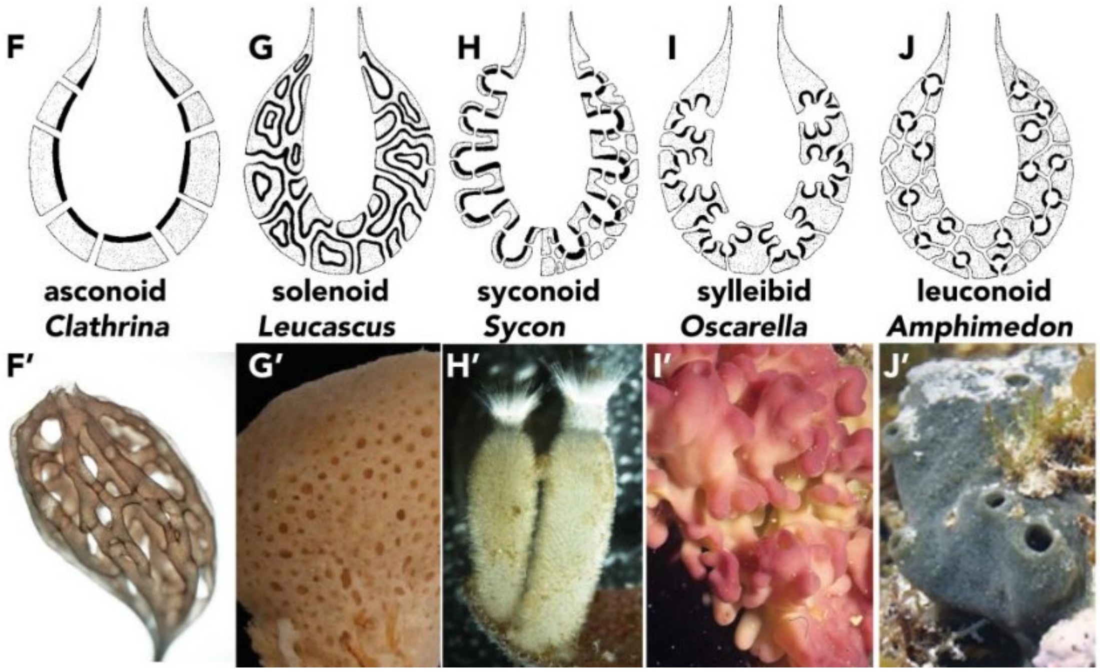


Figure 2. Schematic (F-J) and live (F'-J') examples of the different body organization which can be found in Porifera (edited from Adamski, 2021).

The space between the pinacoderm and the choanoderm is filled with a collagenous matrix, the mesohyl, which harbours various mobile cells and, usually, a skeleton made of spongin, mineral (silica or calcium carbonate) or both. The consistency of sponges varies with the nature and the density of the skeleton from soft crumbly to stiff elastic or stony hard (Rützler, 1986).

Depending on the combination of skeletal elements, organization and characteristics of larvae, sponges are divided into four classes: Calcarea, Hexactinellida, Homoscleromorpha, and Demospongiae, varying greatly in shapes and sizes (Van Soest et al., 2012). Representatives of the class Calcarea (calcareous sponges) are all marine species, including approximately 770 species, characterized by a mineral skeleton built of spicules of calcium carbonate in the form of calcite. Spicules can be of one axis, three rays, four rays and/or multiradiate spicules. A dense basal skeleton, with the main spicules cemented together, is sometimes present. Calcareous sponges are viviparous, with two unique types of hollow larvae.

Representatives of the class Hexactinellida (about 670 species), commonly called glass sponges, are all marine, mainly deep-sea, sponges. They are characterized by a syncytial body plan organization and spicules are represented by hexactins (six rays), while dense spongin or non-spicular skeletons are absent. All glass sponges are viviparous, with a trichimella larvae.

The class Homoscleromorpha represents the smallest class, including only 120 marine species. The inorganic skeleton, if present, consists of small siliceous calthrops (tetraxon spicules with equal rays) and/or their derivatives. All Homoscleromorpha are viviparous with hollow cinctoblastula larvae.

The class Demospongiae is the most representative class of the entire phylum, including about 8850 species inhabiting both marine and fresh waters (Ereskovsky & Lavrov, 2021), with a skeleton made either of spongin fibres only or of spongin fibres in combination with siliceous spicules. In some groups, there are no special skeletal elements at all (e.g., *Halisarca dujardini, Chondrosia reniformis, Thymosiopsis conglomerans*), while in others a hypercalcified basal skeleton develops in addition to other skeletal elements (e.g., *Astrosclera willeyana*; *Acanthochaetetes wellsi*). Larvae are mostly parenchymellae or, in some groups, single-layered larvae. Reproductive strategies within the class are both oviparity and viviparity.

As previously mentioned, sponges can reproduce both sexually and asexually. Porifera can be gonochoric, sequential, or simultaneous hermaphrodites. Fertilization can be internal or external, with at least six major types of larvae (plus secondarily acquired direct development in some species) described to date (Maldonado, 2006). Usually, archeocytes contained in the mesohyl develop into egg cells, and sperm cells develop from the choanocytes. Fertilized eggs develop into a cluster of cells, with small flagellate cells at one end and larger, epithelial-like cells at the other. As the larva grows, the flagellate cells fold into the layer of larger outer cells. Once fully developed, the larvae settle, and transform into young sponges (Rigby, 1983). Conversely, the asexual reproduction can include simple budding, or formation of highly specialized structures called gemmules, produced during stressful periods and remarkably resistant to dehydration. Many sponge species seem also to be able to regenerate from fragments (Rigby, 1983).

1.1.2 Ecological role of Porifera

Sponges are considered crucial ecosystem engineer in many habitats worldwide: with their aggregations, they increase the habitat complexity and consequently the abundance and biodiversity of associated benthic species (Bell, 2008; Folkers et al. 2020). In some habitats, such as coral reefs, they can also act as bioeroders, especially behaving as intermediate disturbers and representing the main driving force in the turn-over of bioconstructions (Van Soest et al., 2012). Sponges are also efficient filter feeders, and they play an interesting role in biogeochemical cycling and in the benthic-pelagic coupling of inorganic nutrients (e.g., dissolved carbon, various nitrogen compounds and silicates) within the ecosystem (Lesser et al., 2006; Maldonado et al., 2012). By pumping large volumes of seawater, sponges are in fact able to process inorganic nutrients and dissolved organic matter through a combination of metabolic processes that include feeding, respiration, egestion, and excretion. Additionally, sponges are a reservoir of microbial diversity, and the associated microbiome play a decisive role in the nitrogen cycle of many habitats and may contribute significantly to the organic production in oligotrophic habitats (Van Soest et al., 2012). In this way, they impact and regulate the availability of nutrients and compounds in the surrounding environments (Maldonado et al., 2012; De Goeij et al., 2013).

However, even though sponges provide a wide range of ecosystem services and cover a crucial functional role in the benthic-pelagic coupling, they still remain one of the most underappreciated marine groups, being poorly represented in many research, monitoring and conservation programs (Bell, 2008).

In the Mediterranean Sea, 785 valid species of sponges have been listed until today, with the Western Mediterranean showing the highest number of species (422) (de Voogd et al., 2022). They are present in several crucial habitats, including *Posidonia* meadows, marine caves and coralligenous assemblages, and in the bathyal zone, where they can create the so-called "sponge grounds", supporting rich biodiversity levels and representing a secondary substrate for a specialized associated fauna (Koukouras et al., 1996; Coll et al., 2010; Bo et al., 2012; Gerovasileiou et al., 2016). Due to the fundamental functional role covered by sponges in marine ecosystems, several species are under the protection of the international legislation. For example, for the Mediterranean Sea, a total of 15 sponge species are listed in the Annexes of the Bern and Barcelona Conventions, as well as many are included in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, being considered severely endangered or threatened (Roveta et al., 2022; IUCN, 2024). Major concerns have been raised regarding Mediterranean Porifera, since massive mass mortality events (MMEs) have brought entire populations of some sponge species to the brink of extinction during the last three decades, probably linked to microbial invasion and to the increased frequence of climatic anomalies (Garrabou et al., 2019, 2022). As a point of fact, the current climate crisis is causing an increase in the frequency and intensity of marine heatwaves (MHWs), and MMEs of marine organisms are one of their main ecological consequences (Garrabou et al., 2022). Elevated seawater temperatures can affect the frequency and severity of disease outbreaks by increasing the prevalence and virulence of pathogens, facilitating invasions of new pathogens or reducing host resistance and resilience (Webster, 2007; Sutherland et al., 2004). The reported MMEs of benthic organisms mainly concerned the Western Mediterranean ecoregion and in terms of taxonomic groups, Cnidaria and Porifera accounted for 85% of the observations. Mortality events for Porifera were recorded in a greater number of geographic areas compared to cnidarians, including areas which have been historically harvested for commercial bath sponges (e.g., Aegean Sea and Tunisian Plateau/Gulf of Sidra) (Garrabou et al., 2019). Shallow benthic communities have suffered a loss of structural complexity, due to the marked decrease of this habitat-formers, and it has been observed an impressive and fast shift of the benthic assemblage from a biocoenosis mainly composed of slow-growing and long-lived species to a biocoenosis dominated by fast-growing and short-lived species (e.g., algae) (Di

Camillo & Cerrano, 2015). Together with climate change, habitat destruction, eutrophication, establishment of alien species, fishing impacts and pollution may represent the others major threats to sponges abundance and biodiversity (Coll et al., 2010).

1.2 The economic value of marine sponges

The importance of sponge protection and conservation is also related to their high economic value. Sponges have been known to mankind since the earliest civilizations (4000 YBP) and with a wide range of uses, spanning from decoration, personal hygiene, food and medicine (Pronzato et al., 2008). The exploitation of sponge populations for their trade dates back to ancient Greeks and Romans, when some species were highly valued and used as bath utensils: the so called "bath sponges". Mediterranean species of bath sponges belong to four species of the genus *Spongia* (*S. officinalis, S. mollissima, S. lamella,* and *S. zimocca*) and one species of the genus *Hippospongia* (*H. communis*), whose softness is due to the complete absence of a mineral skeleton. Their exploitation continued for centuries, reaching a mean production of about 350 tons$\cdot$year^{-1} (reported by the FAO for the Mediterranean Sea in the 1930s), until the 20th century, when the sponge trade suffered a strong decrease mainly related to overfishing and to the so called "sponge disease" (Pronzato & Manconi, 2008).

In fact, in 1986, a severe epidemic affecting horny sponges broke out in several areas of the Mediterranean Sea (Gaino et al., 1992), with bacteria penetrating fibres and causing profound alterations of the skeleton, thus making sponges unsuitable for commercial purposes. Disease events became more frequent and deadly in the following years (Vacelet et al., 1994), enhancing the effects of overfishing, to the point that some species were on the brink of extinction. As a consequence of periodical depletion of bath-sponge stocks, overfishing and repeated MMEs, several mariculture projects were started to prevent the depletion of sponges' natural populations (Duckworth, 2009; Celik et al., 2011; Bierwirth et al., 2022).

At present, bath sponges represent a "niche product" supplied to selected consumers, mainly due to the introduction of synthetic sponges. However, recently, a new commercialization of sponge-derived products has begun, after the discovery of biologically active metabolites produced by many species (Duckworth, 2009). As a point of fact, Porifera are rich in secondary metabolites which perform a wide range of ecological functions, from chemical defence against predation and biofouling organisms to cellular communication and competition for space (Proksch, 1994). Sponges are also well known for containing substantial amounts of symbiotic microorganisms in their body, including bacteria, archaea, cyanobacteria, microalgae, and fungi, which can account for up to 40-50% of the biomass of the sponge itself and perform a wide range of functional roles: production of a diversity of chemical compounds, vitamin synthesis, biochemical transformations of nutrients and waste products (Taylor et al., 2007; Moitinho et al., 2017; Mazzella et al., 2024). Interestingly, there is a considerable debate about whether sponges are the true producers of bioactive metabolites or just hosts to the true producers, the associated microorganisms. Either way, it is important to investigate the possibilities for marine natural products within the holobiont, as they ultimately provide the compound as a whole organism (Folkers & Rombouts, 2020; Cerrano et al., 2022). Thousands of sponge-derived bioactive metabolites have been isolated and identified so far, including acids, alkaloids, esters, fatty acids, terpenes, all of which exhibit a broad array of pharmacological properties such as anticancer, antiviral, antimicrobial and anti-inflammatory drugs (Blunt et al., 2009; Mehbub et al., 2016; Shanmugam & Vairamani, 2016). However, one of the major obstacles for these marine natural products to reach the pharmacological market is the so called 'supply problem', as wild harvests of marine sponge populations to obtain high amounts of substance are not justifiable and offend against the provisions on sustainable use of natural resources (UN, 1992; UNEP, 2000; Nickel & Brümmer, 2003). To address this issue different approaches are being developed for producing sponge biomass, such as *in situ* aquaculture, breeding in aquaria, in vitro culture systems for the cultivation of sponge cells, organotypic culture systems, three-dimensional growing primmorphs or the use of sponge fragments (Brümmer & Nickel, 2003).

