UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO DI SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale in Biologia Marina

La riproduzione dei coralli profondi: il caso di studio del corallo nero Mediterraneo *Antipathella subpinnata*

On the reproduction of deep corals: the case study of the Mediterranean black coral *Antipathella subpinnata*

Relatore
Prof.ssa Martina Coppari

Tesi di Laurea Magistrale di:
Monica Amadori

Correlatore
Dott.ssa Camilla Roveta

Sessione Straordinaria febbraio 2023

Anno Accademico 2021 – 2022

Index

RIASSUNTO ……..…………………………..………………..5

ABSTRACT…………………………………………………..8

1. **INTRODUCTION** 10

1.1 Phylum Cnidaria 10

1.2 Class Anthozoa 13

1.2.1 Phylogeny 15

1.2.2 General morphology 16

1.2.3 Reproductive strategies 20

1.3 Order Antipatharia 26

1.3.1 Anatomy and morphologies 30

1.3.2 Asexual reproduction 32

1.3.3 Sexual reproduction 34

1.3.4 Larvae 41

1.4 The target species: *Antipathella subpinnata* (Ellis and Solander, 1786) …………………………………………………………44

1.4.1 Genetic structure in the Mediterranean Sea 47

1.4.2 Ecological role and vulnerability 48

1.5 Aim of the study 50

2. **MATERIALS AND METHODS** 52

2.1 Literature collection 52

2.2 Study area 57

2.2.1 The Tyrrhenian Sea 57

2.2.2 Giannutri Island 59

2.2.3 Punta Pennello 60

2.2.4 Mezzo Canale shoal 61

2.3 Study of the reproductive cycle of *Antipathella subpinnata* 62

2.3.1 Samples collection 62
2.3.2 Histological analysis 64
2.3.3 Reproductive parameters 68
2.3.4 Statistical analysis 69
2.4 Larval settlement devices 70
2.4.1 Building and set up of the larval settlement devices 71
3. **RESULTS** 74
3.1 Literature analysis 74
3.2 Sexual reproduction of *Antipathella subpinnata* 79
3.3 Statistical analysis 87
3.4 Larval settlement devices 87
4. **DISCUSSION** 88
4.1 Literature analysis 88
4.2 Sexual reproduction of *Antipathella subpinnata* 94
4.3 Larvae 101

BIBLIOGRAPHY 104

SITOGRAPHY 124

RIASSUNTO

Fino ad oggi, lo studio della riproduzione dei coralli si è concentrato principalmente su specie superficiali, mentre per i coralli di ambiente profondo (> 40 metri) gli aspetti riproduttivi sono ancora ampiamente trascurati. La mia tesi di laurea ha come obiettivo approfondire sia la riproduzione asessuale che sessuale di esacoralli ed ottocoralli in ambiente profondo, tramite un'estesa ricerca bibliografica, da cui è emerso che gli esacoralli profondi sono caratterizzati da una preponderanza di specie broadcast e gonocoriche, mentre negli ottocoralli le strategie broadcast e brooder sono equamente distribuite, con specie esclusivamente gonocoriche ad eccezione di due casi di ermafroditismo.

Nonostante il Mar Mediterraneo sia una delle principali aree di studio, le informazioni riguardanti la riproduzione dei coralli neri sono ancora molto scarse. In questo contesto, il mio elaborato di tesi ha avuto come target lo studio della riproduzione sessuale e dell'ecologia larvale di *Antipathella subpinnata,* importante specie strutturante della zona mesofotica, vulnerabile sia a fattori antropici che climatici. Le popolazioni in esame si trovano sulla secca di Mezzo Canale e l'isola di Giannutri (Mar Tirreno) in un range di profondità compreso tra 70 e 80 metri. Campioni di *A. subpinnata* sono stati raccolti da subacquei tecnici ad agosto 2021 e giugno-ottobre 2022, e la riproduzione è stata descritta

attraverso analisi istologiche e inclusione in resina. Tutte le colonie sono risultate essere gonocoriche e di sesso maschile, ad eccezione di una il cui sesso non è stato determinato. Il rapporto maschi-femmine (sex ratio) è fortemente sbilanciato a favore dei maschi, e lo spawning risulta essere ad agosto, anche se colonie fertili sono presenti ad ottobre, suggerendo uno spawning prolungato come riportato per altre specie di corallo nero. La presenza di soli maschi è potenzialmente supportata dalla capacità della specie di riprodursi per frammentazione, o da un possibile contributo di propaguli riproduttivi da popolazioni adiacenti più profonde (deep refugia hypothesis).

Un altro obiettivo della tesi è stato lo studio dell'ecologia larvale di *A. subpinnata*, vista la totale assenza di dati al riguardo. Per questo scopo, sono stati costruiti dei dispositivi con diverse tipologie di substrati per l'adesione delle larve (ceramica, coralligeno ed acciaio inox), posizionati *in situ* nel mese di agosto. Nessuna larva di *A. subpinnata* è stata trovata nei substrati raccolti nei mesi successivi; probabilmente la posizione dei substrati immediatamente al di sotto delle colonie fertili non è adeguata alle caratteristiche della larva. L'assenza di femmine e la potenziale dispersione dei gameti maschili grazie alla direzione antioraria della corrente nell'area di studio, potrebbe suggerire un flusso di propaguli riproduttivi tra diverse popolazioni limitrofe.

Ulteriori studi che indaghino la genetica delle popolazioni di coralli neri nel Mar Tirreno sono necessari per comprendere la connettività tra le popolazioni, così come differenti approcci per lo studio dell'ecologia larvale.

ABSTRACT

To date, the reproductive study of corals is mainly focused on shallow species, indeed for deep corals (> 40 meters) the reproductive ecology is still largely neglected. My master thesis aims to deepen both the asexual and the sexual reproduction of hexacorals and octocorals in the deep environment, through extensive bibliographic research, which highlighted that deep hexacorals are characterized by a predominance of broadcast and brooder species, while in octocorals the broadcast and brooder strategies are evenly distributed, with species exclusively gonochoric except for two cases of hermaphroditism.

Although the Mediterranean Sea is one most studied area, very little is known on the reproduction of black corals. In this context, my thesis dissertation focused on the study of the sexual reproduction and larval ecology of *Antipathella subpinnata*, an important ecosystem engineer of the mesophotic zone, vulnerable to both anthropogenic and climatic stressors. The studied populations are located in Mezzo Canale shoal and Giannutri Island (Tyrrhenian Sea) in the depth range between 70 and 80 meters.

A. subpinnata samples have been collected by technical divers in August 2021 and June-October 2022, and the reproduction has been described through histological analysis and resin inclusion. All the colonies collected were males and gonochoric, excepting for one undefined colony. The ratio males-females

(sex ratio) is strongly skewed in favour of males, and the spawning turns out to be in August, although fertile colonies are still present in October, suggesting a prolonged spawning as already reported for other black coral species. The presence of only males is potentially supported by the possible contribution of reproductive propagules from adjacent deeper populations (deep refugia hypothesis).

Another objective of this thesis is the study of the larval ecology in *A. subpinnata,* considering that, data on this topic are totally absent. For this purpose, different devices provided with various substrate material (ceramic, coralligenous and stainless steel) for the larvae adhesion have been built and placed *in situ* in August. No larvae have been found in the substrates collected in the following months; probably the position of our substrates immediately below the parent colonies was not appropriate to the larval characteristics. The absence of females and the potential dispersion of male gametes following the anticlockwise current circulation in the study area, could suggest a flux of reproductive propagules among adjacent populations.

Further studies investigating the genetic population of black corals in the Tyrrhenian Sea are essential to understand the connectivity among populations, as well as different approaches to study larval ecology.

First chapter

1. INTRODUCTION

1.1 Phylum Cnidaria

Cnidaria is a phylum under the kingdom Animalia with 11894 species of aquatic animals currently accepted by the World of Registered Marine Species (WoRMS, 2022). About the 99% are marine species, while the remaining are typical of freshwater environments.

The name Cnidaria derived from Greek (*cnidos*: stinging nettle), and their distinctive features are the cnidocytes, specialized cells synthetized in the cytoplasm containing stinging organelles, called nematocysts. They are mainly localized in the tentacles (Beckmann and Özbeck., 2012) contributing to the prey capture, defense and adherence to the substratum during locomotion. Of the three categories of cnidocytes (nematocysts, spirocysts and ptychocysts) only nematocysts occur in all cnidarians (Fautin, 2009).

Cnidaria are dipoblastic: in fact, they are formed by two epithelial layers, the external epidermis (derived from ectoderm) and the internal gastroderm (derived from endoderm), with a middle layer called mesoglea formed by an acellular jelly- like substance. The ectoderm has a protection function, while the gastroderm plays a role in the digestive process, with gland cells secreting digestive enzymes.

Cnidaria are the most primitive of animals whose cells are organized into distinct tissues, but they lack organs, cephalization, and a nervous coordination. They possess a diffuse nervous system: nerve cells are distributed in the epidermis and the gastroderm forming two interconnected nerve nets. Nerve impulses are transmitted through neurotransmitters which spread in both directions (Hickman et al., 2016).

While the larval stages present asymmetry (Dunn and Wagner, 2006), the adult stage normally shows a radial or biradial symmetry around a longitudinal axis with an oral region closer to the mouth and an aboral region further from the mouth.

Animals belonging to this phylum display two distinct morphological body plans: a sessile polyp and a floating medusa (Fig. 1).

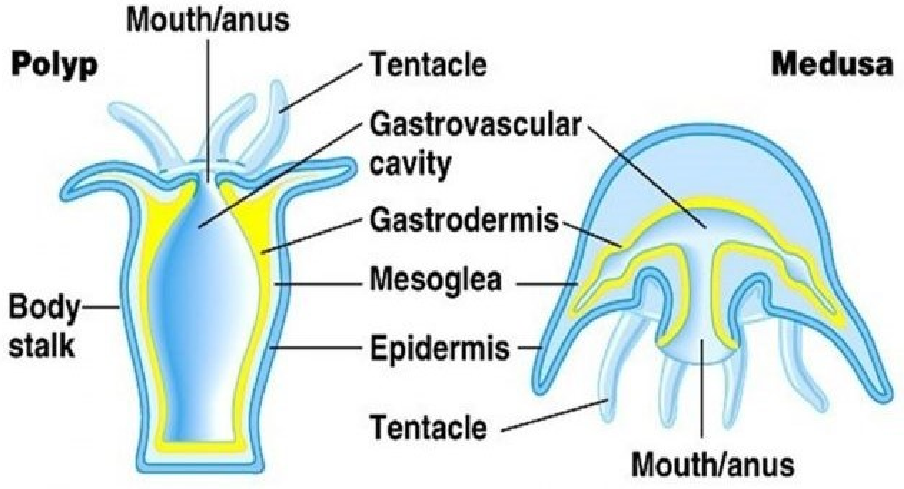

Fig. 1 Polyp and medusa forms (Figure from https://www.bankofbiology.com/2012/03/)

The sessile polyp is sac like, with a single body cavity: the gastrovascular cavity (or celenteron), which communicate with the outside through the mouth, surrounded by one or more rows of tentacles. The aboral region is attached to the substrate by a pedal disc (Fig. 1). Polyp form can be either solitary or colonial. Medusae are motile, with mouth and tentacles hanging down from an umbrella-shaped bell (Fig. 1) (Rogers, 2009). Most of individuals lives exclusively as polyp (all anthozoan, some hydrozoan), while others differentiate the form throughout their lives (many hydrozoans, all scyphozoan and cubozoan).

The phylum Cnidaria includes 5 classes: Anthozoa (sea anemones and corals), Hydrozoa (hydras and other colony forming species), Scyphozoa (jellyfish), Staurozoa (peduncle jellyfish) and Cubozoa (box jellyfish), although, for the purpose of this thesis, only the Class Anthozoa is described in detail. A recent study, based on phylogenomic data, places the origin of this class (and by extension of the entire phylum) in the Cryogenian to Tonian periods (648-894 Ma) (Mc Fadden et al., 2021). This is far earlier than the previous studies dating the origin in the Precambrian (Waggoner and Collins., 2004).

1.2 Class Anthozoa

Anthozoans include 7177 species described so far (WoRMS, 2022), representing about two third of the extant cnidarian species. They occur worldwide in all marine habitats from the intertidal zone up to 8600 m depth at all latitudes. They are among the oldest clades of animals (Fautin et al., 2021) and include some of the ocean’s most important foundation species. The term “Anthozoa” comes from the Greek words *ánthos* and *zóa* meaning “flower animals”, as the polyp aspect resembles a flower.

Morphological traits that have led to their ecological success include a modular colonial growth form, the ability to precipitate an internal or external skeleton of crystalline aragonite or calcite and symbioses with photosynthetic dinoflagellates. Symbiosis occurs in favorable conditions between the host (Anthozoan) and photosynthetic unicellular dinoflagellates, named as zooxanthellae, which are located in the endodermal cells of the host. Symbiosis is based on the transfer of osmotically active compounds with a mutual benefit: the anthozoan receives nutrition and increase the ability of accumulate calcium carbonate thanks to the photosynthetic activity, while the host contribute to endosymbiont's metabolism providing essential nutrients derived from its catabolism of macromolecules and carbon dioxide (Furla et al., 2005). This relation occurs in the euphotic zone in clear and shallow water (up to 60 m), mostly in tropical and subtropical regions (Glynn, 1996). All these traits allowed anthozoans to create biogenic structures (i.e., coral reefs) and to support these ecosystems. However, the class Anthozoa also includes taxa lacking one or more of the abovementioned traits, such as sea anemones presenting a soft body and occurring in a solitary form (McFadden et al., 2021).

Anthozoans are defined as ecosystem engineers, due to their tridimensional structures, increasing the heterogeneity and the complexity of the habitat and providing shelter, food and nursery areas for several associated species. They create the so-called marine animal forests (Rossi, 2013) representing not just high-level biodiversity habitats but also providing many ecosystem services such as the capability of carbon immobilization (Rossi and Bramanti, 2020) as well as supporting recreational and leisure activities.

1.2.1 Phylogeny

The evolutionary relationship of Anthozoan has been long debated. Wells and Hill (1956) proposed three subclasses based on the arrangement of mesenteries: Ceriantipatharia, Alcyonaria and Zoantharia. Afterwards, the division suggested by Hand in 1966 in four subclasses (Antipatharia, Ceriantharia, Zoantharia and Alcyonaria) was rejected since the data available did not support the monophyly of Antipatharia and Ceriantharia (Berntson et al., 1999). In 1974, Smith divided the class Anthozoa in two subclasses based on the composition of nematocysts (Won et al., 2001). Molecular systematics provided evidence to determine the relationship among anthozoans, with the phylogenetic analyses presented by Quattrini et al. (2020), reporting the reciprocal monophyly of subclasses Hexacorallia and Octocorallia, with strong support for Ceriantharia as a sister group to all the other hexacorals.

Nowadays, the class Anthozoa is divided into 15 orders and three subclasses (Hexacorallia, Octocorallia and Ceriantharia).

- Hexacorallia: polyps with unbranched tentacles and mesenteries in pairs multiple of six; non-spicular calcareous skeleton, corneous, chitinous or lacking. They include:

  - Order Scleractinia: madrepore

  - Order Actinaria: sea anemones

- Order Antipatharia: black corals
- Order Zoantharia: zoanthids
- Order Corallimorpharia: mushroom corals.