The environmental and economic potential of sponges continues to rise when we consider their proven biofiltration and ecosystem restoration ability. Given their highly efficient filtration capacity of organic particles, such as dissolved and particulate organic particles, bacteria, phytoplankton and even viruses, their growth leads to the bioremediation of the surrounding environment (Gökalp et al., 2021; Aguilo-Arce et al., 2023). Similarly, they are constantly exposed to contamination from the water column. Various experiments have been carried out to test the accumulation capacity and levels of pollutants, such as trace elements and persistent organic pollutants, in different sponge species, suggesting them as both bioindicators and bioremediation organisms (Batista et al., 2014; Roveta et al., 2021, 2023). In conclusion, the economic value of their seawater filtration rates, as estimated by Pham et al. (2019) being nearly double the market value of the fish catch, further highlights their ecological and economic importance.

1.3 The target species: Chondrosia reniformis

Chondrosia reniformis (Nardo, 1847) is a thick encrusting, smooth, and cushion-shaped sponge belonging to the class Demospongiae (Hooper et al., 2002). Under the effect of light intensity, its body colour varies to white, brown or black. This species was considered to have a worldwide distribution, including the Atlantic, the Pacific, the Indian oceans and the Mediterranean Sea. It lives on shady rocky cliffs or caves at a depth of up to 50 m and it can be found in shallow, mesophotic, and oligotrophic habitats (Di Camillo et al., 2012; Moussa et al., 2022). Unlike the other members of the phylum Porifera, *C. reniformis* lacks siliceous spicules or the reinforcing spongin fibres in its skeleton (Parma et al., 2007). The bulk of the body of *C. reniformis* is mainly made of tightly packed collagenous fibres present in the cortical region of the body of the sponge, called cortex or ectosome, and in the intermediate one, called mesohyl (Figure 3). The ectosome, can also actively incorporate a wide range of foreign materials, including sand grains, silicates and quartz (Bavestrello et al., 1998). The choanosome is of a softer consistency and densely filled with choanocyte chambers (Figure 3).

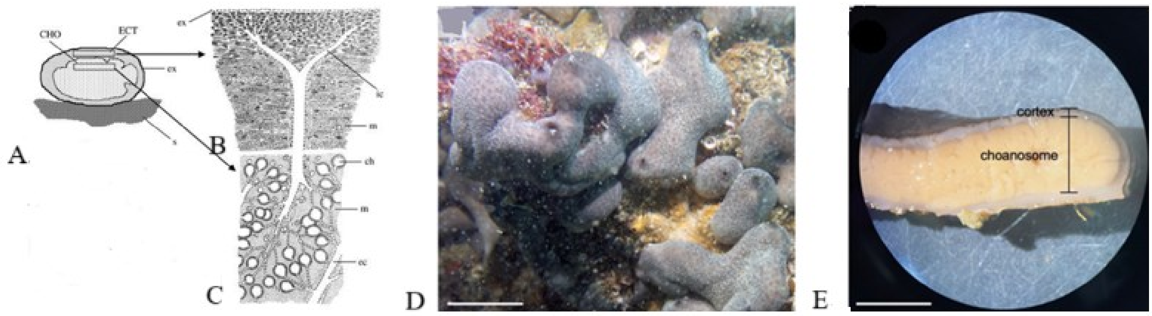


Figure 3. (A) Vertical section through a whole specimen of *Chondrosia reniformis*, showing the location and orientation of choanosome (CHO) and ectosome (ECT), ex (exopinacoderm), s (substrate); (B,C) Ch (choanocyte chamber), ec, (exhalant canal), ic (inhalant canal) and m (collagenous mesohyl) (edited from Wilkie et al., 2006). D) External view of *C. reniformis* in the wild. (E) View of the inside of a *C. reniformis* sample, divided into two body regions, the external cortex and the internal choanosome. Scale bars: 2 cm (edited from Roveta et al., 2022).

Concerning the growth dynamics of *C. reniformis*, a seasonal pattern was observed possibly related to changes in the water temperature as the sponge slightly increases in size from April to December and shrinks from January to March (Di Camillo et al., 2012). However, growth rates may be associated also with the greater variety of microhabitat conditions, such as the presence of competitors and food availability (Garrabou & Zabala, 2001).

It has also been demonstrated a striking tissue plasticity of this sponge body in terms of both shape and mechanical properties (Fassini et al., 2012). For example, after excision, fragments of *C. reniformis* undergo rounding off of cut surfaces and marked bending. The whole sponge is also able to react to mechanical stimulation by stiffening its collagenous body. In natural conditions, *C. reniformis* can undergo to a phenomenon called “creeping”, during which part of the sponge loose from the substrate followed by its slow elongation due to gravity and its eventual separation from the still attached portion. Some authors believe that this phenomenon can be seen as a form of opportunistic asexual reproduction (Wilkie et al., 2006; Fassini et al., 2012), while others relate it to atypical mechanisms of localized locomotion or passive response to environmental stress (Figure 4) (Bonasoro et al., 2001; Fassini et al., 2012). Both the instability of the substratum and the temperature are important factors triggering the start of the “creeping” behaviour, reported to occur mainly from spring to early summer (Parma et al., 2007).

Figure 4. An outgrowth generation from the sponge parental body during the “creeping” phenomenon (photo courtesy: Torcuato Pulido Mantas).

C. reniformis can reproduce also by sexual reproduction, being a gonochoric broadcaster (Di Camillo et al., 2012). Male gametes usually derive from choanocytes, whereas the origin of oocytes is more complicated; in some cases, they can originate from amoebocytes contained in the mesohyl, while in others from the choanocytes (Leys & Hill, 2012). Its reproductive cycle is believed to be influenced by temperature, as usually oocyte production start along with the temperature increase in June and it is completed after 3 months, in late August, concurrently with the annual maximum temperatures (Riesgo & Maldonado, 2008; Idan et al., 2020). Among areas, oogenesis is reported to varied from seasonal to continuous, and around May to August (Di Camillo et al., 2012; Riesgo & Maldonado, 2008). Spermatogenesis seems to be rapid and probably synchronized with the last developmental stage of the oocytes (Di Camillo et al., 2012). Both the dispersal capability of the lecithotrophic larvae and the gametes' dispersal are probably low.

The interest for this sponge has grown over the past years, especially for its ability to synthesize large amounts of collagen, characterized by a unique dynamic plasticity and the ability to reversibly alter its viscoelastic properties in an extraordinary short-time span (Pozzolini et al., 2018). Thanks to its low immunogenicity and mechanical properties, it can also serve as an alternative source of collagen, replacing the one derived from bovine and porcine skin and bones (Pozzolini et al., 2012). In the past, this collagen was partially biochemically characterized (Garrone et al., 1975) and, more recently, some of its gene sequences were identified (Pozzolini et al., 2012). Its biocompatibility on human skin has already been evaluated (Swatschek et al., 2002) and its use in the form of nanoparticles as carriers and coatings for drug preparations also described (Kreuter et al., 2003; Nicklas et al., 2009). The potential applications of collagens extracted from this sponge include Tissue Engineering and Regenerative Medicine (TERM), wound healing, drugs and gene delivery/carrier; cosmetic, food industry as well as nutraceutical (Nicklas et al., 2009; Fassini et al., 2017; Pozzolini et al., 2018; Orel et al., 2021; Tassara et al., 2023). Furthermore, thanks to the rich community of microorganisms hosted inside the sponge body (ca. 10^8 to 10^{10} microbial cells/g tissue), this species can produce a variety of unusual chemical compounds for its defence mechanisms against biofouling and predators (Taylor et al., 2007). These compounds are considered possible resources to provide future drugs with anti-bacterial, anti-cancer, anti-fungal and anti-inflammatory activity (Altuğ et al., 2012).

As already mentioned, the problem that may arise from the exploitation of this sponge for biotechnological purposes are that enormous sponge biomasses are required to perform acceptable preclinical and clinical trials and to obtain a sufficient amount of products (Folkers et al., 2020). As harvesting the enormous sponge biomass from the environment would not be sustainable, some studies have explored the in vitro sponge fragments or cellular culture, which avoids the complex environment necessary for whole organisms (Müller et al., 2000; Nickel & Brümmer, 2003; Osinga et al. 2003). However, long-term in vitro cultivation of marine sponges has been found to be extremely difficult and mass production of sponge biomass under completely controlled conditions has not been realized so far (Brümmer & Nickel, 2003; Duckworth, 2009). Land-based culture needs further research and is not likely to be commercially feasible in the near future. Until now, in situ sponge aquaculture remains the easiest and least expensive way to obtain sponge biomass in large amounts. A recently developed method to culture *C. reniformis* in an integrated mariculture seems to be a promising approach, to enable a sustainable and high-yielding marine collagen production process that is adaptable to seawater environments combined with organic matter sources such as fish culture or sewage outfall (Gökalp et al., 2022).

1.4 Photogrammetry and 3D reconstruction in sponge monitoring

Traditionally, approaches to estimates sponge growth and biomass have been destructive and often not suitable for certain morphologies. Wet weight, dry weight, ash-free dry weight, radiocarbon dating methods are often fatal for the organism and not adapted to all sponge growth habits (e.g., encrusting, rope-like, tubular, or massive) (Çelik et al., 2011, Pulido Mantas et al., 2024). Other common methods for measuring the volume and surface area of sponges, such as water displacement (Jokiel et al., 1978) and paraffin dipping (Veal et al. 2010a), require removal of the subject from its natural environment and its relocation typically resulting in the death of the organism, thus preventing continuous observations over time (Lavy et al., 2015). Further methods, although non-invasive and non-destructive, such as planar projection photography and the use of digital imaging software to measure sponge surface area (van Treeck et al., 2003), mainly record two-dimensional metrics even though sponges often present complex three-dimensional (3D) structures (Pulido Mantas et al., 2024). Recently, technological and methodological advancements have helped to develop new techniques to capture 3D features in underwater environments, such as photogrammetry (Pulido Mantas et al., 2023). Photogrammetry can be defined as "the art, science, and technology of obtaining reliable information about physical objects and the environment through processes of recording, measuring, and interpreting images and patterns of electromagnetic radiant energy and other phenomena" (McGlone et al., 2004). The first attempt to accurate measure volume of sponges with photogrammetric technique was made by Abdo et al. (2006), using a stereovision system. However, with the continuing software and imaging technology developments and the optimization of pattern recognition algorithms, it was possible to give birth to one of the most popular and applied procedures today: Structure from Motion (SfM) photogrammetry. Its main difference with stereo photogrammetry technique is its highly automated nature: the whole 3D geometry of the scene, including the camera orientation and positions, is solved through the implementation of a series of algorithms over a dataset of 60-80% of consecutive overlapping imagery (Westoby et al., 2012; Pulido Mantas et al., 2023). Furthermore, SfM-photogrammetry can be implemented using imagery from a wide range of cameras, from single lens reflex (SLR) to consumer-grade sport cameras, enhancing its accessibility and versatility for an array of uses (Burns et al., 2015). The application of SfM-photogrammetry technique to monitor sponges has allowed to advance the study of sponge distributions (Prado et al., 2021), age and growth estimations (Olinger et al., 2019), volume and biomass (Pulido Mantas et al., 2024) as well as its interactions with other organisms (Olinger et al., 2021). This non-destructive, repeatable technique provides a permanent record and allows for measurements that were previously unattainable with 2D methodologies. Moreover, it does not disrupt benthic communities, making it ideal also for the monitoring of vulnerable ecosystems and marine protected areas (MPAs) (Prado et al., 2021).

1.5. Aim of the study

Marine Porifera perform a wide range of ecological functions and can provide services and economic benefits also for humans, thanks to the diverse array of the bioactive compounds produced. The ERA-NET Blue-Bio Cofund project MedSpon (https://bluebioeconomy.eu/characterization-of-new-antibiotic-principles-against-who-priority-pathogens-of-sustainable-produced-marine-sponges-for-nutraceutical-applications/), concluded in August 2023, aimed to explore the biotechnological potentials of the sponge species *Chondrosia reniformis* and *Axinella polypoides*, through a sustainable exploitation of these organisms. Within the project, the Polytechnic University of Marche was involved to study the sponges *in situ* biology, habitat specifications and providing information for *ex situ* or *in situ* cultivation. Since sponge populations have been decreasing over the past decades, due to a synergy of climate change and human induced pressures, the rising of commercial interest for this species creates the urgency to properly evaluate sponge growth and regeneration rates, before considering any kind of industrial exploitation or mariculture purposes.

Within this thesis, a systematic review was conducted to summarize the current knowledge on *C. reniformis* and understanding how the scientific interest about this sponge has shifted over the years, paying special attention to *in situ* cultivation studies. Additionally, this thesis aimed to understand and assess growth and regeneration rates, biomass and collagen production of *C. reniformis* cultured, by means of SfM-photogrammetry technique and 3D analysis.

Therefore, the research questions at hand were:

1) Which is the state of the art on *C. reniformis* and the *in situ* cultivation techniques regarding this species?

2) Which are the annual growth rates, average biomass and collagen production of *C. reniformis*?

3) Is SfM-photogrammetry an effective approach for the long-term monitoring and assessment of this sponge's growth?

4) Is this sponge suitable to be sustainably reared for nutraceutical applications?

The outcome of the current study provided new insights in the mariculture of a very common sponge species, targeted for biotechnological purposes, promoting a sustainable harvesting of sponges, contributing with a baseline in the transplantation and monitoring techniques, thus paving the way for the set-up of successful *in situ* aquaculture systems.

2. Material and methods

2.1 Literature review

2.1.1 Data collection and literature analysis

To summarize the current knowledge about *Chondrosia reniformis,* a systematic review following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; Page et al., 2021) statement as a guide was conducted. The data were collected from an extensive bibliographic research on Web of Science (www.webofscience.com) and Elsevier's Scopus (www.scopus.com) databases, using the keyword "*Chondrosia reniformis*" in the option "all fields" in Web of Science and in the option "Article title, Abstract, Keywords" in Scopus, in all years until the cut-off date of 16th September 2024. Only peer-reviewed articles were considered for this study, while systematic reviews, editorials and congress proceedings were not included by choice. To select which papers to include in the analyses, a three-step process was implemented as follows: (i) duplicates were excluded, (ii) title and abstract screening was performed to identify potentially relevant manuscripts, and (iii) a full-text screening of the selected documents from step (ii) was conducted. Only documents matching our inclusion criteria were considered eligible, while the others were excluded. The inclusion and exclusion criteria are summarized in Table 1, while the flow chart of the searching strategy and the eligibility process is given as Figure 5.

Table 1. Research procedure and inclusion and exclusion criteria.

Years	1975-2024
Search terms	“*Chondrosia reniformis*”
Database	Scopus and Web of Science
Inclusion criteria	Journal articles Studies concerning the species *C. reniformis* Studies conducted both on field and laboratory Published in English, Italian, French or Spanish
Exclusion criteria	Duplicated manuscripts Systematic review, editorials, proceedings papers Studies not concerning the species *C. reniformis*

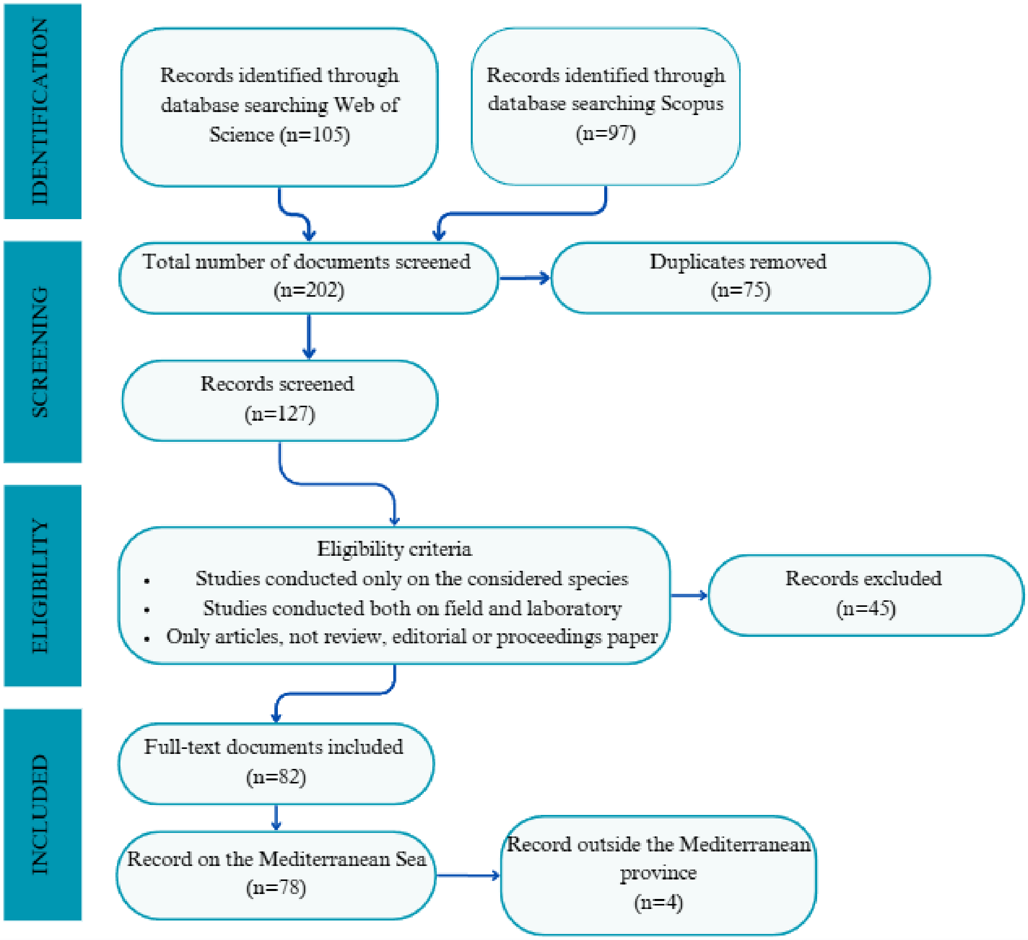


Figure 5. Flow chart illustrating the steps for documents screening and selection on the sponge *Chondrosia reniformis* from Scopus and Web of Science databases.

From each of the included manuscripts, different information was obtained (Table 2), specifically: (i) the discipline (Biochemistry, Biotechnology & Blue economy, Ecology & Evolution, Microbiology, Molecular biology and Physiology); (ii) some discipline were further divided in subdisciplines, in particular Biochemistry (i.e., Biochemistry, Ecotoxicology, Biomineralogy, Biomonitoring), Biotechnology & Blue economy (i.e., Biotechnology, Biomaterials, Pharmacy, Mariculture), and Ecology & Evolution (i.e., Ecology, Evolution biology, Population genetic); (iii) the setup of the study (Field, Laboratory or Field & Laboratory); (iv) the geographic coordinates (when present) of the sampling point(s); (v) the ecoregion of the sampling site(s); (vi) the objective of the study.

Table 2. Parameters used to classify studies on *Chondrosia reniformis* during the data extrapolation phase.

Category	**Definition**
(A) General features of the manuscript	
Year	Year of publication
Authors	Authors of the publication
Title	Title of the publication
DOI	DOI of the publication
Country	Ecoregion where the study was conducted
(B) Features extracted from each survey	
Branch of science	Discipline in which the study was framed
Work setup	Setup in which the survey has been performed: (i) Field; (ii) Laboratory; (iii) Field & Laboratory
Location	Geographic coordinates of the sampling point(s) of the survey

Objective	Specific research objective of the study

The creation of the map of documents distribution in the Mediterranean Sea was carried out following the bioregionalization of coastal and shelf areas proposed by Spalding et al. (2007) and the geographic coordinates of the sampling point(s), if available. When the given coordinates ended up in land, they were adjusted to the nearest coastal points.

2.2 Mariculture system

2.2.1 Study site

The in-situ mariculture system for *C. reniformis* explants was set up along the coast of Alassio (Liguria, Italy; Figure 6A, B), over already present artificial reefs, deployed in 1998–1999 to prevent trawl fishing at a depth of around 20 m (Figure 6C, D). Each module consisted of a concrete cube of 2 m side, with modules organized in groups of five blocks, forming a pyramidal system (Figure 6D) (Relini et al., 2007).

From an oceanographic perspective, the large-scale circulation a few miles offshore the study area is known to be characterized by the presence of the anticlockwise Liguro–Provençal Current (or Northern Current), while the circulation of the coastal area is characterized by a clockwise current, directed from southwest to northeast (Capello et al., 2014).

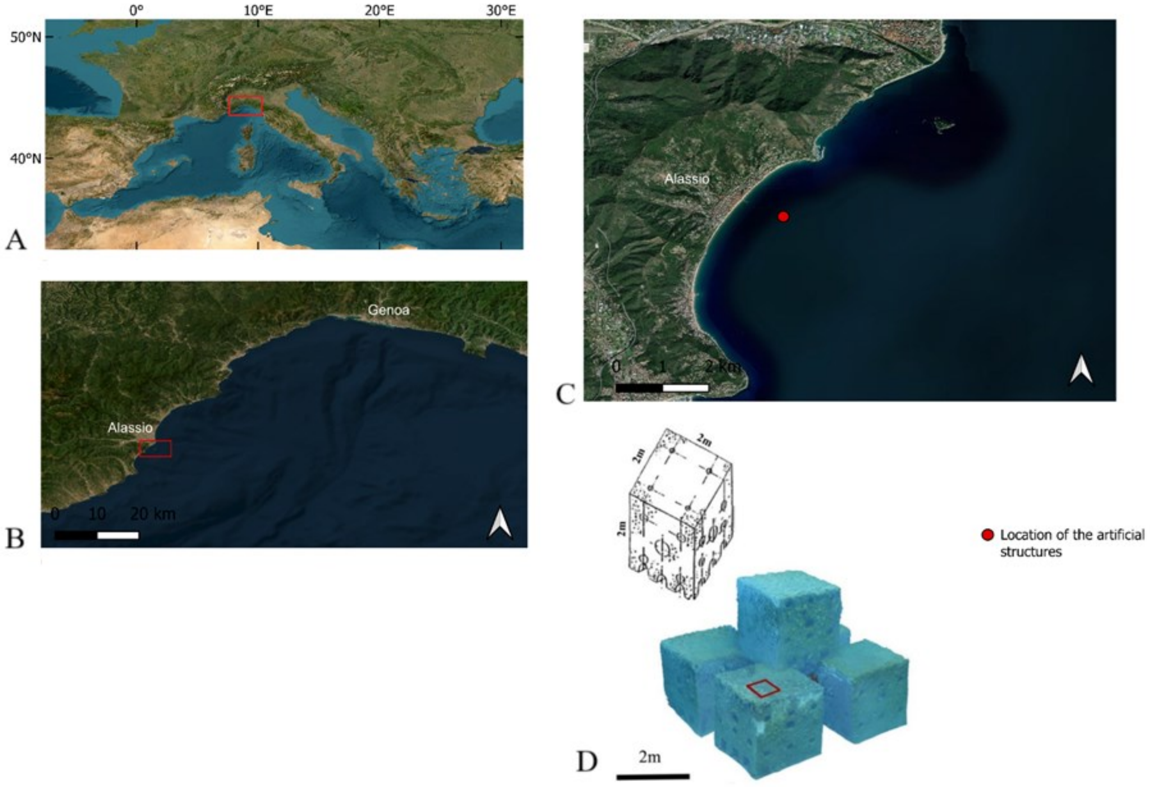


Figure 6. (A-C) Location of the study site and (D) artificial structures used as substrate for mariculture implants of *Chondrosia reniformis.*

2.2.2 Data collection and photogrammetric analysis

A total of 85 *C. reniformis* fragments were transplanted on the horizontal faces of 16 blocks and monitored through SfM-photogrammetry from May 2021 (t_1) to July 2024 (t_{14}). *C. reniformis* individuals were collected in the area nearby the artificial reef and transplanted in groups of 2-6 individuals under a plastic mesh fixed to the cube surface with a two-component epoxy (Subcoat S, Veneziani Yachting; https:// venezianiyachting.com/) to ensure the attachment of the sponge individuals to the surface. The plastic mesh was removed from the transplanted groups after 2/3 months, considered as the minimum time to guarantee the complete attachment of the sponge to the cube. From the 85 individuals, only the explants surveyed for a minimum of 9 months were considered for the yearly growth assessments, for a total of 48 sponges, organized into 12 explants (Figure 7). Additionally, two explants were cut during the monitoring period to investigate the potential regeneration of the sponge and to quantify its rates. Periods and number of explants surveyed for the study are defined in Table 3.