- Octocorallia: polyps with eight pinnately branched tentacles, eight complete mesenteries not in pairs. Nearly all colonial with an internal skeleton. They comprehend:
  - Order Pennatulacea: sea pens
  - Order Alcyonacea: gorgonians and soft corals
  - Order Helioporacea: blue corals.
- Ceriantharia: solitary polyps with two sets of tentacles that form felt-like tubes of mucus and nematocyst like organelles, they burrow in soft sediment.
  - Order Penicillaria
  - Order Spirularia.

1.2.2 General morphology

Anthozoans completely lack a medusa stage: they live as polyps throughout their life cycle. The polyp presents a cylindrical shape surmounted by an oral disc surrounded by tentacles (Fig. 2). Although they are described as a phylum characterized by radial symmetry, all anthozoan polyps exhibit bilateral symmetry, at least internally, either as adults or in earlier developmental stages (Genikhovich and Technau 2017). The bilaterality of polyps derives from the internal arrangement and development of mesenteries and from the lateral compression of the actinopharynx.

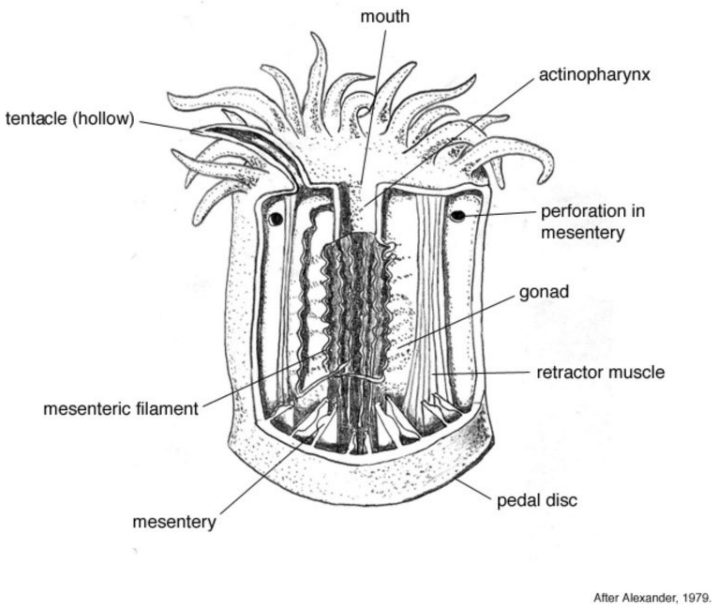

Fig. 2 Anatomy of anthozoan polyp (Figure from http://www.taxateca.com/claseanthozoa.html)

Mesenteries are radial tissue sheets extending from the body wall to the gastrovascular cavity, defined complete if they extend until the actinopharynx and fuse with it. They are site of digestion, absorption and development of gametes and their functions consist in providing support, increasing surface area for respiration and absorption of food. The number of mesenteries coupled with tentacles have been used in the past for taxonomy (Daly et al. 2007).

The actinopharynx, also called stomodeum, is an irregularly shaped tube extending from the mouth of the polyp into the digestive cavity. It represents the muscular part of the digestive system, allowing the animal to ingest food. The siphonoglyph, a densely flagellated channel in the pharynx wall, drives water into the coelenteron. It can be single, or paired, hardly ever absent (Hickman et al., 2016).

The presence of longitudinal, transverse, and radial muscles along the tentacles' walls allows the organism to inflate and deflate, bend, twist, shorten or elongate, as well as extend or contract tentacles. The movement ability is obtained by expulsion and incorporation of water through the cavity, in this way the polyp can extend when it looks for food or retract when it is disturbed (Hickman et al., 2016).

The tentacles bear cnidocysts, that, once used, are replaced in a process lasting some hours. Sweeper tentacles have been observed in stony corals and in several black corals, with the function of protection and competition with other organisms (Bo et al., 2012).

Most anthozoan orders occur in colonial form with genetically identic polyps communicating with each other through living material tissue: the coenenchyma and the gastrovascular cavities are indeed, linked by canals or tubes. The skeleton is present only in the Scleractinia, Octocorallia and Antipatharia orders.

In the Scleractinia order, it is secreted by the ectoderm in the form of calcium carbonate. It attaches to the surface of pre-existing skeleton and remain amorphous a few hours, until crystallizing into aragonite (Mass et al., 2017).

Octocorals display an internal skeleton composed of calcium carbonate, gorgonin (iodinated fibrous protein) or both. They also produce sclerites, small carbonatic skeletal plates, with function in structure and defense (Conci et al., 2021).

Antipatharians possess an internal skeleton with spines, but much more elastic due to the presence of antipathin, a protein similar to chitin (Ehrlich, 2019). Alcyonacea and Actinaria lack a hard skeleton, however they possess a hydrostatic one: they introduce or remove water from the polyps to inflate or deflate the colony. Some of them can enclose sand grains or shell fragments into the epidermidis thanks to their stickiness, a similar strategy is adopted by most zoanthids to obtain the reinforcement of the polyp’s wall (Cerrano et al., 2010).

1.2.3 Reproductive strategies

Reproduction is the biological process by which new organisms are produced from their parents, and anthozoans can reproduce asexually, sexually, or even both.

Asexual reproduction occurs when an individual produces new offspring that are genetically identical to the ancestor at all loci in the genome, except at those sites that have experienced somatic mutations. It is normally used to maintain a population within the parental habitat (Richmond, 1997) and it is an adaptation to unstable and unfavorable local conditions (Foster et al., 2007). Animals can reproduce asexually in two main ways: agametic reproduction and parthenogenesis. Agametic reproduction is common in Anthozoa and can occur in different modalities: fission, fragmentation, budding (Fautin, 2002). Hexacorals, and in particular sea anemones, commonly experience transverse and longitudinal fissions with pedal laceration. Another strategy adopted by hexacorals is the polyp bail-out, represented by the detachment of a single, non-skeletonized polyp from the colony under stressful conditions, followed by a complete tissue and cells rearrangement and in some cases in a regression into a simple, ciliated form (Coppari et al., 2020). The bail-out can occur naturally in the field, while it can be induced in laboratory at any time. It proceeds in 3 steps which includes: (1) withdrawal of coenosarc and isolation of individual polyps; (2) detachment of polyps with zooxanthellae from the skeleton and release, (3) dispersal, re-attachment to the bottom and secretion of a new skeleton (Sammarco et al., 1982).

Hexacorals and octocorals reproduce also by fragmentation, a process in which a live portion of a colony becomes physically separated (fragment), due to the breakage of the skeleton from the rest of the colony and after its reattachment produces a new colony (Highsmith, 1982). It is carried out by tentacles, by group of polyps or by portions of colonies (Coppari et al., 2019). Fragmentation has a notable importance for colonial invertebrates living in environments exposed to disturbance. In stoloniferous zoanthids, fragments can be generated by 2 processes: stolonal degeneration, which is endogenous and the exogenous storm-related turbulence. In species that undertake fragmentation, small colonies experience higher mortality rates than the larger one (Karlson, 1986). New individuals may arise from portion of the tissue that are budded off from a parent, or by a parent dividing lengthwise or crosswise into two smaller individuals. Budding is common in cnidarian (jellyfishes, corals, sea anemones) being normally used for the growth of the colony with the related increase in size. Parthenogenesis involves the production of individuals from diploid oocytes that do not undertake meiosis, or for which meiosis does not lead to segregation or recombination, producing females identical to the mother (de Meeûs et al., 2007). In anthozoans is rare, occurring in some or octocorals (Fautin, 2002)

Sexual reproduction is a process that creates offspring by combining the genetic material of two organisms in a process starting from meiosis. Afterward, gametes mix each other forming a larval stage (planulae) that after a free-living phase, will undergo metamorphosis establishing a new colony. Gametogenesis in corals takes place in gonads present on mesenteries. Sexual reproduction is important to maintain genetic diversity of a species. Most coral species are hermaphrodites having both female and male gonads within the same colony. On the other hand, one third of corals are gonochoric, presenting separated sex, with colonies either males or females. Corals exhibit two modes of reproduction: brooding and broadcast spawning (Fig. 3).

Brooding species display an internal fertilization and the development of larvae occur within the parental polyp. Gametogenesis in this case is less synchronous, being not essential for successful fertilization. Brooding corals show an extended reproductive season from several months up to continuously over a year (Harrison and Wallace, 1990).

After, larvae, released from the polyp, usually disperse over relatively short distances (< few kilometers), and are competent to settle and undergo metamorphosis. Regarding the species showing the stable symbiosis with dinoflagellates of the genus *Symbiodinium*, brooded larvae are generally already provided with zooxanthellae (i.e., vertical transmission), unlike those derived from broadcast species that mostly acquire the zooxanthellae at a later stage from the sea water (i.e., horizontal transmission) (Karako-Lampert et al., 2004).

Broadcast spawners are more common and show considerable variation in their pattern of spawning among reefs. They release sperms and eggs in water column during one or a few discrete periods. As the fertilization is external, female and male colonies must be synchronous in the release of gametes. Larvae may persist as plankton in the water column for days to weeks, reaching large distances (> several kilometers), ensuring genetic connectivity among reefs. Eventually, the larva swims to the sea floor and searches for a suitable substrate to settle (Gilmour et al., 2016). Larval settlement occurs when the larva finds an appropriate place to settle and undergoes metamorphosis, becoming a tiny coral polyp (not bigger than one millimeter). In the following weeks, the polyp grows up quickly to avoid being buried by sediment or covered by algae. The surviving corals begin the early life stages by dividing into clones, closely connected each other. The early developmental stages are characterized by an enormous loss of offspring, which is compensated by the release of huge quantities of gametes.

The timing of coral spawning is regulated by several factors such as environmental factors that act as cues for the release of gametes. These factors include sea temperature and sunlight, in fact most of the corals spawn before or during the warmest and calmest months. Lunar cycle as well, controls spawning and planulation for broadcast and brooding species. Corals may spawn at any period of the lunar cycle, but year after year there is a periodicity for every species. For broadcasters, spawning occurs during the week following full moon, between sunset and midnight. On the other hand, brooders may release planula at any time during day or night (Guest et al., 2010).

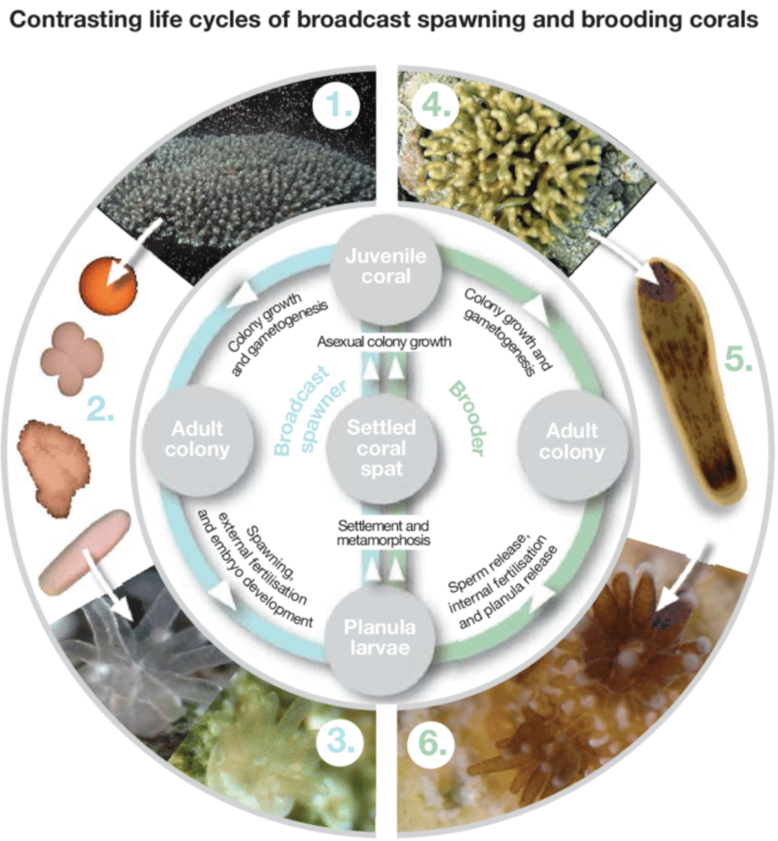

Fig. 3 Scheme comparing broadcast and brooder reproductive strategy (Figure from Guest et al., 2010).

The early life history processes (dispersal, settlement and recruitment) of a habitat-forming sessile species strongly affect the recovery and resilience of population. The dispersal capability is influenced by reproductive mode (broadcast vs. brooding), larval development (sedentary vs. pelagic larval phase), nutritional mode (lecithotrophic vs. planktotrophic) and behavior (benthic vs. planktonic). These characteristics determine the exchange of individuals among populations and, when they contribute to the gene pool of receiving population, we talk about population connectivity. Population connectivity is fundamental in the resilience and persistence of a population after an impact and its study is essential to conserve populations. Most studies have been carried out in shallow tropical reefs, only a few studies are present in mesophotic environments. (Costantini et al., 2017).

1.3 Order Antipatharia

Antipatharia is an order belonging to Hexacorallia subclass, accounting for 294 species divided in 43 genera (WoRMS, 2022). Although they are commonly known as black corals due to the color of their skeleton, they show a variety of colors from white to yellow, red and green. The name of this order derives from the Greek words "*anti*" and "*pathos*", literally meaning against evil or disease. Since ancient times they were treasured by many cultures for medicinal purposes or to make amulets against illness and as an aphrodisiac. Nowadays, except the use in Chinese traditional medicine, some species are still adopted to produce jewelry. As a result, they are targeted by commercial fisheries in many regions of the world such as Asia, Latin America, and the Caribbean (Wagner et al., 2012). Moreover, they are affected by the indirect effect of demersal fishing practices. Therefore, Antipatharians are protected by the Convention on International Trade in Endangered Species of Wild Flora and

Fauna (CITES) (GFCM, 2009, OSPAR), which restrict their exploitation and importation/exportation.

In the past, they were included in the taxon Ceriantipatharia, but after recent molecular study based on mitochondrial DNA, Antipatharia is recognized as a distinct order (Bo et al., 2011b). Antipatharians can be divided in 7 families (Opresko, 2001; WoRMS, 2022):

- Antipathidae (Ehrenberg, 1834)

121 species organized in 10 genera: *Allopathes, Antipathes, Arachnopathes, Blastopathes, Cirrhipathes, Pteropathes, Eucirripathes, Hillopathes, Stichonopathes, Pseudocirrhipathes.*

- Aphanipathidae (Opresko, 2004)

37 species organized in 2 subfamilies that diverge by the spines' morphology: Aphanipathinae (5 genera: *Aphanipathes, Asteriopathes, Phanopathes, Pteridopathes, Tetrapathes*) and Acanthopathinae (4 genera: *Acanthopathes, Distichopathes, Elatopathes, Rhipidipathes*).

- Cladopathidae (Kinoshita, 1910)

22 species organized by their branching type into 3 subfamilies: Cladopathidae (3 genera: *Chrysopathes, Cladopathes, Trissopathes*), Hexapathinae (3 genera *Heliopathes, Heteropathes, Hexapathes*), Sibopathinae (1 genus *Sibopathes*).