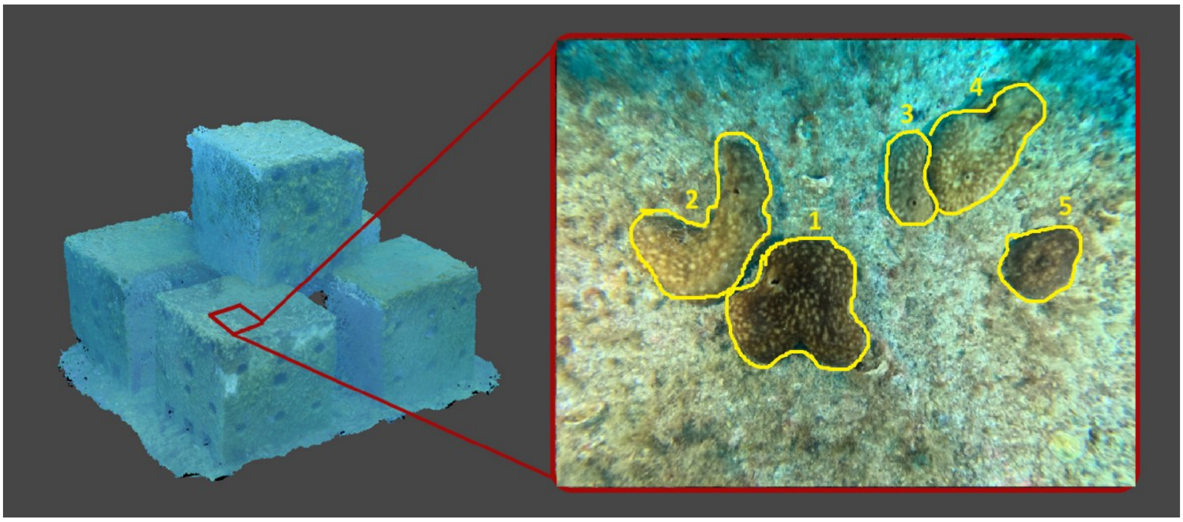


Figure 7. Transplanted *Chondrosia reniformis* fragments on the horizontal faces of the blocks.

Table 3. Summary of the monitoring activities.

Start Date	*End Date*	*Explant Code*	*Number of individuals*	*Cut*
20 May 2021	24 July 2024	Explant 0_1	6	Yes
19 November 2021	24 July 2024	Explant 0_I	3	No
19 November 2021	24 July 2024	Explant 0_II	4	No
19 November 2021	24 July 2024	Explant 0_III	4	No
17 October 2021	24 July 2024	Explant 1_3	4	No
17 October 2021	24 July 2024	Explant 1_4	6	No
19 November 2021	24 July 2024	Explant 1_6	4	Yes
28 February 2022	24 July 2024	Explant 3_I	3	No
28 February 2022	24 July 2024	Explant 3_II	2	No
28 February 2022	24 July 2024	Explant 3_III	5	No
9 April 2022	24 July 2024	Explant 1	3	No
9 April 2023	24 July 2024	Explant 2	4	No
Total		*12*	*48*	*2*

For the photogrammetric analysis, images were collected using a Sony RX100 V camera system equipped with an underwater housing and a couple of Akkin 5000. The photographic protocol consisted, in a first place, in the placement of metric references close to the sponge individuals, and subsequently a series overlapping images (40 to 60 pictures; Figure 8A) were taken around each sponge at an approximate distance of 0.5 m, covering in the process any possible perspective and ensuring a minimum of 60% overlapping among consecutive images (Bayley and Mogg, 2020).

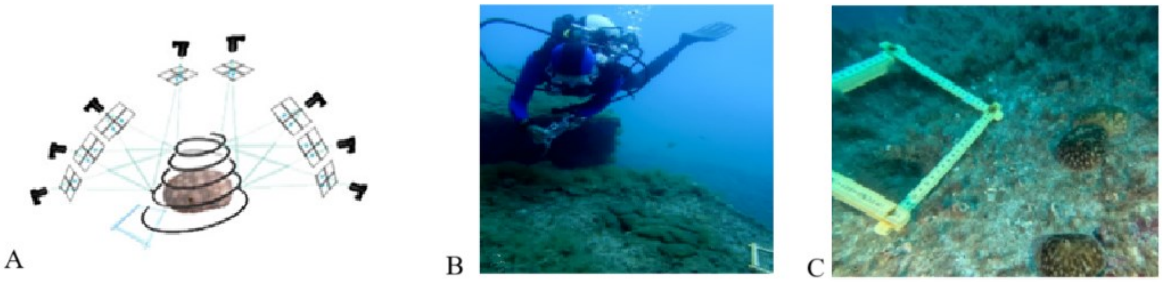


Figure 8. (A) Scheme of the image acquisition strategy. (B) Photographic sampling conducted by a diving operator. (C) Metric reference positioned next to the sponge individuals to be surveyed (photo courtesy: Torcuato Pulido Mantas).

To produce the digital reconstructions of the sponges, the software Agisoft Metashape (Agisoft LLC, St. Petersburg, Russia) was used. In the first place, a set of images was aligned using medium accuracy generic pair selection settings to produce the point clouds, limiting the key points identification to 10,000 common features. From this sparse cloud, the dense point cloud was obtained, and a mesh was produced by the arbitrary 3D surface type, high face count. Once the 3D models of the sponges were texturized, the 3D reconstructions were scaled up through the deployed metric references, manually detected in the imagery dataset and used to create scale bars in the reference settings. Substrate and references were removed from the model during the cleaning process. Subsequently, by using the "close holes" tool inside the mesh toolbox of Agisoft, it was possible to close the mesh of the reconstructions, allowing surface area and volume calculations. The overall photogrammetric process to generate, clean and measure each 3D reconstruction took around 60 minutes of processing time per sponge plot, using a HP Laptop 15s-eq2xxx with an AMD Ryzen 5 5500U with Radeon Graphics 2.10 GHz processor, 8 GB RAM.

2.2.3 Analysis of Chondrosia reniformis morphological changes

To assess potential changes in the morphology and shape of the monitored sponges, distance-based mesh comparisons were performed using CloudCompare v2.12 (2022) open-source software. To do so, the models corresponding to two subsequent sampling events of the same sponge individual were imported into the software; a preliminary manual mesh alignment was performed and finished using the "fine registration" tool, limiting the number of iterations to 99. Once the two model meshes were aligned, using the substrate level as a reference to control the alignment, distances between the two times meshes were calculated by the "Cloud/Mesh Dist" tool.

2.2.4 Biomass and collagen estimations

To estimate the biomass changes in each group of explants monitored by means of SfM-photogrammetry, sponge samples of individuals present in the surrounding area of the study site, at a similar depth and of similar size to the population assessed in the study were collected in the field. Samples were taken to the laboratory, and their volume was calculated by water displacement method (i.e., ml of displaced water in a graduated cylinder). Secondly, all sponge fragments were dried at 60ºC for 48 hours inside an oven and weighted to obtain the dry weight (DW) biomass of each of the samples. Additionally, *C. reniformis* collagen content was estimated using the average yields defined by Pozzolini et al. (2018), based on the volume changes obtained by SfM-photogrammetry:

$$\text{Collagen content (DW)} = \text{Biomass sponge (DW)} \cdot 0.30$$

2.3 Statistical analysis

The volume extracted from the 3D models of *C. reniformis* was expressed in cm^3 and calculated as the sum of the volume of each individual of each explant (generally 2 to 6 individuals per explant). Yearly changes in volume (reported as % $year^{-1}$) were calculated as the differences in volumes between the first available record (depending on the transplantation date) and the final time in July 2024 (t_{14}) and standardized per year.

Biomass data were expressed in dry weight grams per dm^3 of sponge (g DW dm^{-3}), and yearly changes in biomass (reported as g DW dm^{-3} $year^{-1}$) were calculated as the differences in biomass between the first available record (depending on the transplantation date) and the final time in July 2024 (t_{14}) and standardized per year and sponge volume.

The relationship between the volume and the DW biomass of the collected samples was investigated by performing a regression. A linear regression was then performed using the *stats* package in the R software v. 4.3.2 (R Core Team, 2024). The obtained equation was used to estimate the biomass of the explants of *C. reniformis* using the volume calculated from each of the 3D models.

The experimental designs, aiming at testing differences in the volume and biomass changes in time of *C. reniformis* explants, included one factor:

(i) Experimental design 1: time (fixed, 2 levels: t_1 and t_{14}), to compare the initial and final time points.

(ii) Experimental design 2: time (fixed, 9 levels: t_2, t_3, t_5, …t_{14}) for five explants; time (fixed, 7 levels: t_3, t_4, t_{10}, …t_{14}) for the others five explants. This experimental design considers the variability in data across explants, with different time points used depending on the availability of measurements for each explant, to analyse the changes throughout the monitoring period.

Prior the analyses, data were tested for normality (Shapiro's test) and homoscedasticity (Levene's test); then, a paired t-test and a repeated-measures Analysis of Variance (ANOVA) were performed, respectively. After the repeated measure ANOVA, if statistical differences were found, a Tukey's post-hoc test was performed. Statistical differences were considered when alpha=0.05. Statistical analyses were carried out using the free software PAST version 4.17 (PAleontological STtatistics; Hammer et al., 2001).

3. Results

3.1 *Literature review: Chondrosia reniformis surveys in space and time*

A total of 202 documents (Figure 5), including duplicates, have been found using the keyword "*Chondrosia reniformis*". Duplicates were removed (75) and the remaining documents were screened by reading the title and abstract. Those not following the eligibility criteria were then discarded (45), leading to the final number of 82 works, retained for quantitative analyses (Figure 5). Most of the works about *C. reniformis* have been conducted at Mediterranean scale (78), while only 4 considered sites outside the Mediterranean province. Inside the Mediterranean Sea, most of the studies presented the sampling site(s) located in the Western Mediterranean ecoregion (37), mainly along the Ligurian (Portofino Promontory), French and Spanish coasts, followed by the Aegean (10) and the Levantine (7) seas (Figure 9A). Only a few works presented the collection sites in the Alboran (2), Adriatic (2) and Ionian seas (1) and the Gulf of Sidra (2) (Figure 9A). Nonetheless, up to 16% of the total documents have not been considered for the creation of Figure 9 since they did not give information about the sampling site(s).