- Leiopathidae (Haecklel, 1896)

9 species belonging to the genus *Leiopathes.*

- Myriopathidae (Opresko, 2001)

34 species divided in 5 genera*: Antipathella, Cupressopathes, Myriopathes, Plumapathes, Tanacetipathes*.

- Schizopathidae (Brook, 1889)

60 species organized in 14 genera: *Abyssopathes, Alternatipathes, Bathypathes, Dendrobathypathes, Dentropathes, Diplopathes, Lillipathes, Parantipathes, Saropathes, Schizopathes, Stauropathes, Taxipathes, Telopathes, Umbellapathes.*

- Stylopathidae (Opresko, 2006)

11 species and 3 genera: *Stylopathes, Triadopathes, Tylopathes*.

In this subdivision 5 new species of black corals are also included, which were recently described by Horowitz et al. (2022), collected from the Great Barrier Reef and the Coral Sea. Two species belong to the family Antipathidae, two to the family Aphanipathidae and one to the family Cladopathidae, respectively.

Antipatharians are the least known among anthozoans, due to their inaccessibility, approximately 75% of the recognized species occurs deeper than 50 meters (Wagner et al., 2011). Black corals typically have a wide bathymetric distribution and their habitat characteristics such as dim light, low sedimentation level and moderate current are not fully understood (Gaino et al., 2008). They can be found in all the oceans, from polar to tropical latitudes. They are more abundant with increasing depth, probably to avoid competition with obligate photosynthetic organisms. They are able to compete for space by using sweeper tentacles and secondary metabolites. Antipatharians are predated by a few animals such as gastropods and green sea turtles (Wagner et al., 2012).

Black corals have been traditionally considered an exclusively azooxanthellate order, however, there are many records in literature of the 90's regarding algal symbiosis in Antipatharia (Brook, 1889; Pesch, 1914). These studies reported algal cells in the gastroderm or ectoderm of the tentacles, in the body wall, in the actinopharynx and in the mesenteries. One of the latest records is from Wagner et al. (2011), that reported dinoflagellate algae in gastrodermal tissue of 10 species of Hawaiian Antipatharians collected at depths between 10 and 396 m. Another study conducted by Bo et al. (2011b) confirmed the presence of symbiosis between *Symbiodinium* and black corals of the genus *Cirrhipathes*, with the presence of zooxanthellae in the gastroderm of the tentacles and of the mesenteries in the oral region.

1.3.1 Anatomy and morphologies

Antipatharia have been considered the most primitive group of hexacorallians for their simple morphological organization, comprising polyps with six tentacles, a spiny endoskeleton and a reduced muscular system that allow only partial contraction (but not retraction) of the tentacles. Generally, polyps are no more than a few millimeters in width and no more than a few centimeters in height (Brugler et al., 2013). They produce a thorny, proteinaceous axial skeleton composed of chitin fibrils (14.5% glucosamine), and non-fibrillar scleroprotein (34.5% glycine, 14.9% alanine, 13% histidine). The skeleton is covered by spines of variable forms: from simple to thorn like, to spherical and bubble-shaped, to long and bifurcated (Brugler et al., 2013). Their vast array of morphologies can be summarized in five main typologies, in their turn characterized by different coloration:

- Fan shape: colonies display a ramification on the principal axis, with branches very close to obtain a fan shape. E.g., *Trissopanthes pseudotristicha* (Opresko, 2003) (Fig. 4a).
- Bush or arborescent shape: it is the more common shape, colonies possess big branches with many plans of branching. E.g., *Antipathella subpinnata* (Ellis and Solander, 1786) (Fig. 4b).

- Bottle-rush shape: it is characterized by branching on several levels around a principal axis. E.g., *Cupressopathes* sp. (Fig. 4c).
- Feather shape: colonies have their branching mostly in one plane around a main branch, ending with a feather like shape. E.g., *Bathypathes* sp. (Fig. 4d).
- Wire like shape: colonies do not branch and present a wire like shape. E.g., *Cirrhipathes, Pseudocirrhipathes* and *Stichopathes* (Fig. 4e).

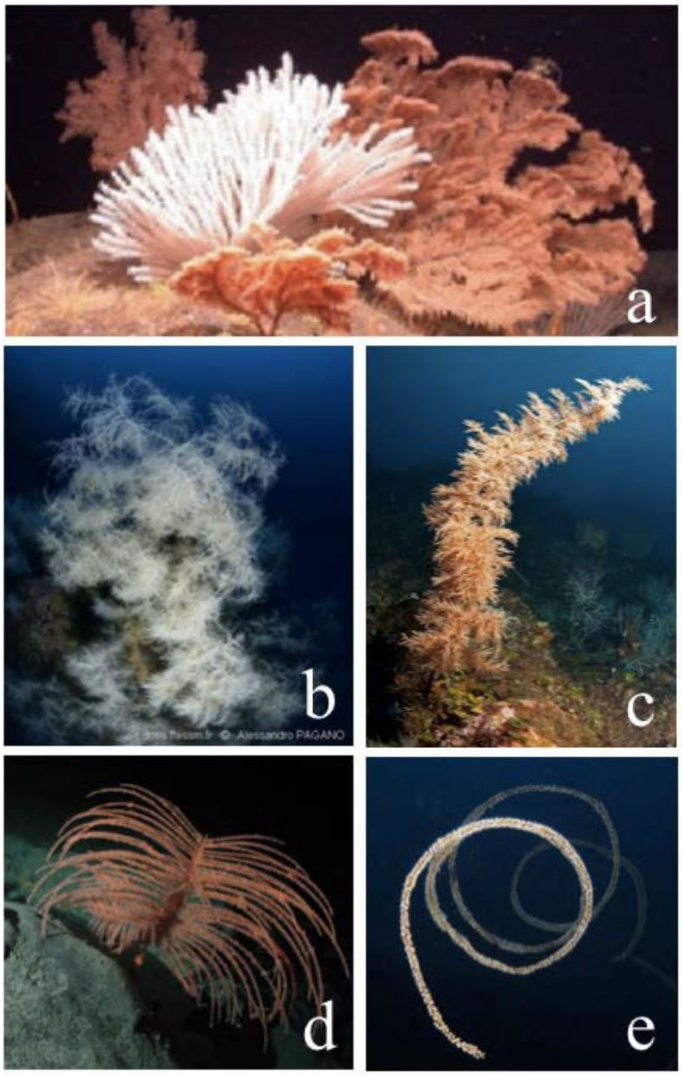

Fig. 4: Examples of the five main morphologies found in Antipatharia: a) Fan shape (*Trissopanthes pseudotristicha*); b) Arborescent shape (*Antipathella subpinnata*); c) Bottle-rush shape (*Cupressopathes* sp.); d) Feather shape (*Bathypathes* sp.); e) Wire like shape (*Stichopathes lutkeni*). (Figure from Msc Andruccioli, 2019)

They are slow growing organisms, long living and with an old age of maturity. Longevity ranges from decades to millennia. The growing rate for a shallow specie: *Antipathes dichotoma*, 12-32 years old, is reported to be 130-1140 µm/ year, while for the deep specie *Leiopathes glaberrima*, 2377 years old, the rate is less than 10 µm/ year (Roark et al., 2006). Referring to growth in height, *Antipathella aperta* in shallow waters display a rate of 2,9-6,4 cm/ year, while *Antipathella fiordensis* 1,6 cm/ year (Bo et al., 2009b).

1.3.2 Asexual reproduction

As already reported in the previous sections (1.2.3), asexual reproduction is extremely important in Cnidaria (Fautin, 2002) and it is also present in Antipatharians (Miller and Grange, 1995). It was observed for the first time in controlled conditions by Coppari et al. (2019) in the Mediterranean species *A. subpinnata*. In this study, spontaneous fragmentation of the mother colonies occurred shortly after their sampling, and positioning in aquaria, probably due to synergetic effects and stress of the collection, transport and rearing condition per se. The fragments survived and reattached to different substrates, small rocks and coralligenous substrates but also the glass wall of the tank. A schematic description of the fragmentation process is reported in Fig. 5: 1) detachment of the fragment from the mother colony; 2) initial adhesion mediated by the contact of apical or intermediate polyps or cnidocysts; 3) a new colony originates through the creation of new branchlets.

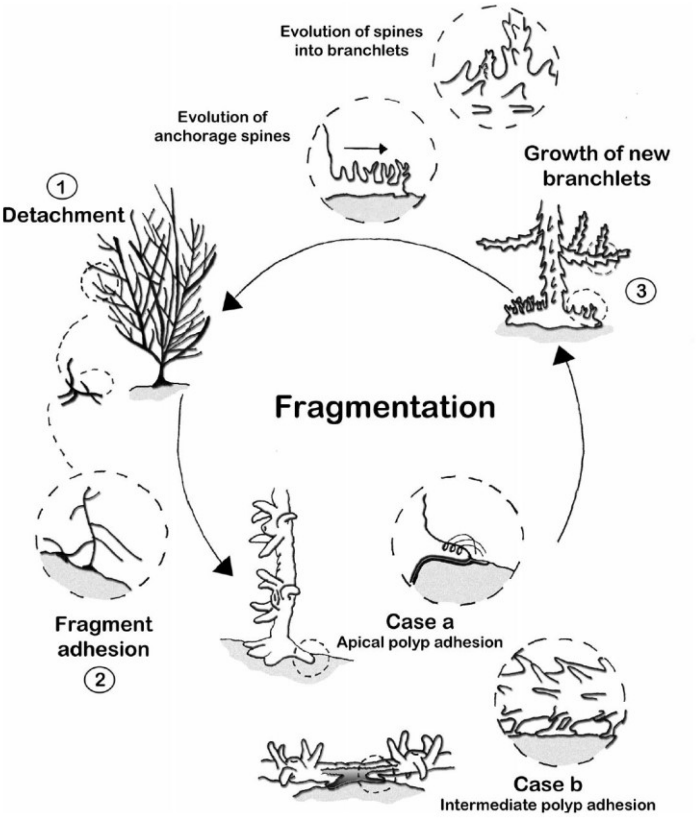

Fig. 5 Scheme of the fragmentation cycle (Figure from Coppari et al., 2019).

In natural conditions the apical fragmentation may result from heavy currents, or as a control mechanism of colony's growth (Bo et al., 2009). Other studies suggested the potential of food availability and food typology as triggering factors (Orejas et al., 2008).

Polyp bail-out has been originally hypothesized in Antipatharians, based on the observation in aquarium of pseudo larvae of *Antipathella fiordensis* (Grange, 1990). Recently, this phenomenon has also been observed in the black coral *A. subpinnata* in controlled conditions (Coppari et al., 2020). One month after the sampling and the transport to aquarium, colonies started to produce hundreds of motile and negative buoyant propagules on a daily basis. The portion of the colony that released propagules has experienced necrosis and then death. The settlement of the bail-out propagules has not been observed in this study.

In Antipatharians, new polyps can be generated through budding, leading to the growth of the colony. Polyp buds can develop directly on the polyp or more frequently in the space between polyps (Pax et al., 1987).

1.3.3 Sexual reproduction

In modular organisms the production of gametes has an important function in increasing genetic diversity, necessary to adapt to environmental changes. Understanding the reproductive pattern in corals is crucial for their conservation. Despite the importance of gamete production, the sexual reproduction in black corals remains poorly studied, mainly due to their inaccessibility.

Wagner et al. (2012) reviewed the information on sexual reproduction for 56 species of Antipatharians (>20% of described species). Generalities on the reproduction of black corals are reported below:

- Of the six mesenteries, only the two in the transverse plan, bear gametes, which are usually the longest.
- Individual polyps are gonochoric, indeed the presence of hermaphrodite polyps has been observed very occasionally. Females and males did not show morphological differences. For some species, female polyps, before the spawning, become swollen and appeared orange due to the presence of oocyte (Parker et al., 1997, Gaino and Scoccia, 2010). In general, male polyps did not change their color with the development of gonads, however, a single male colony with a light creamy-orange color when approaching the spawning is reported in literature (Parker et al., 1997).
- Colonies are either males or females, except for *Stichopathes saccula.*
- Fertilization occurs in the water column (broadcast species) as there is no evidence of internal fertilization. Structural and ultrastructural investigations by Gaino and Scoccia (2010), described the gametes flow

through the pharynx and from the apical region of tentacles (Fig. 6). Gametes were enveloped by parental tissue, derived from the breakage of the mesenterial septa. The presence of pro-acrosomal vesicles, considered adapted to external fertilization, highlights the broadcast strategy of the species (Gaino and Scoccia, 2008).

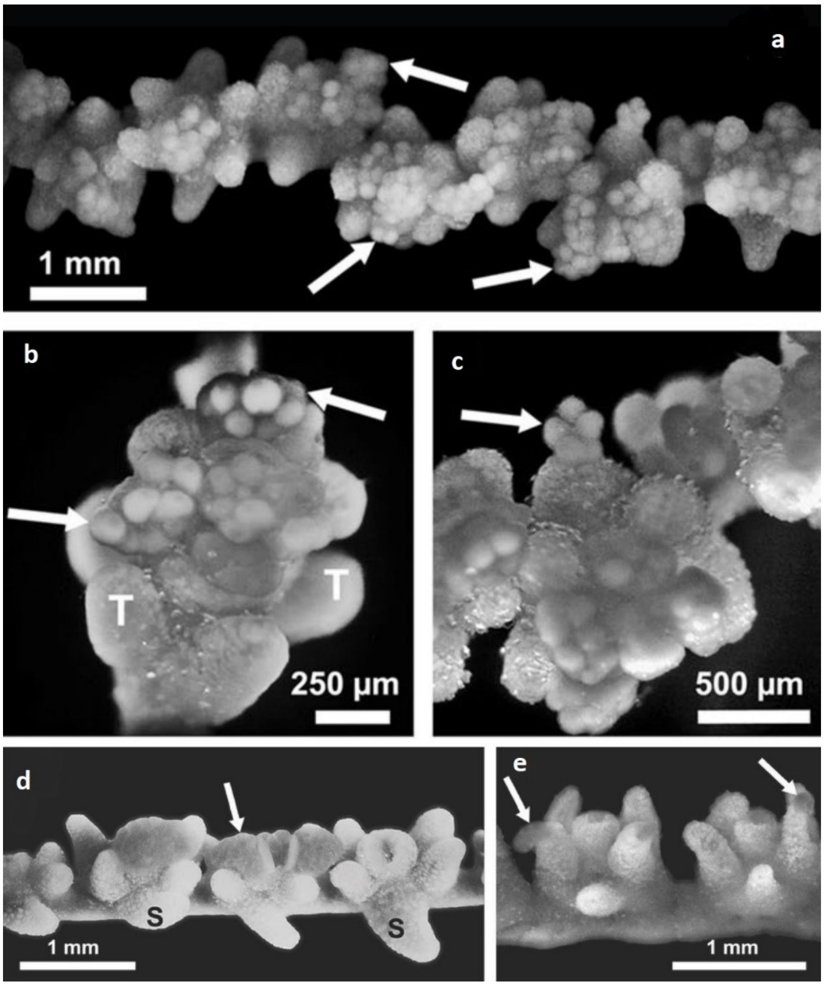

Fig. 6 Spawning of *A. subpinnata*. Female polyps with egg masses (a-b-c) and male fertile polyps (d-e) (Modified from Gaino and Scoccia, 2010).