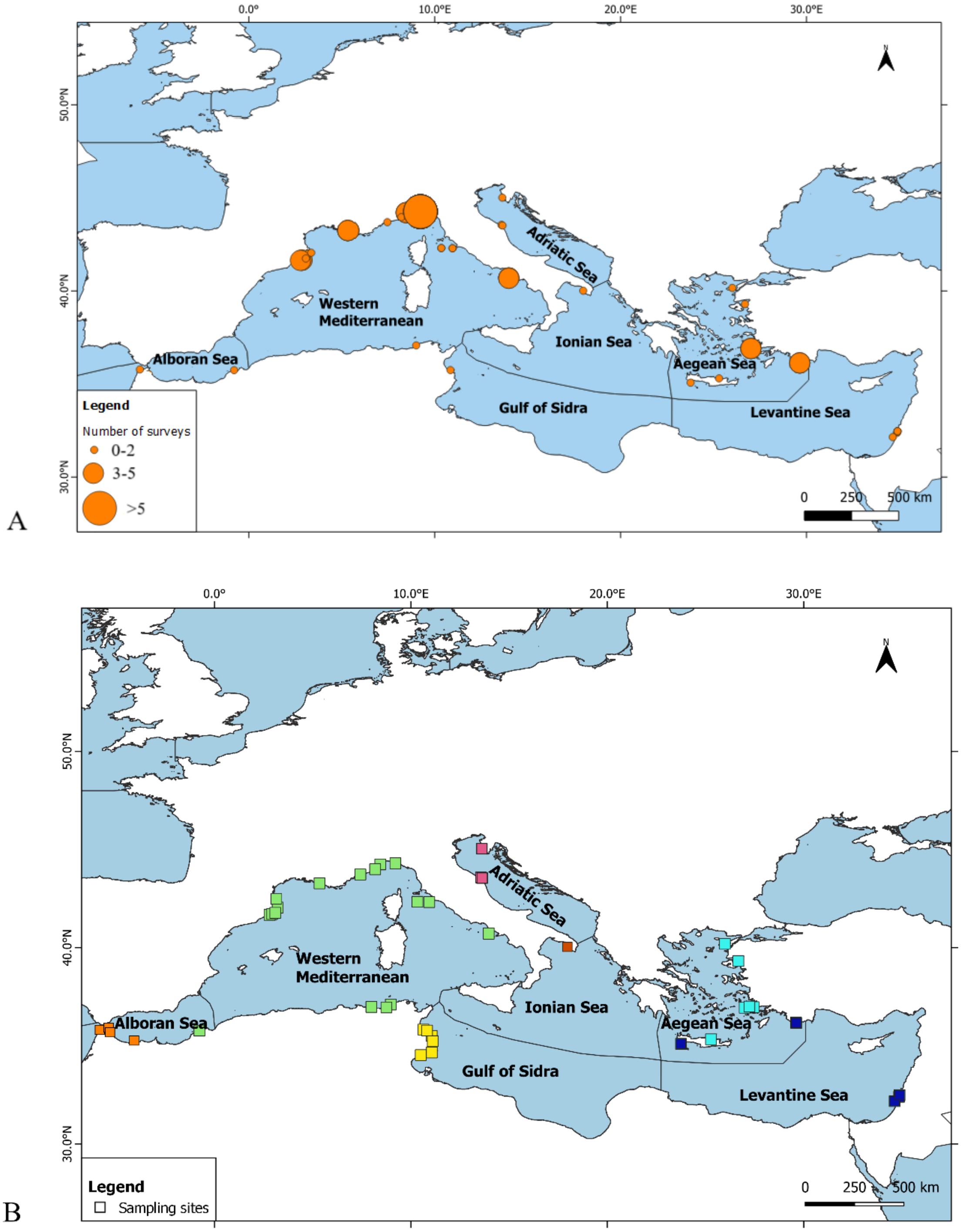


Figure 9. (A) Map showing the number of studies in each sampling location. (B) Map showing the distribution of the sampling site(s) present among the articles in the different Mediterranean ecoregions.

The first document found was published in the decade 1971-1980, precisely in 1975. In general, it is evident an upward trend in the number of documents through time, with a peak in the decade 2011-2020 (Figure 10).

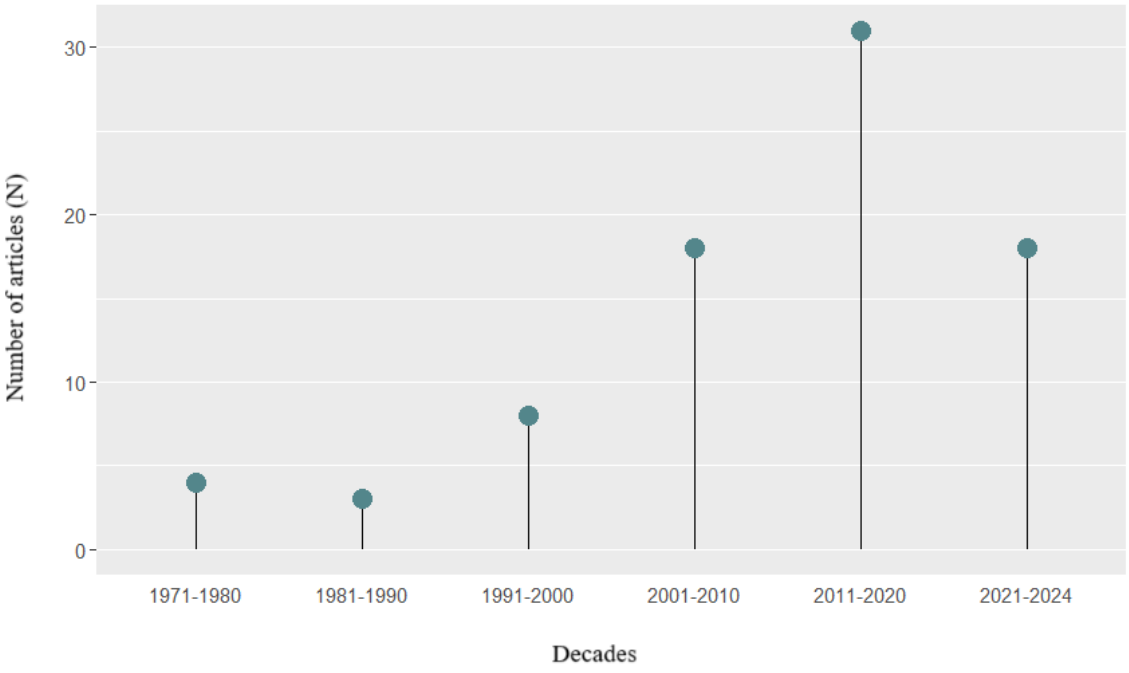
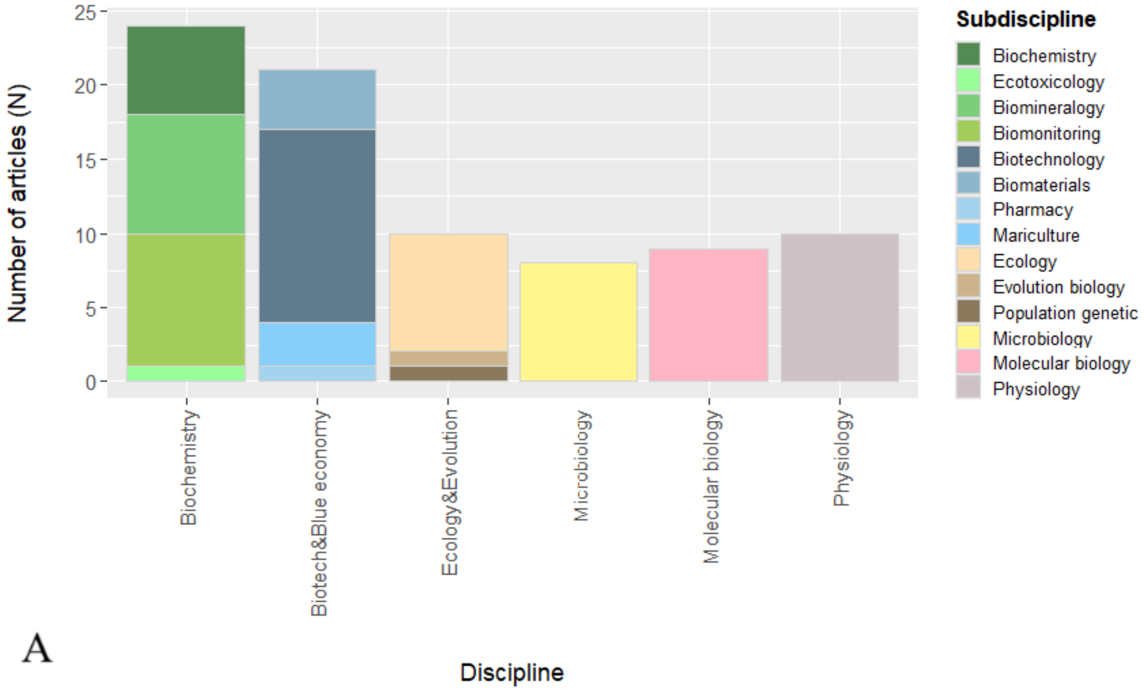


Figure 10. Lolly plot showing the number of articles per decade.

From the full-text screening of the selected documents, most of the studies were framed in the discipline Biochemistry (24), inside which most of papers belong to the subdisciplines Biomonitoring (9) and Biomineralogy (8) (Figure 11A). Other well studied fields were Biotechnology & Blue economy (21), with the subdiscipline Biotechnology including most of papers (13), and Mariculture being the least explored (3); Ecology & Evolution and Physiology (10, respectively). Inside Ecology & Evolution most of works are referred to the Ecology subdiscipline (8). Conversely, Microbiology and Molecular biology studies represented a smaller component (8 and 9, respectively).

To determine temporal trends and the evolution of study branches over time, a heatmap was created (Figure 11B). Early studies on *C. reniformis* were mostly biochemical studies, while after the 2000s, the topic suffered a reduction, leaving space for research in other disciplines, including Physiology and Ecology & Evolution. However, in recent years this discipline has gained renewed attention, due to the increase in biomonitoring research. Studies regarding the biotechnological potential of the sponge started in the decade 2001-2010 and reached a peak in the following one (Figure 11B), together with Microbiological and Molecular biology studies.



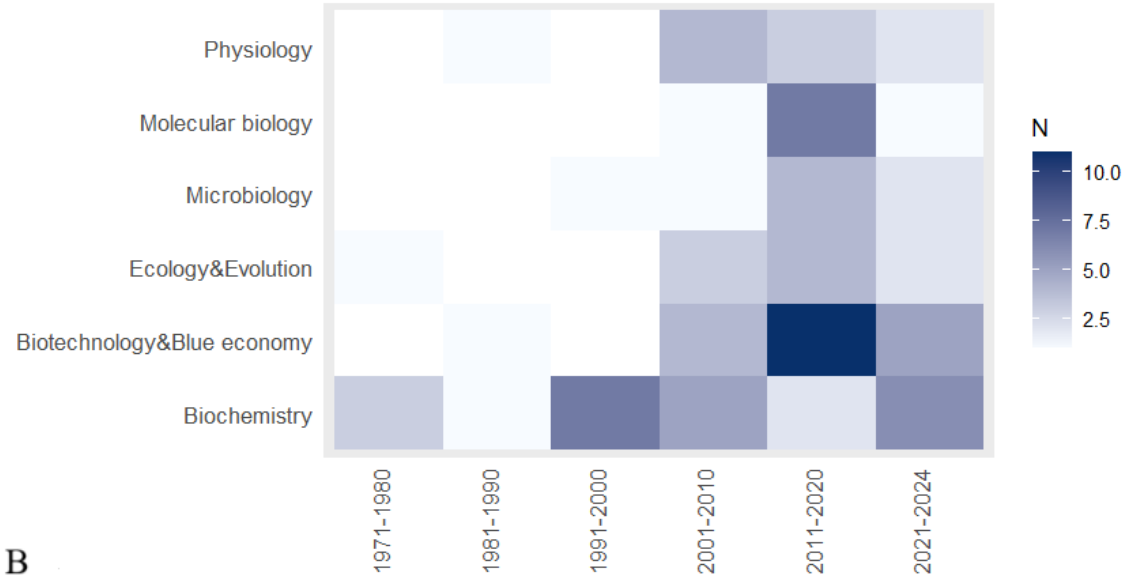


Figure 11. A) Stacked bar chart showing the number of articles (N) included in each identified discipline, subdivided in the respective subdisciplines. B) Number of articles (N) per decade and the discipline in which the study was framed.

The 88% of the studies about *C. reniformis* were conducted in laboratory, and only the 5% in the field. The remaining 7% was a combination of the two (Figure 12A). The Donut chart in the Figure 12B shows the percentage of experiments conducted in field or in the laboratory in relation to the discipline in which the study was framed.

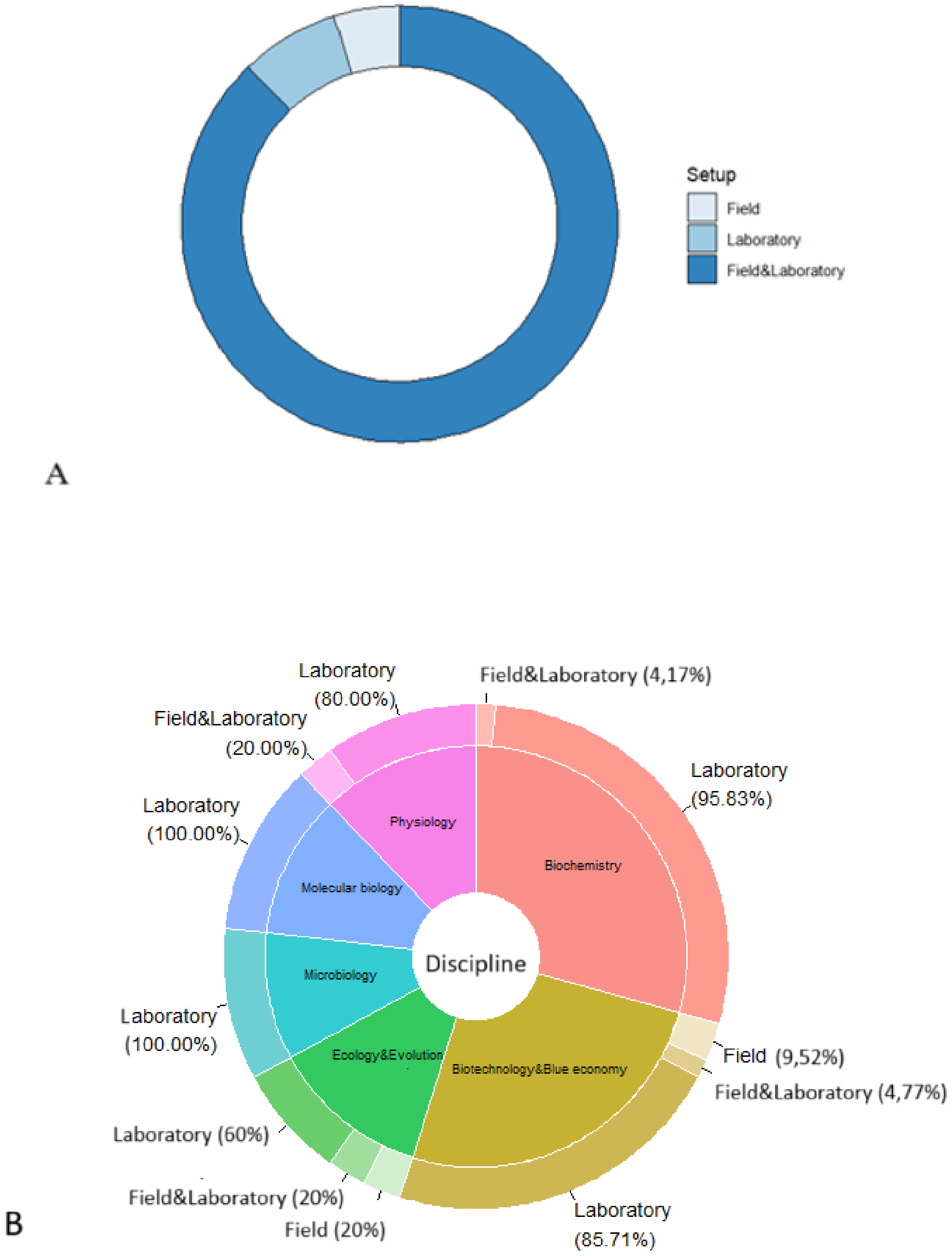


Figure 12. (A) Donut chart showing the percentage of studies conducted in Field, Laboratory and Field & Laboratory. (B) PieDonut chart showing each discipline and the percentage distribution of each experimental setups.

3.2 SfM-Photogrammetry Assessment of transplanted Chondrosia reniformis fragments

The digital reconstructions produced (Figure 13) for three-time steps of the monitored period had averages (±SD) of 79 ± 27 images. A total of 86 3D models were produced and analyzed. The reconstructions' averaged scale error was 0.02 ± 0.014 cm, never exceeding an error of 2 mm.

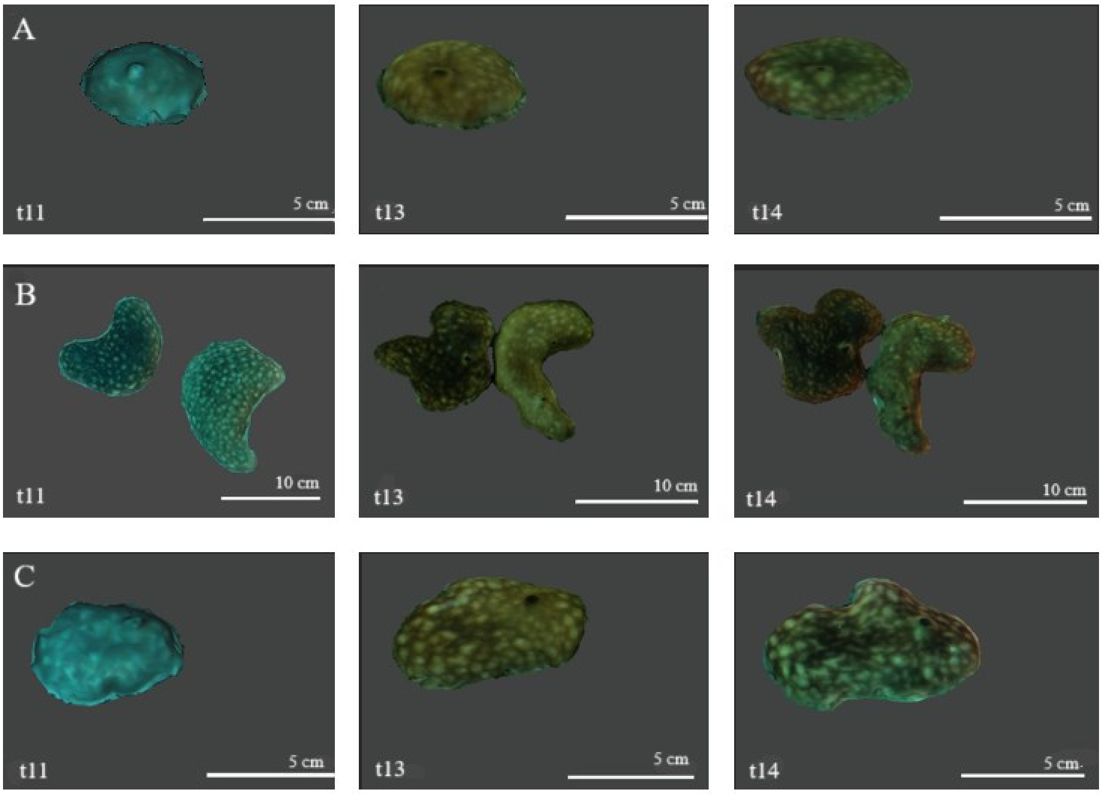


Figure 13. (A–C) Examples of the 3D reconstructions produced for *Chondrosia reniformis* explants over three monitoring time steps (May 2023, t_{11}; January 2024, t_{13}; July 2024, t_{14}).

3.2.1 Changes in the estimated volumes of transplanted Chondrosia reniformis fragments

The mean sponge volume increased significantly from the initial time (68 ± 45 cm^3) to the final time (195.37 ± 150.32 cm^3) (paired t-test, $p < 0.01$). Volume measurements also showed statistical differences over the entire monitoring period (repeated-measures ANOVA: df = 8, F = 8.531, p-value < 0.001), particularly when comparing the final monitoring times (January and July 2024) with the earliest (November 2021 and February 2022), and between the intermediate and final surveys (between September 2023 and July 2024) (Tukey's post hoc, $p < 0.05$) (Figure 14). The average increase in volume of the surveyed explants of *C. reniformis* for the whole monitored period corresponded to an increase of 108 ± 1 % $year^{-1}$. However, by considering the three years of monitoring period separately, a slight decline in sponge volume production in 2023-2024 was recorded, with an average volume increase of 100.4 ± 0.6 % · $year^{-1}$ in 2021-2022, 81.5 ± 0.9 % · $year^{-1}$ in 2022-2023 and only 56.1 ± 0.5 % $year^{-1}$ in 2023-2024 (Figure 15). There were also some cases of explants of *C. reniformis* between 2023 and 2024, which suffered a steep decrease in volume, up to -12.9%, after a previously regular growth phase during the first considered time period (2021-2022).

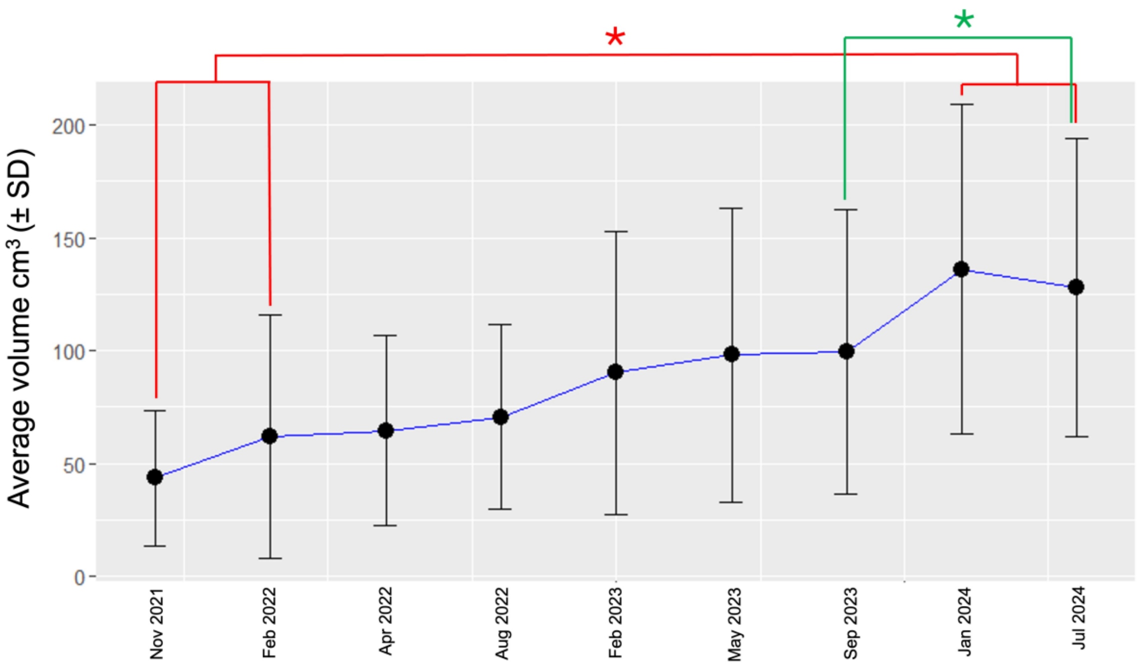


Figure 14. *Chondrosia reniformis* average volume increase throughout the monitoring period. Asterisks (*) indicate statistical significant differences highlighted by the Tukey's post hoc.

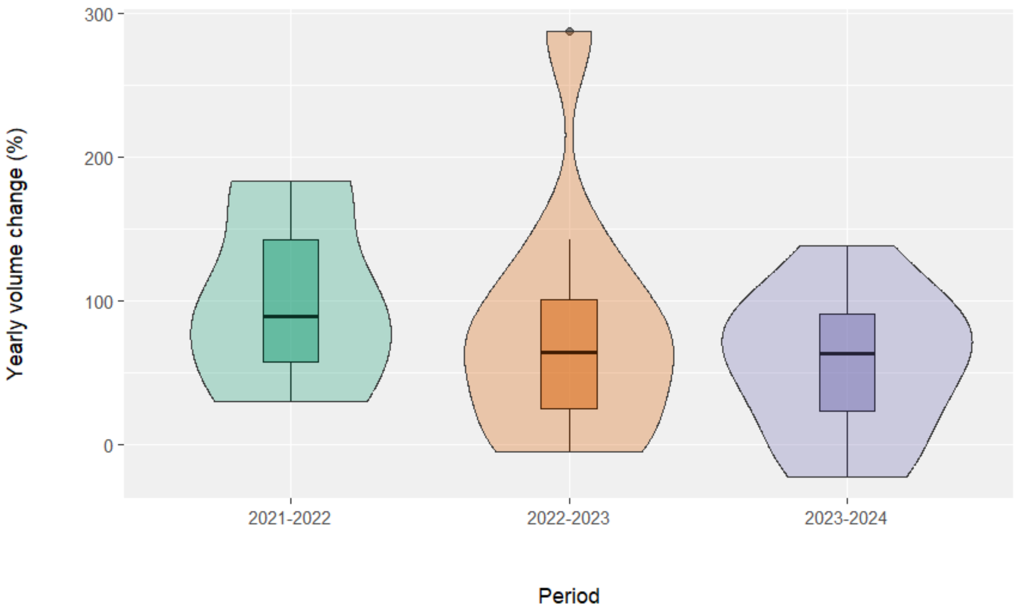


Figure 15. *Chondrosia reniformis* estimated yearly volume changes (%) for the three years

monitoring period of this study: 2021-2022, 2022-2023 and 2023-2024.

3.2.2 Changes in the estimated morphology of transplanted C. reniformis fragments

Sponge changes measured in terms of mesh distances with the “cloud/mesh dist.” Tool of CloudCompare v2.12 (2022) using the models from two sampling events (t_{13}, January 2024 and t_{14}, July 2024) gave linear growth values up to 1 cm and showed how *C. reniformis* focus its growth mostly in its peripheral area (Figure 16).