- Gametes exhibit a seasonal appearance and disappearance in shallow waters (<70 m).
- Polyps with gametes are distributed uniformly among branches in the medial section of most colonies, the distribution in basal and apical part is patchy. Tips of pinnules are occupied by unfertile polyps (Rakka et al., 2017).
- Gametes number varies from less than 5 to more than hundreds, according to polyp dimension and depth (Gaino and Scoccia, 2008).
- Gametogenesis is affected by abiotic condition such as temperature, photoperiod, lunar cycle.

The importance of temperature in gametogenesis has been confirmed by a positive correlation between sea surface temperature, maturity stage and polyp fecundity for *Antipathella wollastoni*, in Azores Archipelago. However, at the maximum temperature did not correspond the high frequencies of the mature stages, suggesting that temperature might be a cue for final gamete maturation rather than spawning. In *A. wollastoni*, the presence of oocytes at different stage of maturation within a polyp, may suggest the hypothesis of repetitive spawning, within a prolonged spawning period (Rakka et al., 2017). Spawning in successive events was also reported for a Hawaiian black coral, *Antipathes griggi* (Wagner et al., 2012). This strategy might be adopted to enhance the synchronization in gamete fertilization whereas the environmental conditions are favorable. In temperate areas, the annual sea water regime, ensures a synchronous spawning that occurs in a single event. On the other hand, in tropical environments, characterized by stable sea water temperature, some species (i.e., *Cirrhipathes* sp.) show multiple spawning events (Gaino et al., 2008).

- Larval stage has been observed only for one specie: *Antipathella fiordensis,* in laboratory (Miller, 1996).

Only for four species, *Antipathella fiordensis* (Parker et al., 1997), *Antipathella wollastoni* (Rakka et al., 2017), *Antipathes griggi* (Wagner et al., 2012) and *Cirrhipathes anguina* (Terrana and Eeckhaut, 2019), the annual reproductive cycle has been studied. Gametogenesis starts in spring (immature oocytes), reaching the peak in late summer (immature and mature oocytes) through the production of different cohorts.

Oogenesis in *A. fiordensis*, consists of six stages in which the diameter of oocytes increases, up to reach the maximum in term of diameter and number (Fig. 7). After the spawning, a few oocytes persist, that will be reabsorbed before starting the new cycle.

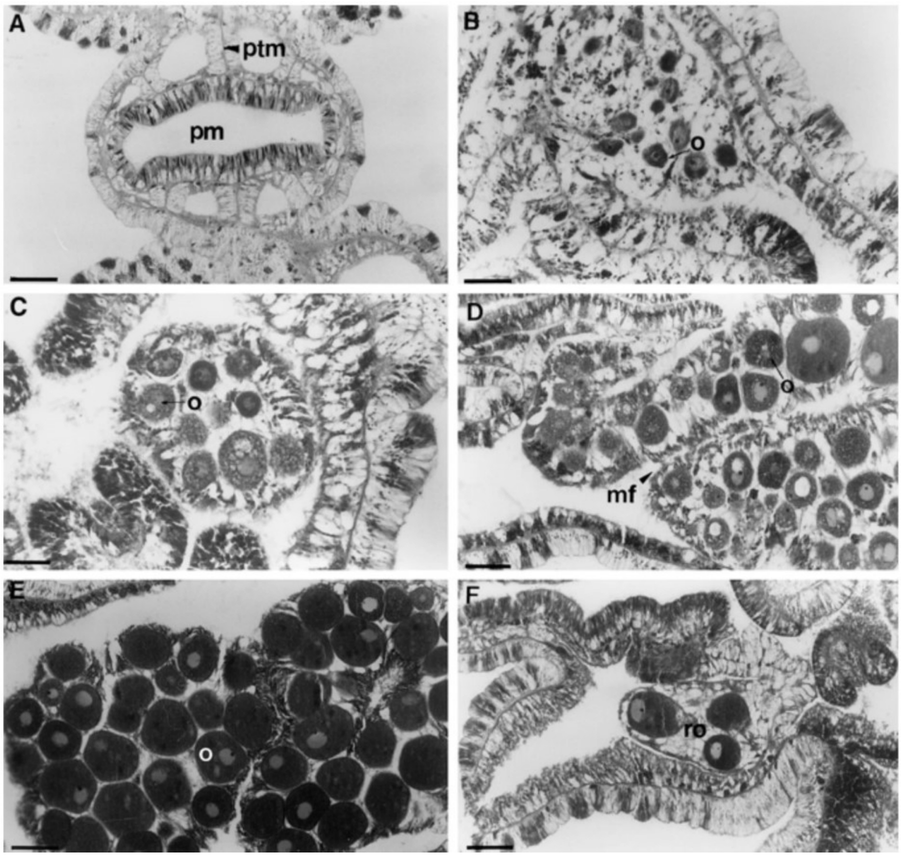

Fig. 7 Stages of oogenesis in *A. fiordensis*. A: stage 0 (unsexable); B: stage 1 (early); C: stage 2 (growing); D: stage 3 (maturing); E: stage 4 (mature); F: stage 5 (spent) (Figure from Parker et al., 1997).

In the same species, spermatogenesis includes 5 stages (Fig. 8). In the mature stage, spermatocytes show a drop shape with tail of spermatozoa oriented in the same direction and heads visible (Parker et al. 1997).

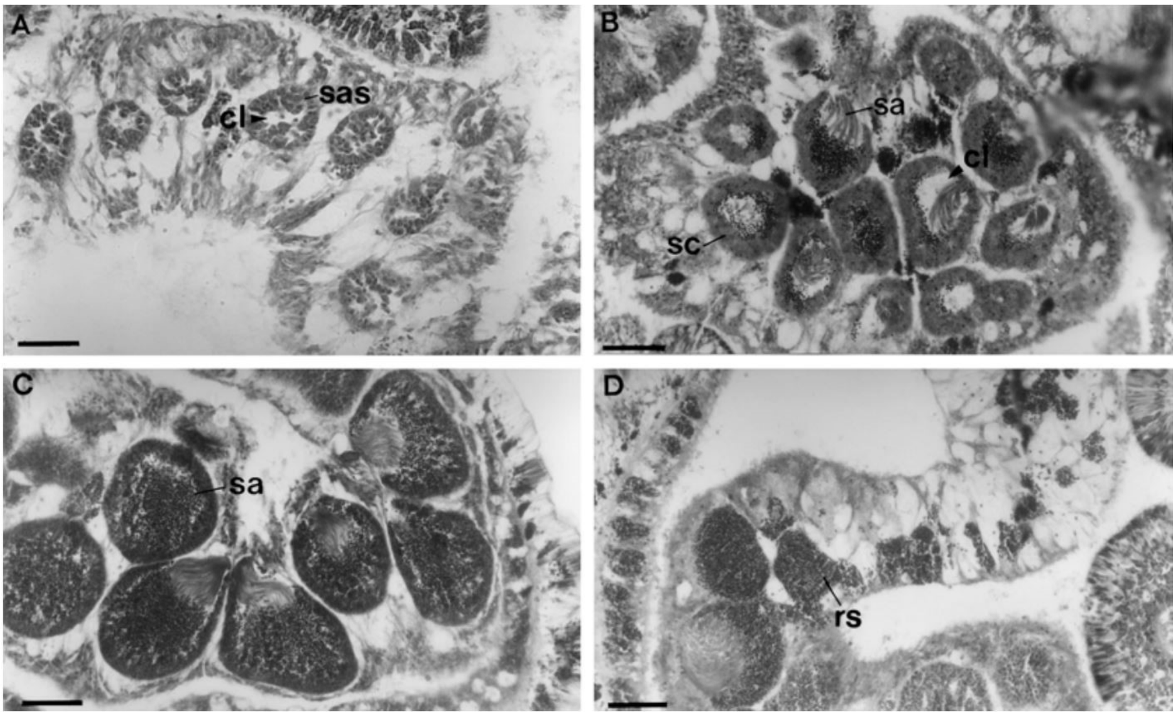

Fig. 8 Stage of spermatogenesis in *A. fiordensis.* A: stage 1 (early); B: stage 2 (maturing); C: stage 3 (mature); D: stage 4 (spent) (Figure from Parker et al., 1997).

The reproductive cycle of *A. subpinnata* has not been entirely understood, with only one study been published so far. Colonies were sampled in Adriatic and Tyrrhenian Sea. The colony from Adriatic (42 cm tall) was sexually immature, while the 5 colonies from Tyrrhenian Sea (120 cm tall) showed a sex ratio in favor to males: 4:1. *A. subpinnata* reproduces once a year and the spawning event is likely to occur at the end of summer (August), where water temperature is higher (16°), while no fertile colonies were found between September and November (14°) (Gaino and Scoccia, 2010).

Unpublished data on the reproduction of 78 *A. subpinnata* colonies in several Mediterranean localities, reported a severe imbalance in favor of females with 61 samples, against 6 male samples. Sex ratio have been evaluated in two Ligurian localities of Bordighera and Portofino, with a result of 35:0 and 10:0 respectively. The spawning event occurred in both females and males in August, only Punta Spadillo (Sicily) presented fertile colonies in October and Liguria 2 fertile colonies in September and October. Number and size of gametes in both sexes increased during summer months, reaching 225 µm for oocytes and 200 µm for spermatic cysts. All the samples were gonochoric, except for one, exhibiting in the same polyp both female and male gametes (Msc Andruccioli, unpublished data, 2019). This seasonal trend has been reported as well for one female *A. subpinnata* sample collected in the Ligurian Sea. The presence of gametes has been observed starting from June, reaching the maximum in term of number and diameter in August, with a few oocytes until October (Msc Mestice, unpublished data, 2018).

1.3.4 Larvae

As mentioned in the paragraph above, the first and only successful attempt to raise and follow the development of a black coral larva has been conducted by Miller in 1996 in aquaria. The specie under consideration was *A. fiordensis*, which occurs in New Zealand fjords at 15-30 m depth. The spawning has never been observed *in situ*, in fact it has been induced artificially. The development of the swimming larvae or planulae from the fertilization of the eggs happened after 36 hours, a shorter period compared to other coral species, probably due to the cold water. Larvae were small, less than 0.2 mm long, ciliated, with tiny external hairs that through coordinated movement, allowing them to be mobile (Fig. 9). They were weak swimmers, likely non-feeding, negative phototaxic and slightly negative buoyant; it is not clear if they alter their behavior in relation to the research of a settlement site. These larvae survived for 10 days in aquaria, but none settled, so it is unknown how long it can survive before settling. The hypothesis of restricted larval dispersal capabilities has been supported by molecular studies in New Zealand, through the measure of gene flow. The results showed different genetic structures among populations separated by several kilometers, moreover, colonies less than 5 m distant were closely related than colonies more distant. It suggested that many larvae may settle within 5-10 meters from the parent colony (Miller, 1996).

Fig. 9 Ciliated *A. fiordensis* larva of 5 days, the diameter is approximately 150 µm (Figure from Miller, 1996).

Similar larval characteristics in term of negative phototaxic and buoyancy, have been reported as well for the deep scleractinian coral *Lophelia pertusa*. These are in contrast with the characteristics of many scleractinian coral species of tropical shallow reefs, which exhibit an upward swimming behavior. This positive buoyancy might increase the dispersal potential of coral propagules, in particular from deep to shallow environment. On the other hand, *Lophelia pertusa* and black coral's larvae remain trapped at depth (Terzin et al., 2021).

1.4 The target species: **Antipathella subpinnata** *(Ellis and Solander, 1786)*

Antipathella subpinnata (Ellis and Solander, 1786) is a species of Antipatharia, belonging to the family Myriopathidae. The inclusion of this species in the family is recent, after molecular analyses performed by Lapian et al. (2006). Among the seven antipatharians species (*Antipathes dichotoma*, *A. subpinnata, A. wollastoni*, *Leiopathes glaberrima*, *Parantipathes larix*, *Parantipathes* sp., and *Phanopathes* sp.; Bo et al., 2018, 2020; Bo and Bavestrello, 2019; Corbera et al., 2019) of the Mediterranean basin, it is the commonest of Mediterranean Temperate Mesophotic Ecosystems (TMEs): communities found below PAR 1%, approximately between 40 and 260 m depth (Bo et al., 2019a; Cerrano et al., 2019).

A. subpinnata is characterized by a Mediterranean-Atlantic distribution. In the Mediterranean Sea, it is present particularly in the northern western basin, where it creates forest-like aggregations (i.e., the so-called animal forests; Rossi et al., 2017), both along the coast and in offshore locations such as seamounts (Bo et al., 2011c) (Fig. 10).

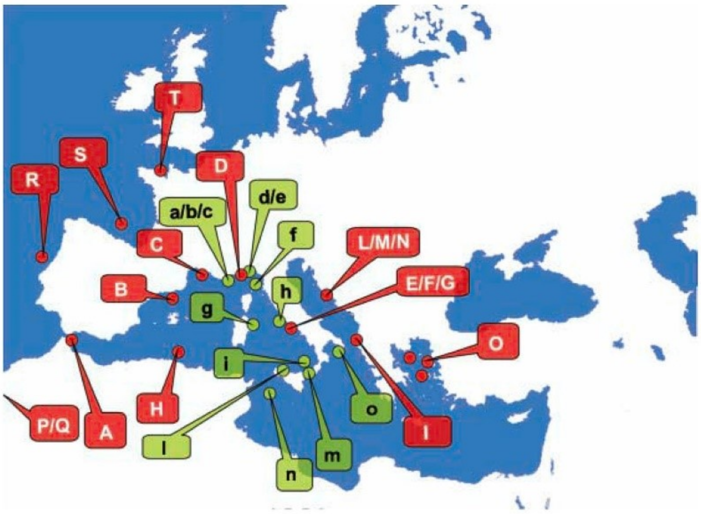

Figure 4. Distribution map of *Antipathella subpinnata* in the Atlantic-Mediterranean region. Legend of colours: Dark grey/red: historical record sites; light grey/green: current record sites. A, Near Gibraltar. B, Spanish coast. C, French coast. D, Ligurian Sea. Gulf of Naples, Tyrrhenian Sea: E, Bay of Naples; F, Capri Island; G, Nisida Island. H, Tunisian and Algerian coasts. Adriatic Sea: I, Albanian coasts of Otranto Strait, Croatian islands of L, Lastovo; M, Lissa and N, Lagosta. O, North Aegean Sea. Eastern Atlantic (not shown in the map): P, Josephine Seamount; Q, Great Meteor Seamount. R, West Coast of Portugal. S, West Coast of France: Biskaya Bay and Gascogne Gulf. T, Brest, Atlantic France, English Channel. Gulf of Genoa, Genoa, Ligurian Sea: a, Bordighera; b, Wreck 'Ravenna', Imperia; c, Capo Mele. Portofino Promontory, Genoa, Ligurian Sea: d, Secca dell'Isuela; e, Punta di Portofino. f, Secca fonda della Civitata, Capraia. G, Capo Comino, Sardinia. h, Ponza, Latina. i, Dorsale della Sciara del Fuoco, Stromboli, Sicily. l, Scoglio della Formica, S. Flavia, Palermo, Sicily. m, Secche di Favazzina, Bagnara, Strait of Messina, RC, Sicily. n, Pantelleria Island, Sicily. o, Gallipoli, Lecce.

Fig. 10 Distribution map of *Antipathella subpinnata* in the Atlantic-Mediterranean region (Figure from Bo et al., 2008).

Recently, *A. subpinnata* colonies have been found in Adriatic, between 52 and 80 m, in three different sites at Tremiti Islands Marine Protected Area (Chimienti et al., 2020). In the eastern Mediterranean the only record is in the Greek archipelago, in the north Aegean Sea.

A. subpinnata is present mainly in the circalittoral showing higher abundances with increasing depth and becoming more prevalent in cold water coral assemblages and seamounts. It shows a peak of abundance between 60 and 150 m depth (Bo et al., 2009a), despite it can be present down to 600 m depth (Deidun et al., 2015). This species is found on hard substrates, mostly on rock, even if covered by fine muddy sediments. It can also colonize artificial substrates such as wrecks, (e.g., the “Ravenna wreck” in the Ligurian Sea) (Bo et al., 2008).

A. subpinnata shows a white, large and branched morphology with elongated pseudopinnules arranged irregularly in 1-4 rows. It can reach more than 1 m in height (Chimienti et al., 2020). Each polyp presents a mouth surrounded by 6 tentacles: 2 sagittal that are longer and 4 lateral (Fig. 11).

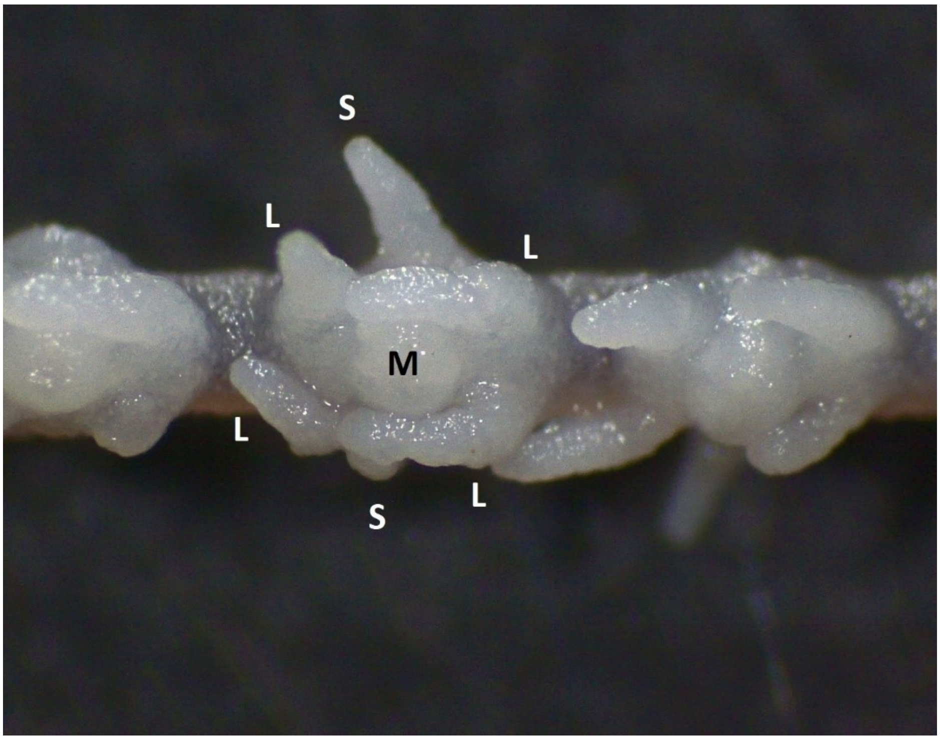

Fig. 11 Polyp morphology of *A. subpinnata* colony, characterized by the mouth (M) encircled by 6 tentacles, 2 sagittal (S) that are longer and 4 lateral (L).

A. subpinnata is a gonochoric, broadcast spawner with a spawning period observed at the end of the summer by Gaino and Scoccia (2010) in the

Tyrrhenian Sea. Asexual reproduction occurs through fragmentation (Coppari et al., 2019) and polyp bail-out (Coppari et al., 2020), both observed only in aquaria, but they may potentially occur under natural conditions as well (Terzin et al., 2021).

1.4.1 Genetic structure in the Mediterranean Sea

The genetic connectivity of a population in benthic marine species is influenced by many biological factors such as sexual and asexual reproductive strategies, the pelagic larval duration of offspring, larval buoyancy, motility behavior and life-stage mortality. Broadcast species have a higher mixture rate compared to brooders. The objective is to study the genetic connectivity between coastal and offshore populations to apply proper management strategies.

Genetic connectivity among *A. subpinnata* populations in Mediterranean was investigated for the first time only recently by Terzin et al. (2021), providing insight in the process of population maintenance and gene flux. Colonies from offshore localities (Ligurian seamount and a Tyrrhenian canyon) and Sicilian and Ligurian coastal areas have been genotyped. Results showed genetic differentiation between coastal (upper mesophotic) and offshore areas (lower mesophotic), with the coastal genotype exhibiting a high degree of admixture: panmixia, with the gene flow maintaining connectivity. The connectivity of coastal population can be explained by asexual reproduction, as the population could be partially clonal, as it was verified from the analysis, with the coastal populations being less susceptible to human impacts. Conversely, the offshore colonies were genetically distinct from each other, suggesting a limited or absent ongoing gene flow. These last were also characterized by a limited influx of larvae from adjacent reef, resulting in less resilience to tackle human impacts.

1.4.2 Ecological role and vulnerability

These forests are damaged by commercial and recreational fishing activities, due to their presence on elevations such as seamounts. Their arborescent morphology causes the entanglement in the fishing net and lost fishing gears, resulting into the partial or total removing of the colony and in the colonization of the skeleton by fast growing species (Mortenses et al. 2005; Bo et al. 2014).

A. subpinnata acts as ecosystem engineers, enhancing the spatial heterogeneity of the seabed, attracting numerous species, many of them of commercial interests, including sedentary (*Palinurus elephas* and *Homarus gammarus*) and migratory species (*Seriola dumerili* and *Thunnus thynnus*), as well as squid egg masses (Chimienti et al., 2020). Another important ecological role comprises the capture of particulate organic matter and plankton suspended in the water (Terzin et al., 2021).

In the study conducted by Godefroid et al. (2023), the effect of ocean warming on *A. subpinnata* in temperate region was investigated for the first time. The specie has been exposed for 15 days to different temperatures, including the current seasonal range to the forecasted one for 2100, measuring biological endpoints. The results showed no stress at cellular or organism level suggesting a low susceptibility of this specie to thermal increase, *A. subpinnata* is unlikely be affected by ocean warming.

The slow growth rate coupled with the damage of fishing nets make this species vulnerable. Indeed, *A. subpinnata* specie has been listed as “near threatened” in the Red List by IUCN (Otero et al., 2017). It is also included in Annex II of a Barcelona Convention protocol for the Specially Protected Area of Mediterranean Interest (SPAMI) and listed as representative specie that contributes to the formation of hard bottom coral gardens by the International Council for the Exploration of the Sea (ICES) (FAO 2009).

Despite this, there are not many management actions in the Mediterranean Sea to protect cold water coral ecosystems. At Italian initiative involves Southern

Tyrrhenian Sea for the protection of *A. subpinnata*, promoted by the Calabria regional deliberation. In Santa Maria di Leuca, Apulia region, a Fishing Restricted Area (FRA) has been introduced in a site hosting black coral forests. However, so far, the only Italian MPA, containing black coral forests, is in the Tremiti Islands Marine Protected Area (Ingrassia and Di Bella, 2021).

The importance of protecting mesophotic coral ecosystems has been discussed recently by Soares et al. (2020). These ecosystems provide refuge against human stressors, ecosystem services and ecological connectivity, underlining that they are the least protected systems in the ocean world. The authors suggested protecting these mesophotic environments to avoid unnecessary declines in biodiversity and ecosystem goods and services, on which the society rely on.

1.5 Aim of the study

The main aim of this thesis is to shed light into the reproductive strategies of mesophotic and deep corals. To reach this objective a first analysis of the available literature regarding the sexual and asexual reproduction of Hexacorallia and Octocorallia in mesophotic and deep environments, was performed to identify potential gaps of knowledge. As a point of fact, the reproduction of shallow corals has been deeply investigated while the knowledge of deep cnidarians reproduction is still limited.

In this context, the Mediterranean black coral *Antipathella subpinnata* was selected as target species to explore unknown aspects of its life history such as the sexual reproduction and the larval ecology. So far, few publications on this topic are available for the western Mediterranean Sea, therefore this study aims to deeply investigate, in the one hand the sexual reproduction of this species, through histological analysis, describing the fecundity, the number and size of germinal cells in relation to the sampling period. On the other hand, considering that the larvae of *A. subpinnata* has never been observed nor in the field or in aquaria, this work aims to describe its larval ecology highlighting morphology, behavior, metamorphosis, and substrates preference for settlement. *A. subpinnata* is one of the most important ecosystem engineer species of the mesophotic zone. Knowing the reproductive aspects is of crucial importance to understand the life cycle, the connectivity among populations and the potential resilience against climatic or anthropogenic stressors. Ultimately this study aims to provide a baseline to develop management and protection strategies.

Second chapter

2. MATERIALS AND METHODS

2.1Literature collection

The literature on sexual and asexual reproduction in corals of mesophotic and deep environments was obtained thorough Elsevier’s Scopus database (https://www.scopus.com/). The research was conducted entering different keywords: [“cnidaria” or “polyp” or “coral”] AND [“mesophotic” or “deep”] AND [“reproduction”] in the option “Article title, Abstract, Keywords” considering all years until December 2022. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement was followed (Page et al., 2021) and the flow chart is reported in Fig. 12. The literature considered in the review included both journal articles and grey literature, and the criteria used to select the bibliography to be included are reported below; reviews were not included by choice, but their bibliography was screened using the eligibility criteria. Additional articles were also added as expert opinion.

The criteria used to establish the eligibility of each document were:

- ✓ The specie must belong to Hexacorallia or Octocorallia subclass.
- ✓ The study must contain information on either asexual or sexual reproduction.

- ✓ The bathymetrical range of the studies includes studies performed from the mesophotic (> 40 m) to the abyssal depth. Many studies were carried out from shallow to deep environments; in these cases, only data concerning mesophotic or deep environment were considered. Species records were divided in bathymetric ranges, according to their sampling depth: upper mesophotic (40-100 m), lower mesophotic (101-260 m), upper bathyal (261-500 m), lower bathyal (501-3000 m), abyssal (>3000 m).

A total of 145 articles from Scopus and 22 articles from expert opinion, have been screened, by reading title and abstract: the articles that did not match the criteria were excluded, while the others were analyzed in detail reading the full text. Eighty articles were then not included since they did not satisfy the criteria, leading to a total of 87 publications included in the analysis.

Table 1 summarizes the research procedure through Scopus and the inclusion-exclusion criteria.

Table 1 Table reporting the research procedure through Scopus and inclusion and exclusion criteria.

Years	All years until 2022
Keywords	“cnidaria” or “polyp” or “coral” AND “mesophotic” or “deep” AND “reproduction”
Search option	“Article title, Abstract, Keywords”
Database	Scopus
Inclusion criteria	-Hexacorallia or Octocorallia subclass. -Information on either asexual or sexual reproduction. -Mesophotic (>40 m) or deep environment.
Exclusion criteria	Articles not concerning reproduction of Hexacorallia or Octocorallia in mesophotic or deep environment.

Studies were analyzed based on the two cnidarian subclasses (Hexacorallia, Octocorallia), temporal and geographical distribution, depth range (40-100 m, 101-260 m, 261-500 m, 501-3000 m, >3000 m), reproduction type (sexual, asexual), reproductive strategy (broadcast and brooding for the sexual reproduction; budding, fragmentation, fission, parthenogenesis for the asexual reproduction), reproductive condition (gonochorism, hermaphroditism, gynodioecism). In all the analyses, every document could be included in one or more categories.

For the creation of the map of the geographical distribution of documents on Hexacorallia and Octocorallia, geographic coordinates were used. In studies showing only a map of the sampling point(s), geographic coordinates were estimated from it. In the absence of a map, coordinates of the sampling point(s) were estimated using the information across the text, if available. When the given coordinates ended up in land, they were adjusted to the nearest coastal points.

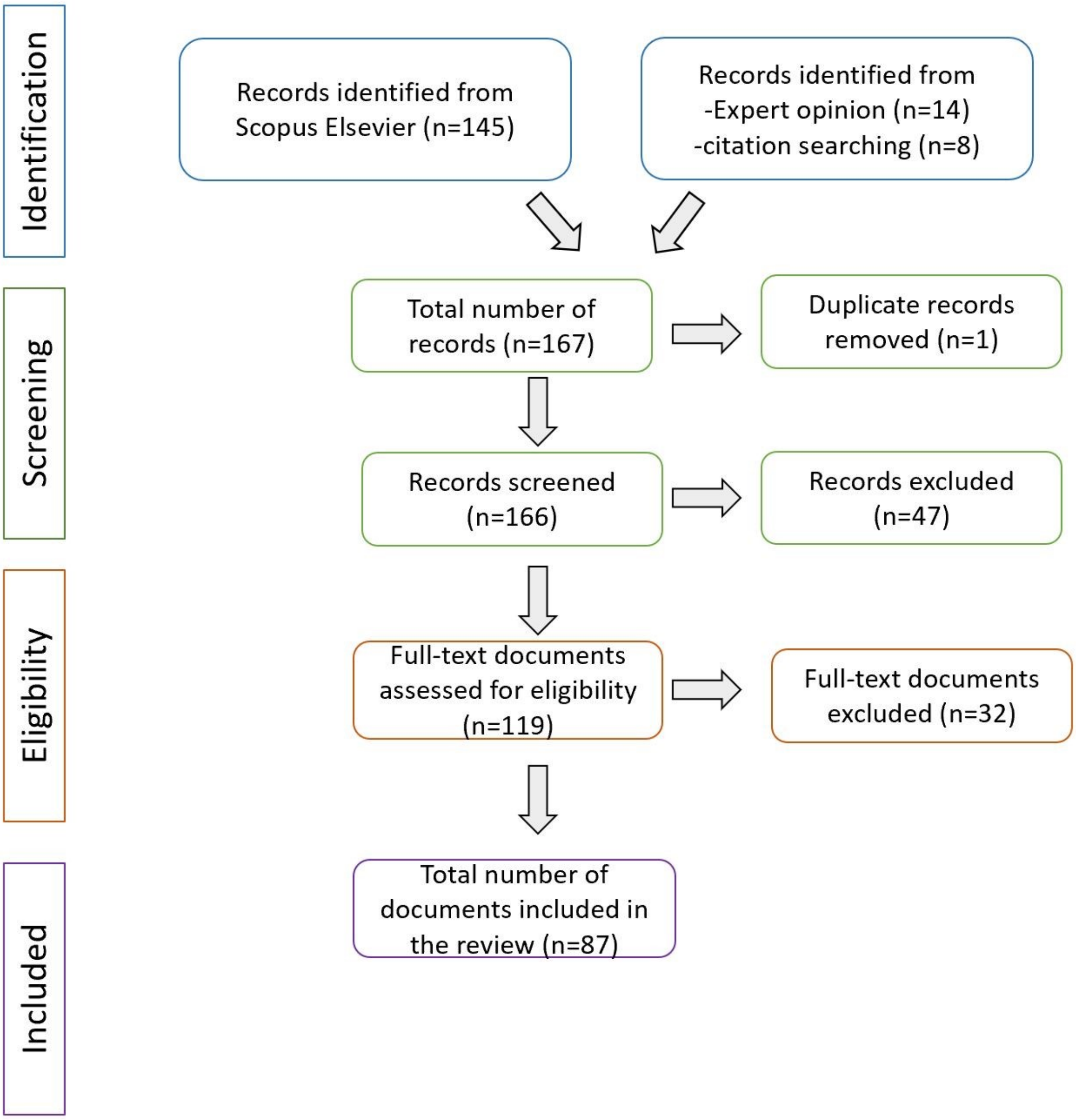

Fig. 12 PRISMA flow diagram of searching strategy and eligibility process applied in this thesis.