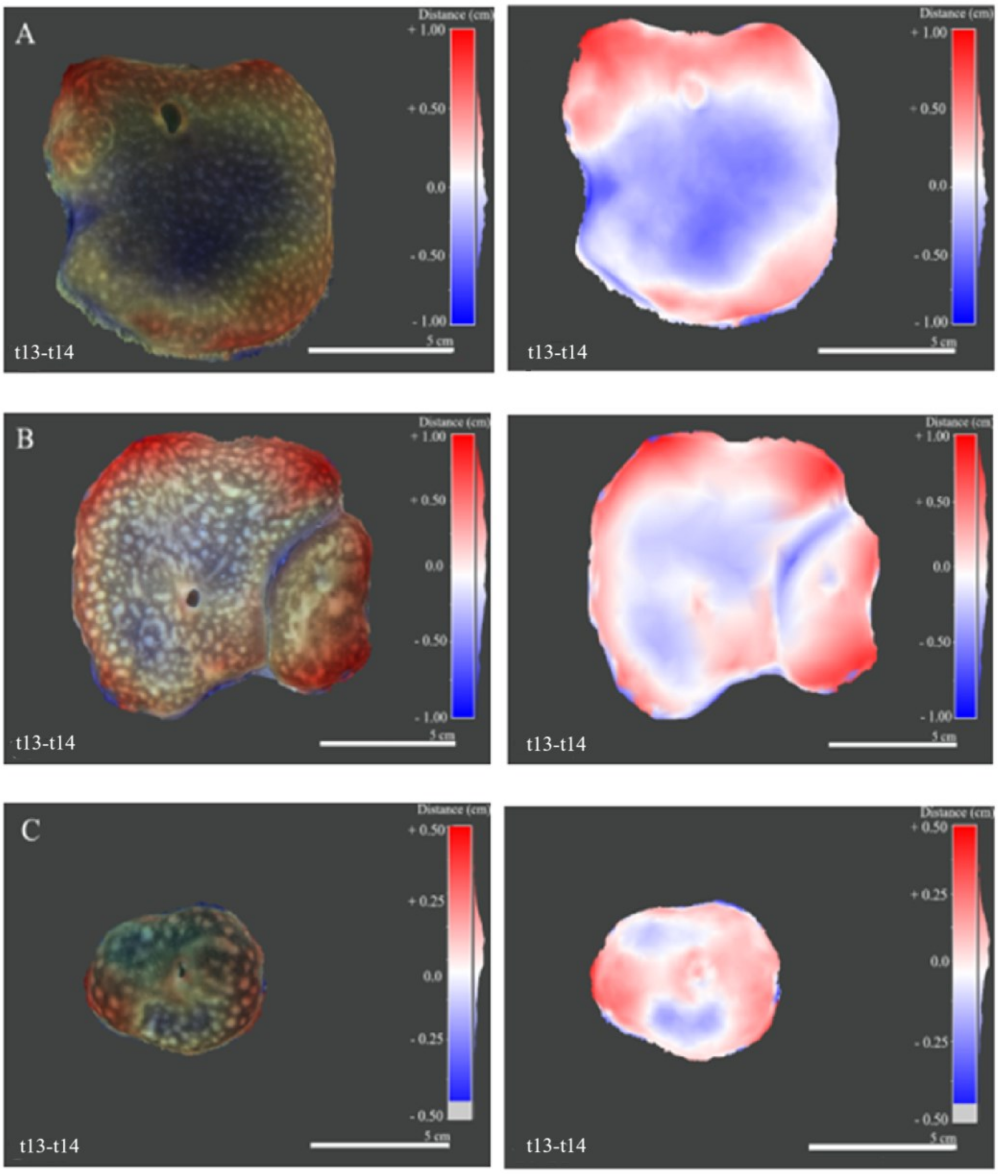


Figure 16. (A-C) Distances calculated between the 3D models of *Chondrosia reniformis* obtained for t_{13} and t_{14} (January 2024–July 2024) using point cloud distances in Cloud Compare.

3.2.3 Biomass and collagen estimations

The linear regression performed to study the relationship between the volume

(x) and the DW biomass (y) found a strong positive correlation ($y = 0.1194x + 0.3731$), with a regression coefficient (R^2) of 0.8269 (Figure 17). Therefore, using the volumes obtained from the photogrammetric approach, the biomass yearly production was assessed. *C. reniformis* explants presented an average estimated biomass production of 129 ± 116 g DW dm^{-3} $year^{-1}$ (Figure 19). Similarly to the volume, also the mean sponge biomass increased significantly from the initial time (8 ± 5 g DW) to the final time (24 ± 18 g DW) (paired t-test, $p < 0.01$) and biomass measurements showed statistical differences also during the entire monitored period (repeated-measures ANOVA: df = 6, F = 4.111, p-value < 0.05), in particular between April 2022 and July 2024 (Tukey's post hoc, p=0.01) (Figure 18). Furthermore, an average collagen production was estimated, representing a rate of 39 ± 35 g DW of collagen dm^{-3} $year^{-1}$ (Figure 19).

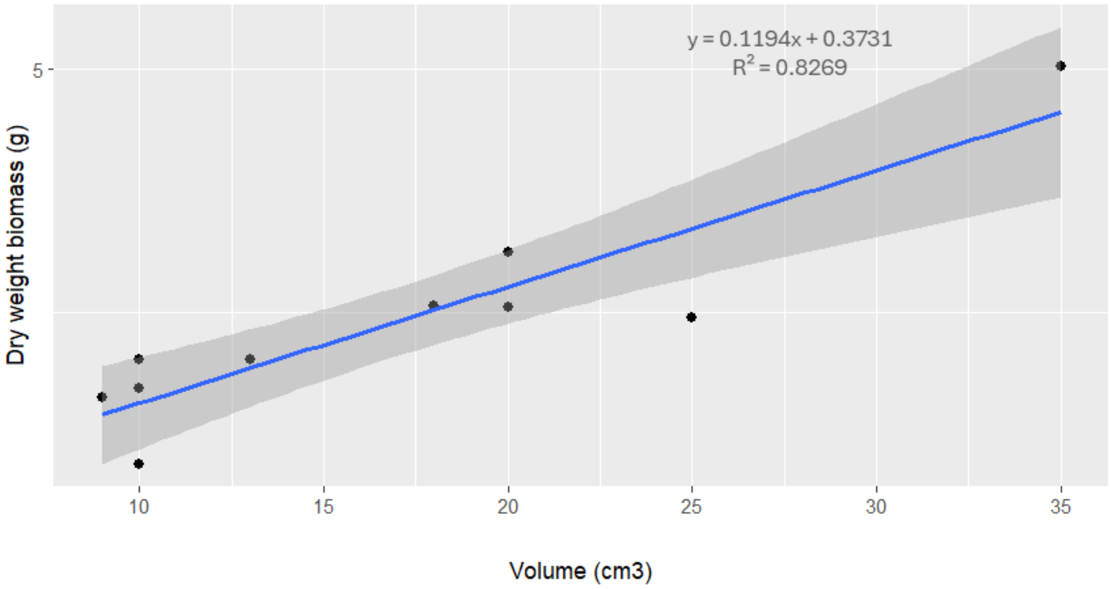


Figure 17. Relationship between the volume and the dry weight biomass of *Chondrosia reniformis* sponge samples.

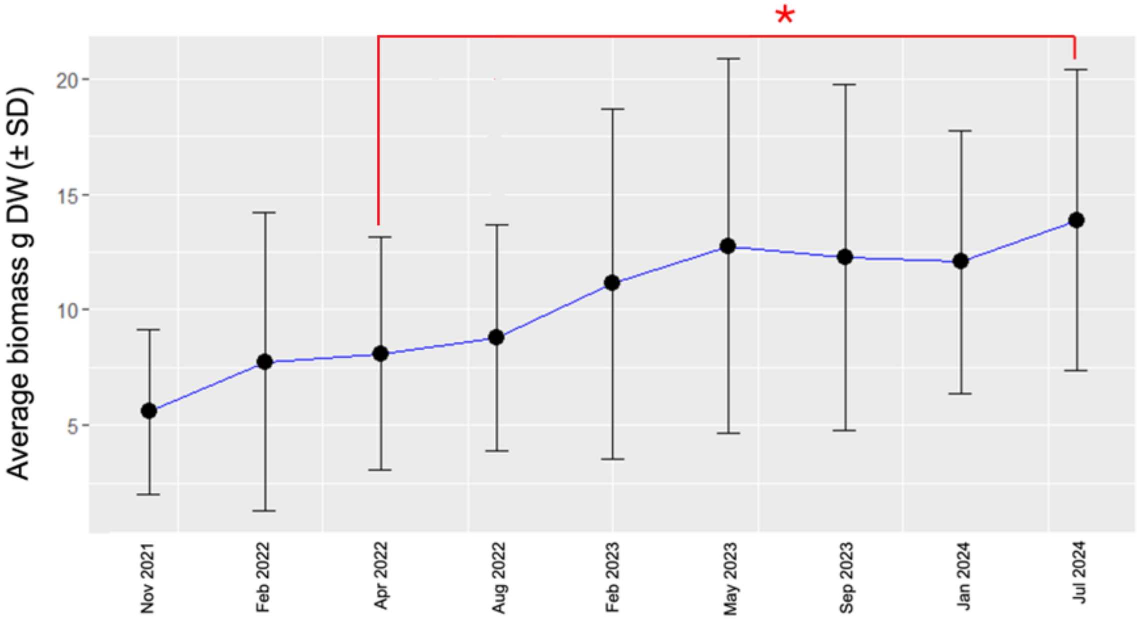


Figure 18. *Chondrosia reniformis* average biomass increase throughout the monitoring period. Asterisk (*) indicates statistical significant differences highlighted by the Tukey's post hoc.

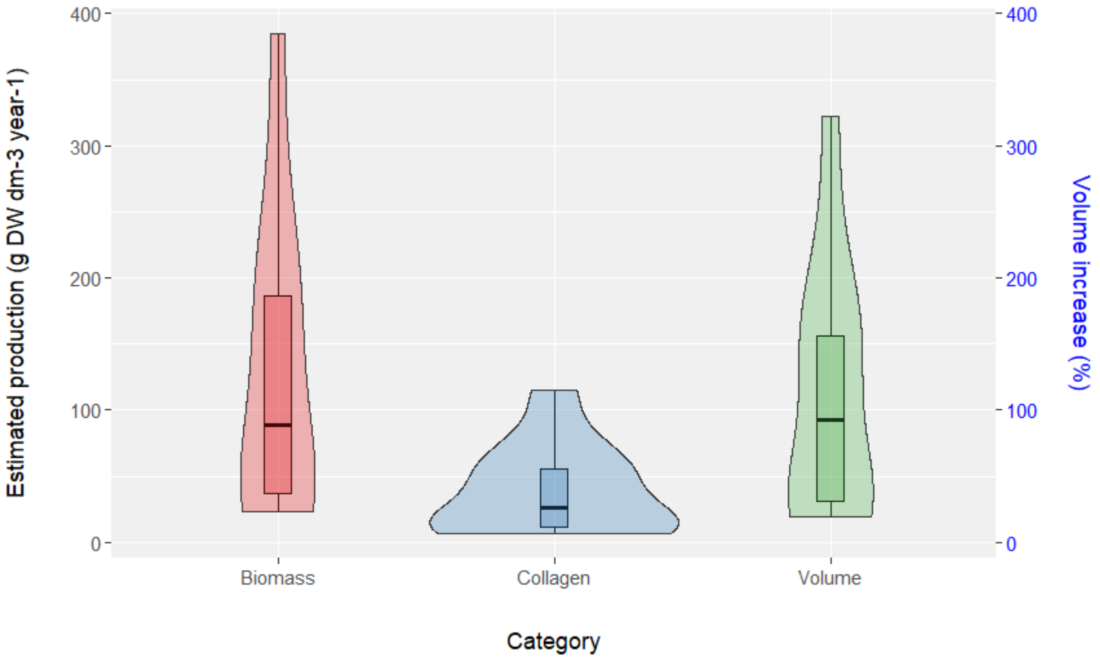


Figure 19. *Chondrosia reniformis* estimated yearly production (dry weight, d.w., of biomass and collagen) and volume increase. Note that the colour of the axis matches the colour of the related variable.

3.2.4 Regeneration rates

Regarding the explants that were cut during the monitored period to investigate the regeneration rates (Figure 20), we obtained two different results. In one case, from an explant with 6 fragments, 4 got lost either for the death of the individuals or for detachment. Nonetheless, the 2 still attached showed a positive regeneration rate, with a volume increase of 138 % year^{-1} (although lower than the growth rate before the cutting, 322 % year^{-1}). In the second case of the 4 fragments cut and transplanted, only 2 were lost, but the 2 that remained showed a significant reduction in the post-cut growth rate (up to -21 % year^{-1}) respect to the volumetric increase rate before cutting (104 % year^{-1}).