2.2 Study area

2.2.1 The Tyrrhenian Sea

The Tyrrhenian Sea is located in the Western Mediterranean ecoregion, lying between the Italian peninsula and the islands of Sicily, Sardinia and Corse. The continental shelf extends only for a relatively short distance. The depth exceeds 2000 m throughout the entire basin, with a maximum depth of 3840 m. Input from the continental waters (Ombrone, Tevere, Garigliano and Volturno river) is minimal, and salinity remains constant at approximately 38 PSU. The recorded temperature during winter is homogenous from the surface to the bottom of the basin, around 13°C. With the surface temperature increasing in the summer season (up to 24-25°), there is also the formation of a thermocline.

The Tyrrhenian area is geologically active due to the presence of various seamounts and volcanos (with more than 14 seamounts described so far in the bathyal plain of this area; Bo et al., 2011c).

The surface circulation is counterclockwise: Atlantic Water (AW), after following the African coastline, crosses the Sardinia Channel and the Levantine Water (LW) enters from the Sicily strait. Then they move together northwards, along the coastline of the Italian peninsula and they come out through the Sardinia Channel (Fig. 13). This general pattern may change in relation with the wind regimes and their influence on the currents. The intermediate circulation is characterized by a cyclonic path along the coasts of the basin and the Levantine Intermediate Water (LIW) current flows anticlockwise along the coast of Latium, at depths between 250 and 700 m (Iacono et al., 2021).

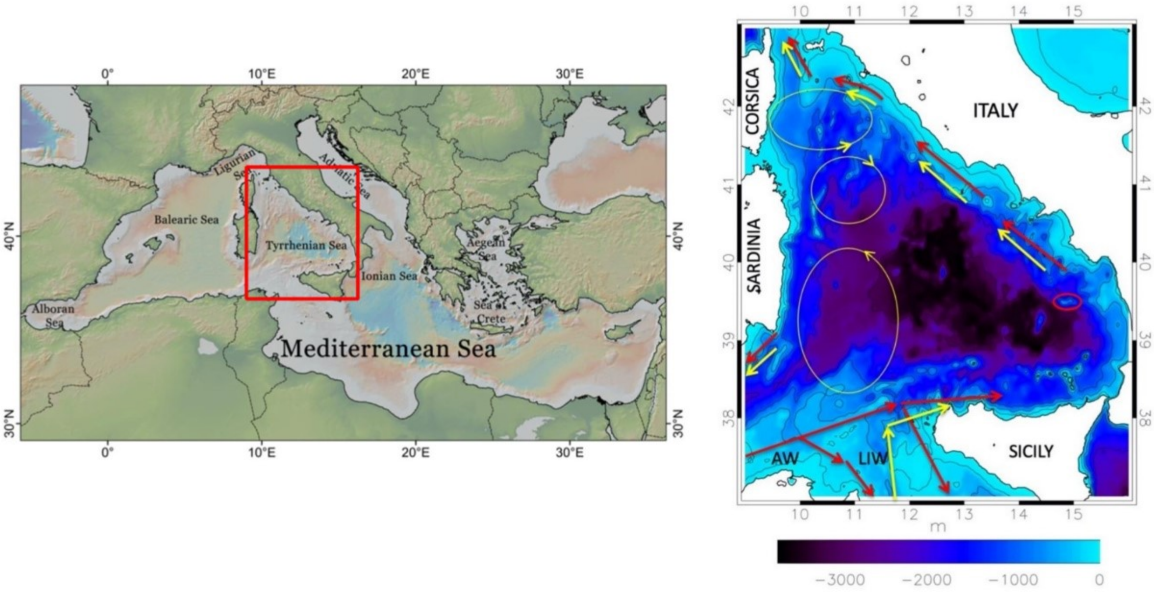

Fig. 13 Left: position of Tyrrhenian Sea inside Mediterranean basin. Right: bathymetry and geometry. Yellow ovals indicate three permanent structures of the basin circulation. The arrows indicate the paths inside the basin of the Atlantic Water (red) and Levantine Water (yellow) (Figures from Iacono et al., 2021).

2.2.2 Giannutri Island

Giannutri Island, with its characteristic half-moon shape, is located in front of Monte Argentario promontory in the Tyrrhenian Sea. Giannutri is the southernmost island of Tuscan Archipelago, located 14 km south-east of the Giglio Island and 12 km south-west of Monte Argentario (Fig. 14A). The island is calcareous, characterized by a rocky and rugged coastal area; it is 3 km long and it has a width of 500 meters.

The area is a regional interest site (SIR) included in the Tuscan Archipelago National Park from 1996. The marine reserve has two different sites of protection: zone 1 in which access, navigation, anchorage, fishing and diving are prohibited, and zone 2 where fishing is regulated by park authority (Fig. 14B).

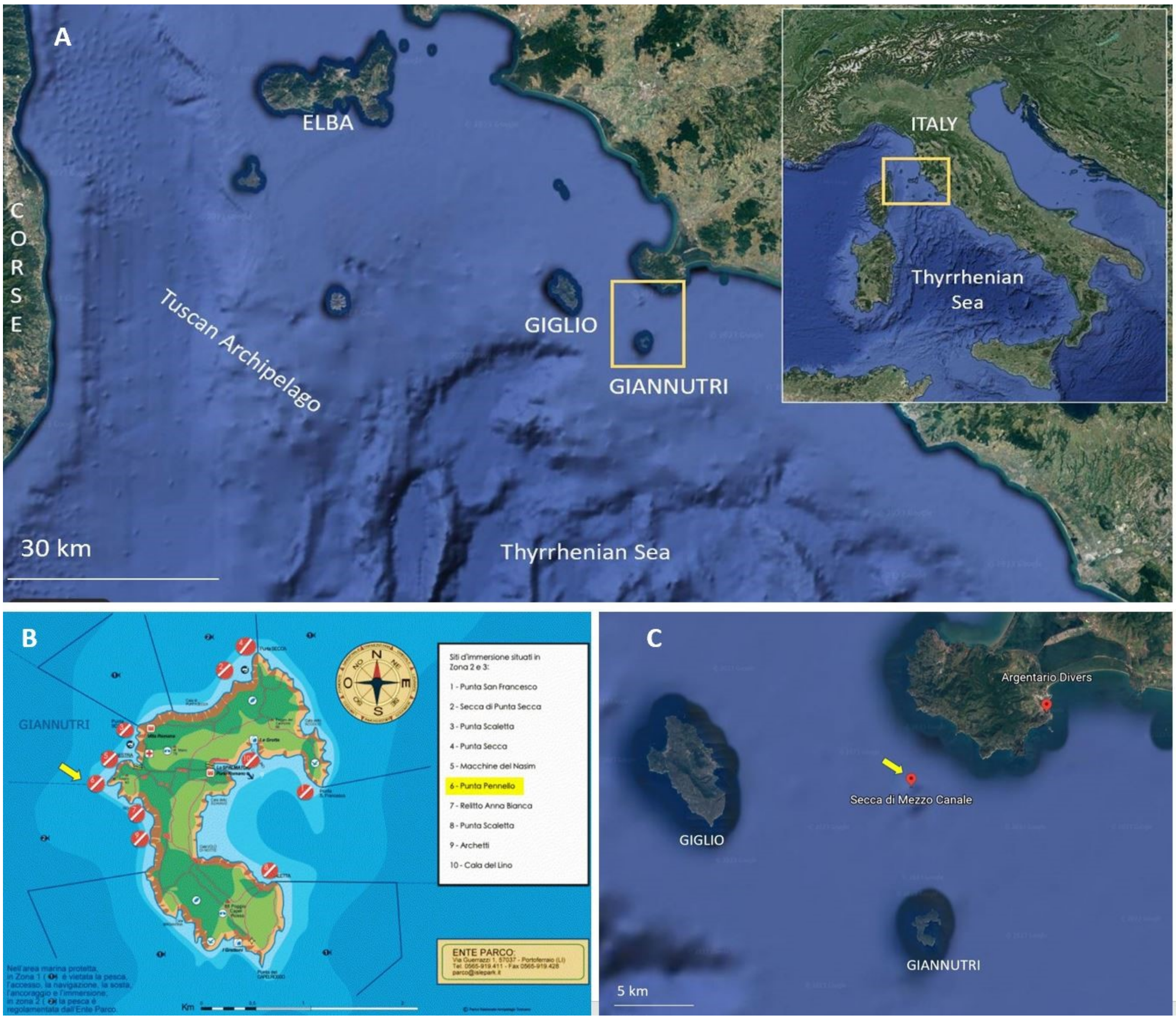

Fig. 14 Figures showing the different sampling areas. **A**: study areas location (Google Earth). **B**: Giannutri Island with the relative level of protection (1-2). The arrow indicates Punta Pennello (Figures from www.argentariodivers.com). **C**: Position of the Mezzo Canale shoal indicated by the arrow (Google Maps).

2.2.3 Punta Pennello

It is a huge shoal with a peculiar geologic conformation, located in the westernmost area of Giannutri (42° 15’ 7.2612’’N; 11° 5’ 10.3812’’E) (Fig.

14B). The slope is smooth and gradual, with a typical environment of a landslide. Punta Pennello continues underwater towards east, shaping a seamount that ends at 60 m. There is a *Posidonia* bed hosting *Pinna nobilis* and along the wall many caves, several of which have hot water springs. Going offshore bryozoan and *Lytocarpia myriophyllum* meadows dominate the communities until reaching a shoal characterized by coralligenous. At higher depth (between 70 and 80 m) the shoal hosts forests of *A. subpinnata*. At the east side, the seafloor smoothly reaches up to 100 m. At 95-100 m a coarse detritus covers the seabed together with Bryozoan, while at 105 m the sea bottom become compact, characterized by the presence of a Crinoid meadow. Other sparse colonies of *A. subpinnata* in Giannutri Island are also reported in a few sites: Secca di Zi Costante and Punta Secca.

2.2.4 Mezzo Canale shoal

This shoal is located in the middle of the canal between the islands of Giannutri and Giglio and the Argentario Promontory (42° 20’ 26.686’’N; 11° 5’ 21.321’’E) (Fig. 14C). The seafloor is mainly muddy and homogeneous, reaching a depth of 90-100 m. The aspect is comparable to a mountain that from 100 m depth, ascend to 24 m, with walls that go down steeply up to 50-60 m, shaping a rockslide. This site, for its position in the open sea, is characterized by strong currents and winds, that makes it inaccessible for most of the year. This shoal hosts many species, such as *Astrospartus mediterraneus, Paramuricea clavata, Savalia savaglia* and *Antipathella subpinnata* among others.

2.3 Study of the reproductive cycle of **Antipathella subpinnata**

2.3.1 Samples collection

To study the reproductive cycle of *Antipathella subpinnata* technical divers of the diving center Argentario Divers were directly involved to participate and collaborate in the scientific research through Citizen Science activities. Prior the start of the activities, technical divers were trained to randomly select colonies of minimum height of 100 cm (i.e., to avoid the unfertile colonies; Gaino and Scoccia, 2010) and tag them at their base with cable ties and labels. Ten colonies were randomly sampled in August 2021 at Mezzo Canale shoal at a depth range of 62-70 m, with a sea water temperature of 16°. As this site is characterized by strong currents being difficult to be reached for a continuous monitoring, during 2022, Punta Pennello (depth of 70 m) was selected as additional study site. A total of 12 colonies were sampled in the period between

June and October 2022. For the entire period, the sea water temperature at the collection site was 14°. Of these, 3 colonies were repeatedly collected and monitored in the same months. The selected period represents the moment when the gametogenesis mainly occurs (Andruccioli, unpublished), ending with the spawning event in August (Gaino and Scoccia, 2010). Due to the bad weather conditions, sampling activities in July and September were prevented, therefore samples of these months were missing.

Fragments of 10-12 cm of *A. subpinnata* branchlets were collected with scissors and placed in falcon tubes filled with sea water (Fig. 15). Once in the laboratory, samples were preserved in 95% ethanol.

Fig. 15 Technical diver collecting *Antipathella subpinnata* branchlets with scissors (Photo courtesy: Argentario Divers).

2.3.2 Histological analysis

We followed the protocol for the cold resin inclusion comprising dehydration, infiltration, polymerisation, blocking, cutting, staining, and mounting (Fig. 16).

For each sample, we selected 5-6 basal fragments or the central portion of 1-2 cm consisting of at least 5 polyps; we did not use the growing apical part or the distal branchlets as they contain very small polyps mostly without gametes (Bo et al., 2009b). In total, at least 10 polyps per sample were processed. Samples have undergone a dehydration process to perform the resin inclusion and the steps are reported in detail below:

- 24 hours in 100% absolute alcohol
- 24 hours in a solution of 50% absolute alcohol and 50% resin (Technovit 8100)
- 24 hours in pure resin (repeated twice)

Infiltration resin: we prepared infiltration resin under the fume hood, mixing 100 cc of pure resin (Technovit 8100) and 1 bag of Hardner I in a beker using a magnetic stirrer for 15 minutes.

Polymerisation: firstly, we made a scheme of samples position in the Teflon plate, then we transferred the samples on a Petri Dish following the disposition of our scheme. To prepare the polymerization resin, we mixed 15 cc of infiltration resin and 0.5 cc of Hardner II in a beker in a magnetic stirrer for 30 seconds/1 minute, until the mixture turned into light blue. With a Pasteur pipette we poured a thin layer of mixture in each form, then we transferred the samples inside the forms, filling it with the remaining mixture until the form was filled. Finally, we placed a plastic cover film in each form avoiding the formation of bubbles. The plate was then placed in the refrigerator for one night.

Blocking: to prepare the glue we mixed 9 ml of universal liquid (Technovit 3040) and 18 ml of powder in a glass jar. We removed the plastic cover films, put a thin layer of glue in each form and a previously labelled plastic histoblock above, then we added further glue. We waited for 12-24 hours for the glue to completely harden under the fume hood.

Cutting: Histoblocks were placed on the microtome, we settled the thickness at 8-9 micron and the blade holder inclination. As soon as we started to cut with the microtome, we prepared a slide with label and a few drops of distilled water, histological sections were placed on the slides being sure to maintain the temporal order of the cut. We transferred the slides on the heating plate.

Staining: we placed our slides in a Hellendhal Staining Jar containing a mixture of distilled water and toulidine blue. We waited for 2-3 minutes, depending on the quantity of the dye, and when they reached the coloration needed, we transferred them in a heating plate again.

Mounting: we placed mounting medium Eukitt on our slides and we covered them with a cover class. We let them dry before starting to observe them.

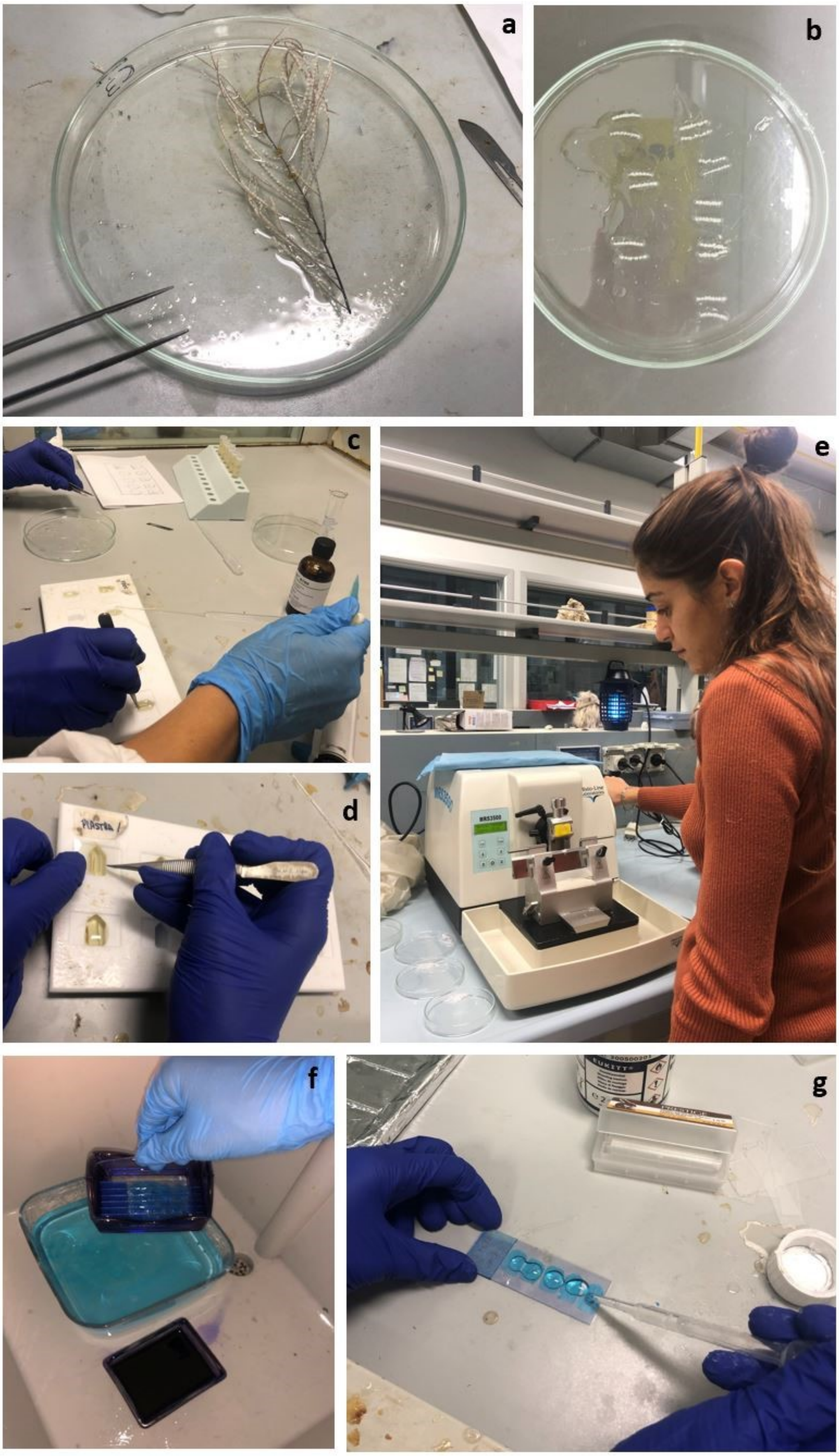
a
b
c
d
PIASTRA 1
e
MRS3500
f
g

Fig. 16 Figures showing the protocol of cold resin inclusion. **a-b**: selection of *A. supbinnata* fragments, **c**: polimerysation, **d:** placing of plastic cover films in each form, **e**: cutting the sample placed on histoblocks through the microtome, **f**: staining with toulidine blue, **e**: mounting.

2.3.3 Reproductive parameters

To evaluate how the gametogenesis progress over months and the change in polyp fecundity, for each colony 10 polyps were processed as explained in paragraph 2.3.2, and analyzed using a LEICA DM 2000 Microscope. For every polyp we evaluated its sex (male/female), the number of germ cells per polyp (polyp fecundity), and the germ cells diameter.

- Sex: when females and males colonies are mature, they show oocytes with a visible nucleus and spermatic cysts bearing spermatocytes with tails noticeable, respectively. Hermaphroditism occurs when in the same polyp both oocytes and spermatocytes are found.
- Number of gametes: with the purpose of defining the stage of gametogenesis of the polyp (and of the colony) over the months, the maximum number of gametes per polyp was counted.
- Diameter: for each germ cell found, the maximum diameter was measured.

When the absence of germs cells inside the polyp was observed, the polyp was noted as unfertile.

Pictures of the germ cells found at different stages of the gametogenesis have been taken with LEICA LAS EZ software.

2.3.4 Statistical analysis

Data of number and diameter of germinal cells, sampled in Punta Pennello, were checked for normality, homoskedasticity and independence, in order to use a parametric test. Levene's test was used for testing homogeneity of the variance and Shapiro's test was used for testing normality of the variance. Since the assumptions for the use of a parametric statistic test were not fulfilled, the non-parametric Kruskal-Wallis test was used to test the significance of the differences in diameter among spermatic cysts, as well as in their number inside the polyp, during the different sampling.

The same test was applied to check for the significance of the differences in the diameter among spermatic cysts of the colonies sampled on the 11th August 2021 and on the 5th August 2022, in Mezzo Canale shoal and Punta Pennello respectively.

2.4 Larval settlement devices

To describe the first stages of larval and primary polyp development, substrates of different materials were positioned in the proximity of adult colonies right before the spawning. Our settlement devices were realized based on previous literature (Fig. 17; Fujiwara et al. 2016), in which ceramic tiles were used due to the similarity of this material to the natural reef. The space between the tiles is small enough to prevent potential attacks by most predators. In this study, in addition to ceramic, we selected other two substrate types: coralligenous and stainless steel. Coralligenous provides a natural substrate, and stainless steel was chosen because several colonies of *A. subpinnata* are known to colonize artificial substrata, such as the wrecks "Haven" and "Ravenna" in the Ligurian Sea (Bo et al., 2008).

Fig. 17 Coral settlement device (Figure from Fujiwara et al., 2016).

2.4.1 Building and set up of the larval settlement devices

- Design: the structure consists in a vertical structure in plastic holding 10 substrates. Each tile is attached through a cable tie to a plastic shelf and this latter is fixed to the vertical structure. The distance between each substrate is 1 cm to reduce the grazing pressure. At the base of the vertical structure, we have a three legs support structure that once in situ is fixed to the sea bottom through weights (Fig. 18). Three structures per typology of substrate have been created for a total of 30 stainless steel, 30 ceramics and 30 coralligenous substrates.

Fig. 18 Vertical structure model with coralligenous substrates.

- Conditioning: Guest et al. (2014) conditioned substrates for 3 weeks prior to settle in situ. Biofilm contains suitable cues for settlement and metamorphosis of larvae, in fact water soluble compounds produced by bacterial biofilms attract coral larvae (Gleason and Hofmann 2011). According to this, on July 8th 2022 substrates were transferred to aquaria tanks filled with seawater. Substrates remained in aquaria for 19 days.

- Transport and in situ deployment: structures were transported from aquaria to the field inside a box with seawater, to maintain the biofilm and colonization of organisms. On August 3rd, the 9 structures were randomly deployed by technical divers inside the *A. subpinnata* forests in Punta Pennello as the spawning is known to occur in this period (Gaino and Scoccia, 2010).

- Collection: 23 days later (26th August) 6 out of 9 substrates, meaning 2 replicates per type, were collected. We decided to leave in situ one series to follow the growing of primary polyp, whenever it attached. These structures were air lifted through a balloon by the technical divers. Cold water (below thermocline at 14°) was collected with a 5 L Niskin bottle to transport the larval settlement devices to the laboratory facilities.

Of the 10 substrates per replicate, 4 substrates were fixed with 70% ethanol for further histological analysis and 6 substrates were maintained in small aquaria (6 L) to follow the fate of the primary polyps attached.

The third replicate was left in situ until the 16th of October to give time to the potential primary polyp to develop. After the retrieval, the same procedure described was followed.

- Aquaria maintenance: Substrates, divided per type, were maintained in box with filtered (0.2 mm) seawater inside an aquarium with a temperature of 14°. Water has been partially changed (1/3 of the total volume) 3 times a week.

- Larval check: substrates stored in 70% ethanol as well as substrates in vivo were monitored under a stereomicroscope. Substrates in aquaria were monitored 3 times per week for 11 days.

Third chapter

3. RESULTS

3.1 Literature analysis

A total of 87 scientific works, from Scopus and expert opinion, were considered in the literature analysis. Most of the papers have been published in the last two decades, indeed a sharp increase in the number of publications can be noticed from 1993-2002 period. The highest number of documents (38) has been recorded in the last decade (2013-2022) (Fig. 19).

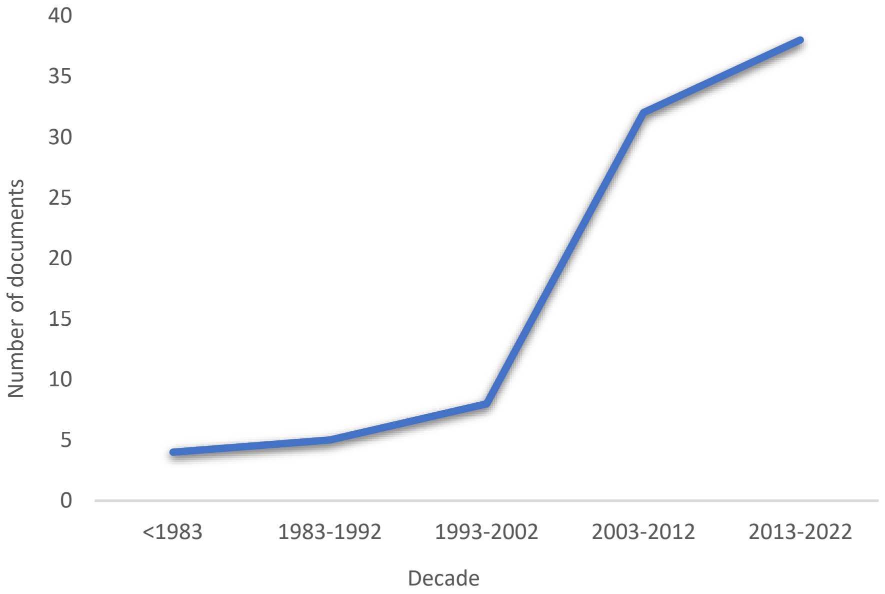

Fig. 19 Number of documents (N) published per decade.

The map below displays the global distribution of Octocorallia and Hexacorallia species considered in the retrieved documents (Fig. 20). Most of the records occurred in the Temperate Northern Atlantic (39) and in the Tropical Atlantic regions (12). The Central Indo Pacific and Tropical Atlantic regions were characterized only by records of Hexacorallia. Samples were performed in the Artic and Southern Ocean regions as well (11).

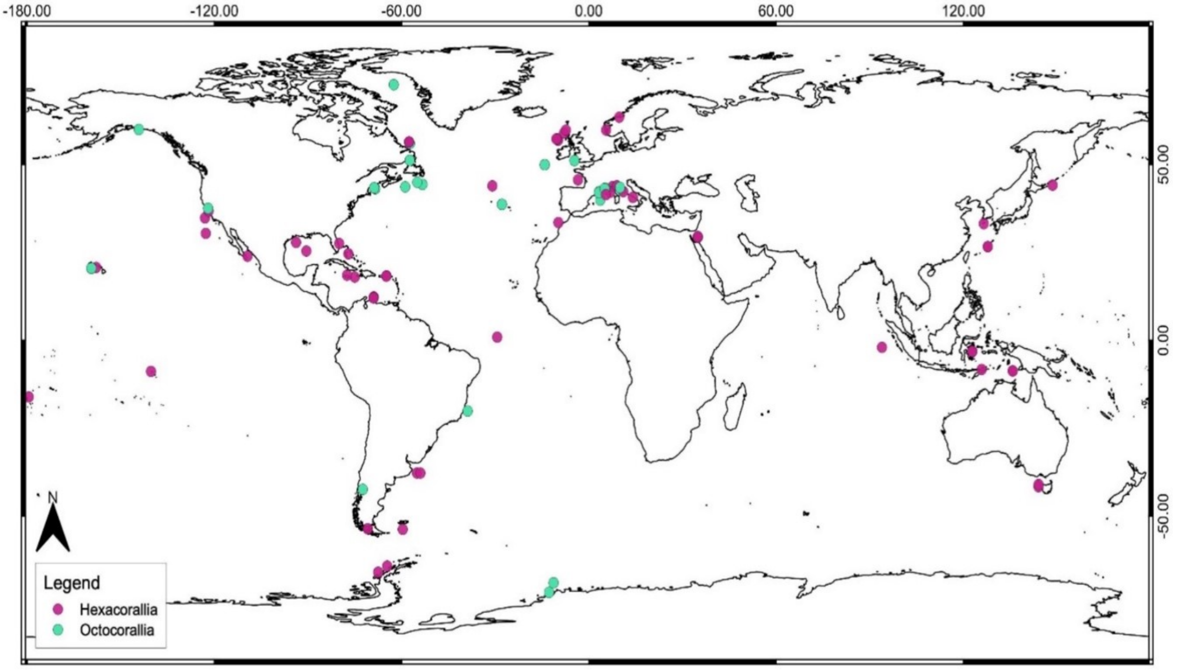

Fig. 20 World distribution of species recorded, divided per subclass: Hexacorallia and Octocorallia.

From the literature, species belonging to Malacalcyonacea (19), Scleralcyonacea (29), Scleractinia (58), Antipatharia (52), Actiniara (9) and Zoantharia (1) orders have been recorded.

The histograms below show the number of documents for each bathymetric range (Fig. 21). For hexacorals, the highest number of documents is recorded in the upper mesophotic (48), followed by lower bathyal (36). In octocorals, the number of documents is more evenly distributed in the 40-3000 m range. For both subclasses, only a small fraction of documents referred to species below 3000 m (3 for Hexacorallia and Octocorallia, respectively).