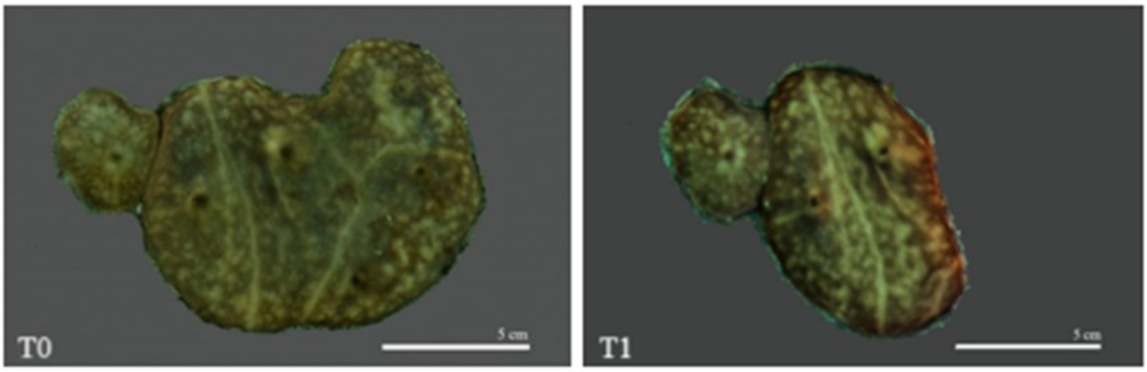


Figure 20. Example of a *C. reniformis* individual who was use as donor specimen to produce an explant (T0, before cutting; T1, after cutting).

4. Discussion

The phylum Porifera is well represented in the Mediterranean Sea, where it occupies several crucial habitats, supporting high biodiversity levels and serving as a secondary substrate for a specialized associated fauna (Koukouras et al., 1996; Coll et al., 2010; Bo et al., 2012; Gerovasileiou et al., 2016). Among these, *Chondrosia reniformis*, a common sponge species found on shallow rocky bottoms of the Mediterranean Sea, is of particular interest due to its biotechnological potential related to collagen production. However, the affection of its shallow populations by MMEs over the past years (Di Camillo et al., 2013; Di Camillo & Cerrano, 2015; Garrabou et al., 2022), pose the necessity to properly evaluate its distribution, growth rates and conservation status, before considering the exploitation of this species for biotechnological purposes.

Overall, our review showed how studies on *C. reniformis* are not equally distributed inside the Mediterranean basin, since research activities on this species mainly focused on the Western Mediterranean ecoregion (37), while the other areas are interested only by a low number of studies. This condition may be explained with the affiliation of the researchers studying the topic, which are mostly present in countries facing the Western Mediterranean ecoregion.

Most of the research conducted on this sponge has been carried out in laboratory settings, while field research has been conducted to a considerably lesser extent. This is probably because most of the scientific effort was framed in disciplines like Biochemistry and Biotechnology, where laboratory analysis is crucial for isolating specific biochemical processes and compounds. However, it is also important to increase efforts towards field research, particularly in disciplines such as Ecology, which can provide critical insights on natural behaviour, environmental interactions, and adaptive strategies of *C. reniformis* in its native habitats.

Our review highlighted how various studies have explored the biotechnological applications of this sponge, especially regarding the high collagen production and its potential pharmacological applications (e.g., Swatschek et al., 2002b; Nicklas et al., 2009a, 2009b; Pozzolini et al., 2015, 2018; Palmer et al., 2016; Fassini et al., 2017; Tassara et al., 2023). A particular aspect on this characteristic is related to the fact that there is an urgent need to find suitable protocols to conduct successful in situ cultivation of the species. In this context, the only five studies found on the topic pointed out the challenges in rearing such species, suggesting that the mariculture of *C. reniformis* is still in its infancy, with a few studies proposing large-scale sustainable production systems (Gökalp et al., 2019, 2022). Preliminary attempts to culture *C. reniformis* for adequate amounts of bioactive compounds date back to the late '90s/early 2000s (Pronzato et al., 1999; Van Treeck et al., 2003), where authors discovered the impossibility of rearing this species suspended on threads or meshes, due to its capability to "escape" through budding (Parma et al., 2007). Wilkinson and Vacelet (1979) reported moderate growth rates of 95% per year (55 weeks doubling time in volume) when *C. reniformis* was cultured under shaded conditions. Osinga et al. (2010) obtained grow rates up to 700% per year when growing *C. reniformis* on the bottom of metal wire cages under pristine conditions. Gökalp et al. (2019, 2022) demonstrates that *C. reniformis* can grow between 79 and 218% in 13 months under pristine and polluted conditions, respectively (Table 4). These growth rates are considerably higher than those reported for naturally growing specimen, as Garrabou & Zabala (2001) reported an *in situ* growth rate of 2.3% per year (extrapolated from two-dimensional areal growth). The significantly higher growth rates observed in aquaculture compared to natural conditions can be attributed to the controlled environment, continuous nutrient availability, optimal water flow, or the absence of predators/competition.

Table 4. List of the reviewed articles with growth rates, cultivation methods and measuring techniques, including the present work.

References	**Cultivation method**	**Measuring technique**	**Time period**	**Growth rate**
Wilkinson & Vacelet (1979)	Mesh system; Mounted on artificial substrate	Volume displacement	November 1976 - October 1977.	95 % increase year^{-1}
Osinga et al. (2010)	Cage system	Projected Surface Area (estimated from length and width of each explant)	October 2006 - June 2007	700 % increase year^{-1}
Gökalp et al. (2019)	Mounted on artificial substrate; Mesh system	Projected Surface Area (estimated from pixel counts with ImageJ® software)	June 2012– July 2013	79-170 % increase year^{-1}

Orel et al. (2021)	Cage system	Projected Surface Area (estimated from pixel counts with ImageJ® software)	May 2019 - January 2020	24-86 % increase month^{-1}
Gökalp et al. (2022)	Mounted on artificial substrate; Mesh system	Projected Surface Area (estimated from pixel counts with ImageJ® software)	June 2019– June 2020	126-218 % increase year^{-1}
Present work	Mounted on artificial substrate	Volume (estimated from 3D models with Agisoft Metashape software)	May 2021- July 2024	108 % increase year^{-1}

Until now, *C. reniformis* growth estimations were expressed solely as % of 2D surface area increase per time. Our study approached for the first time the evaluation of growth rates and biomass production of *C. reniformis* by SfM-photogrammetry, proving to be an effective approach for the long-term sponge growth's monitoring. The application of 3D methodology hindered the comparability of our results with previous studies. Although, considering the correlation between surface area and volume reported by Gökalp et al. (2019) with a fixed conversion factor of 1.1 cm^3 sponge volume per cm^2 of surface area, the average % volume increase per year obtained in our study (108 ± 1 % year^{-1}) falls within the range of % increase in area obtained by previous studies. Additionally, by observing the growth patterns of the species using the cloud-distance of the digital reconstructions (t_{13}-t_{14}), it was possible to depict a general behavioural growth: *C. reniformis* explants firstly attach to the substrate, shrinking during a first phase, in which increases in height, to later grow in surface horizontally, loosing height especially on its central area (Figure 16). These observations suggest that the growth assessment of this species could end up in an overestimation if conducted only by 2D approaches, especially during these first phases of explant re-attachment and growth.

During our monitoring, the observed pattern in volume and biomass increase throughout the considered period suggests that there were periods of gradual growth followed by more marked increases towards the end of the observation period. However, from our data it cannot be inferred a seasonal effect, since the experimental design was not performed to capture seasonal variation for each year. Significant differences in sponge volume were observed between different times, especially between the initial times (such as May/November 2021) and later time periods (i.e., January/July 2024). In contrast, there were a few significant changes between the intermediate time periods until the final phase, suggesting that growth stabilize in the last observation period. There were also some cases of explants of *C. reniformis* which suffered a steep decrease in volume, after a previously regular growth phase. This can be due to the unique ability of *C. reniformis* to undergo the process of budding, known as the 'creeping phenomenon', during which a portion of the parent body is lost (Parma et al., 2007). Other possible explanations include detachment of fragments in response to environmental stresses, such as changes in current or temperature conditions, interaction with predators, infections or diseases (Gökalp et al., 2019). Environmental factors are, in fact, significant in determining sponge’s growth. In particular, it was reported that certain demosponge species grow faster and reach their largest sizes at sites with high water movement, as flow might promote sponge growth through increased food availability or by increasing internal flow through the sponge aquiferous system (Gökalp et al., 2022). Water temperature also was found to be a major factor affecting *C. reniformis* growth, with the temperature at 25°C proving to be optimal for explants growth (Orel et al., 2021). These underlined the importance of carefully selecting mariculture sites with optimal water flow and temperature conditions to maximize the growth and productivity of *C. reniformis*. Some studies demonstrated also how the sponge can grow better in the proximity of fish aquaculture cages, taking advantage of the higher food availability (higher TOC concentration) as a result of fish farm activities and correspondingly lower light levels of the water column (Gökalp et al., 2019, 2022). These findings make the sponge a promising candidate for integrated multitrophic aquaculture, a practice that enables supplying food to the population, but in a more responsible and sustainable way (Khanjani et al., 2022). This point is very important because it offers an environmental-friendly approach for the use of sponges as a source of natural compounds, resulting at the same time in a good system to bioremediate the marine environment, thanks to the filter-feeding activity of these animals, thus combining biomass production and bioremediation purposes (Amato et al., 2024).

Regarding the regeneration rates, we obtained two very different responses to regeneration after cutting *C. reniformis* explants, in particular one of the cuts increased and one decreased substantially in volume. These observations may suggest that the regenerative capacity of *C. reniformis* can be conditioned by different aspects, including not only environmental factors (e.g., nutrient availability, space, light and temperature) but also cumulative stress values, such as cutting and transplantation stress, physiological conditions of the explants themselves (i.e., the pre-cut health status) and genetic variability (Gökalp et al., 2022). For example, Gökalp et al. (2019) reported an initial enhanced surface area increase following explant cutting. However, given the variability of results the effect of cutting on the regeneration of explants needs to be further investigated.

It is important to clarify that the approach adopted here represents the growth of the groups of explants and not of individual sponges. For future experiments, it would be useful to calculate the average annual volume increase also for single individuals to compare the results and evaluate the intra-group variability. The results can be valuable for assessing the growth of explant groups in a mariculture context, where multiple individuals are cultivated simultaneously. However, scaling these findings to a larger-scale cultivation system would require further validation that also considers individual variability.

5. Conclusions

With this thesis we tried to answer to four main research questions:

1) Which is the state of the art on *Chondrosia reniformis* and the *in situ* cultivation techniques regarding this species?

2) Which are the annual growth rates, average biomass and collagen production of *C. reniformis*?

3) Is SfM-photogrammetry an effective approach for the long-term monitoring and assessment of this sponge's growth?

4) Is this sponge suitable to be sustainably reared for nutraceutical applications?

The state of the art on *C. reniformis* spans various fields, including ecology, biochemistry, biotechnology, physiology, molecular biology, and microbiology. However, certain fields have received more attention, such as biotechnology and biochemistry, respect to others, highlighting the need to deepen certain aspects of *C. reniformis* ecology and biology.

Until now, few attempts have been made to develop suitable *in situ* mariculture methods for the sponge *C. reniformis*. Some studies demonstrated how the sponges can grow better in the proximity of fish aquaculture cages, making it a promising candidate for integrated multitrophic aquaculture, combining biomass increase and bioremediation. The culture method proposed in our experiment had never been applied before; one of its advantages is the use of pre-existing artificial reefs, making it a more sustainable option by minimizing environmental impact and reducing the overall costs of installation and material use. However, further studies are needed to determine the best conditions for *C. reniformis* culture and better understand the factors that influence its growth. Future perspectives are also brood stock selection, searching for those individuals that better grow using this culturing method and that better thrive in the selected site.

Although it can hold some limitations linked to water turbidity and sponge structural complexity, our study also demonstrated that SfM-photogrammetry can be an effective approach for the long-term monitoring and assessment of sponge's growth. Its systematic application for the assessment of volume changes in sponges, could represent the establishment of a non-invasive, one size fits all monitoring tool. This would contribute to the standardization of methodologies and units for growth assessments, and would increase the comparability among studies, a recurrent issue found in the literature.

We might conclude that *C. reniformis* showed significant growth in volume and biomass during the study period. Species like *C. reniformis*, which exhibit high growth rates and plasticity, can be promising candidates for *in situ* mariculture for biotechnological purposes. However, it is essential to develop sustainable management strategies to avoid negative ecological side effects and to promote a local blue economy, while contributing to the conservation of natural populations.

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