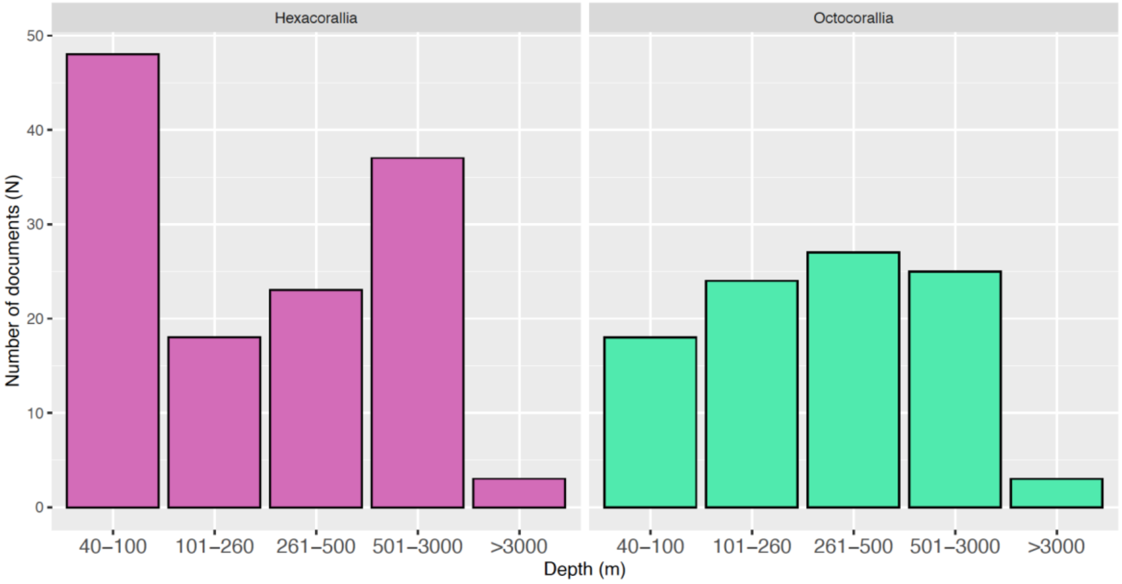

Fig. 21 Number of documents (N) divided per bathymetric range for Hexacorallia and Octocorallia subclass.

The reproduction type (sexual and asexual), adopted by Hexacorallia and Octocorallia, is illustrated in the Pie-Donut chart below (Fig. 22). Nearly the total of works (~90%) concerned the sexual reproduction, divided into ~60% for Hexacorallia and ~30% for Octocorallia, respectively. Asexual reproduction accounted only for a 10%, of which the majority were hexacorals (9%).

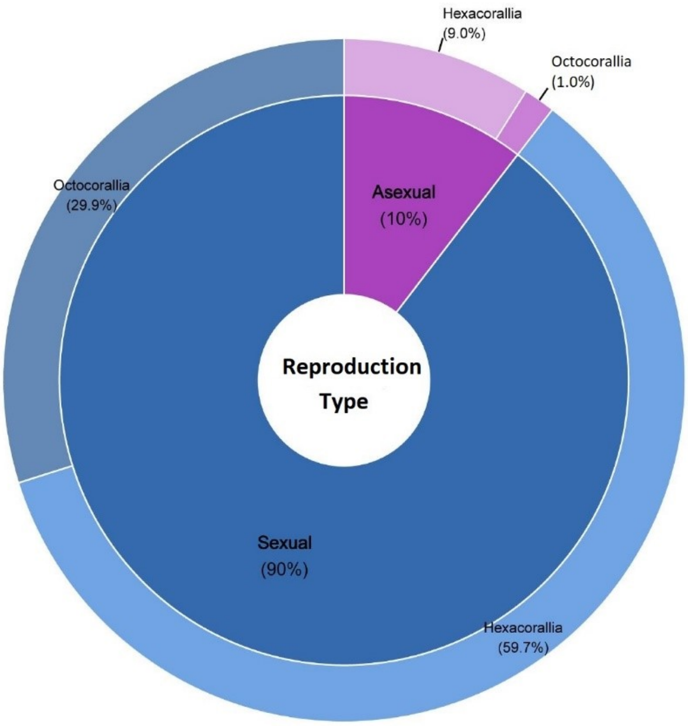

Fig. 22 Type of reproduction (sexual or asexual) adopted by Hexacorallia and Octocorallia subclasses.

For the sexual reproduction, two main strategies, broadcast (65%) and brooding (35%), and three main conditions, gonochorism (82%), hermaphroditism (17%) and gynodioecism (0.9%), were identified. The majority of hexacorals were broadcast species (81%), mostly gonochoric (54.8%), followed by hermaphroditic (23.8%) and gynodioecy (2.4%) (Fig. 23A). Brooding species accounted only for a 19%, of which half were hermaphroditic and half gonochoric (Fig. 23A). On the other hand, octocorals showed approximately a similar percentage of broadcast (54.8%) and brooding (45.2%) species. All the broadcasts were exclusively gonochoric, as well as the majority of brooding species (38.7%) (Fig. 23B).

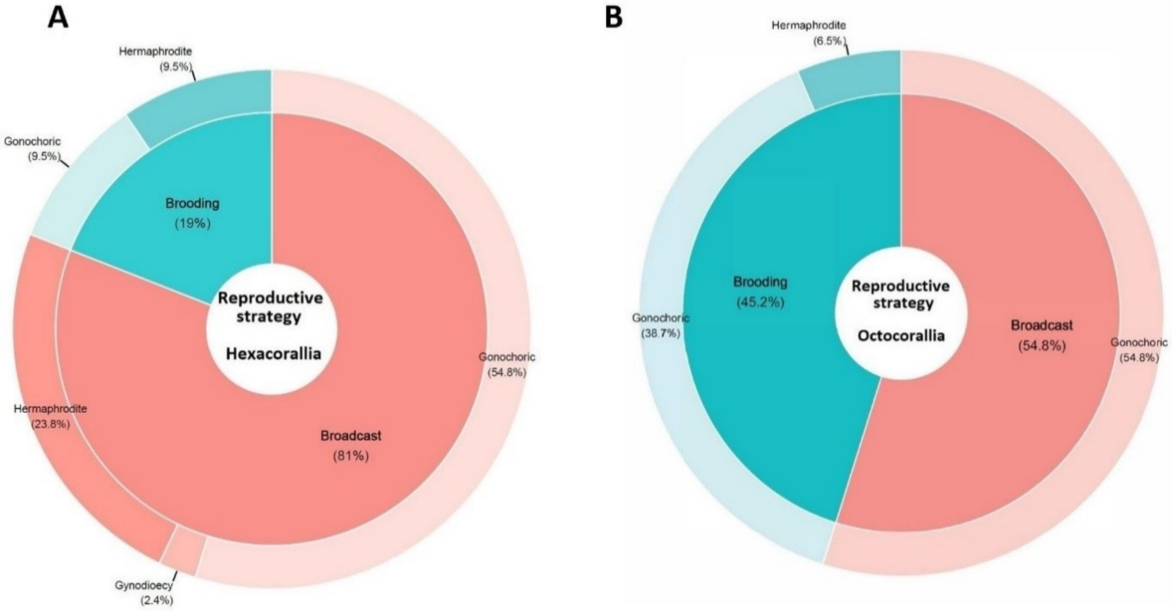

Fig 23 Sexual reproductive strategies in Hexacorallia (**A**) and Octocorallia (**B**).

Asexual reproduction in Hexacorallia subclass has occurred with different strategies including budding (8.3%), fragmentation (33.3%), fission (25%) and fusion (8.3%). In the Octocorallia, only one record of parthenogenesis is reported.

3.2 *Sexual reproduction of* **Antipathella subpinnata**

Both the colonies collected in Punta Pennello (n=12) and Mezzo Canale (n=10) were males, with the ones sampled in the first site presenting spermatic cysts at different stage of maturity during the whole study period. All the samples analyzed confirmed the gonochoric condition of the species with a skewed sex ratio in favor of males in both Punta Pennello (12:0) and Mezzo Canale (9 males and 1 not sexed). A color variation to brown-orange was observed in some colonies, in proximity of the spawning event. Both polyp fecundity and size of gametes were analyzed from Punta Pennello colonies, showing a gradual increase from June, a peak in August, and a slight decline in October, suggesting the occurrence of the spawning event during August. Polyps presenting the spermatic cysts in the gastrovascular cavity and in the proximity of the mouth were observed in August (Fig. 25b) confirming the previous statement. However, this condition was also recorded in October (Fig. 25d) suggesting an asynchrony in the spawning at least for male colonies.

In early June, polyps showed a fecundity of 60% (Fig. 24), with a small number of germinal cells found scattered in the reproductive mesenteries of some of the polyps (Fig. 25a). Moreover, few immature polyps were present interposed to the mature ones. In August, the fecundity increased, reaching 100% of the polyps monitored at the end of the month and in October the fecundity experienced a slight decrease (Fig. 24). Concurrently with the peak of fecundity, larger and more numerous spermatic cysts were found in the gastrovascular cavity, in the tentacles and in the oral region of the polyps. The mature spermatic cysts appeared with a drop shape with spermatozoa tails visible inside (Fig. 26).

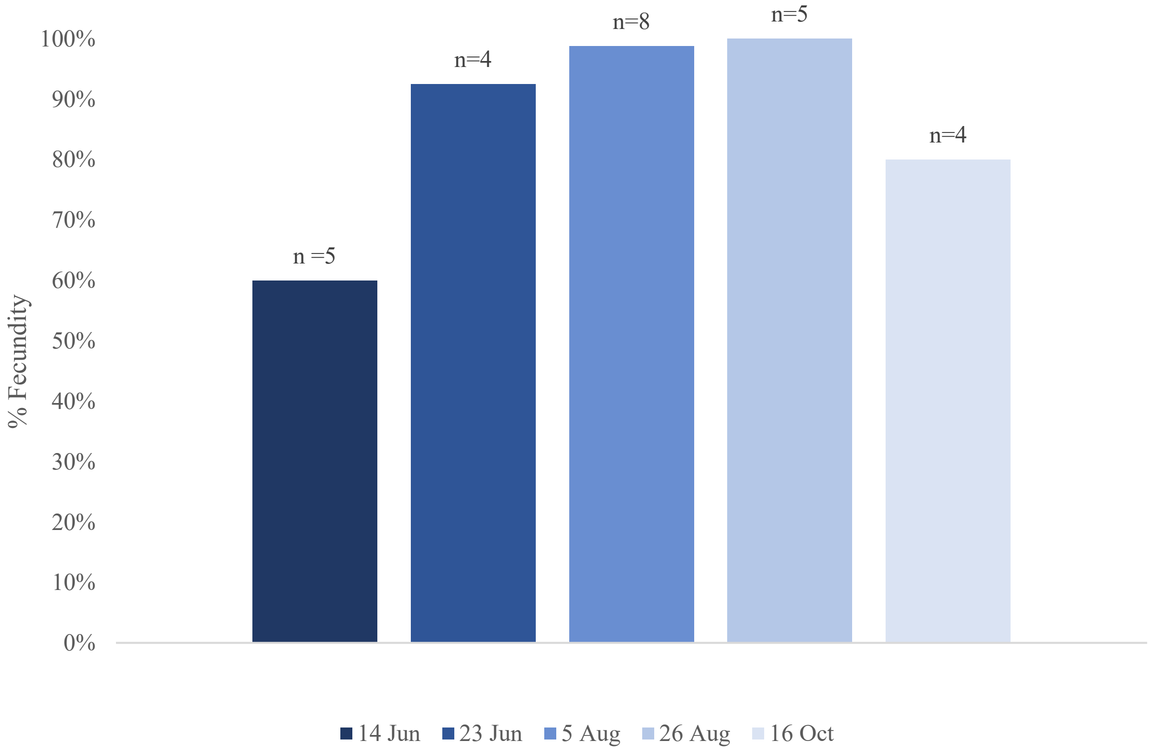

Fig. 24 Fecundity in *A. subpinnata* colonies (expressed in % of the 10 polyps analysed). n: indicates the number of colonies sampled in the respective months.

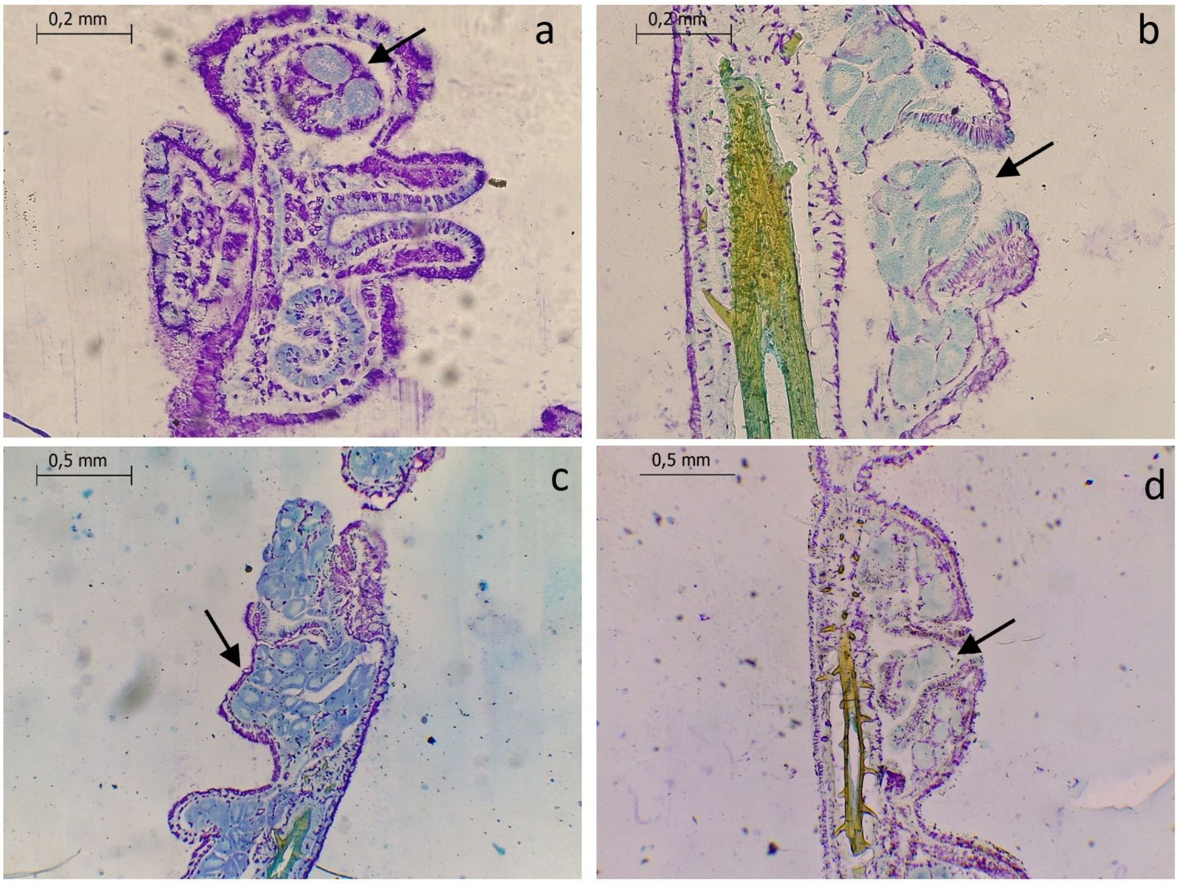

Fig. 25 Spermatic cysts during sampling months indicated through the arrows. **a**: few spermatic cysts in June; **b**: large spermatic cysts in polyp's mouth in August; **c**: many spermatic cysts in the gastrovascular cavity in August: **d**: spermatic cysts in proximity of the mouth in October.

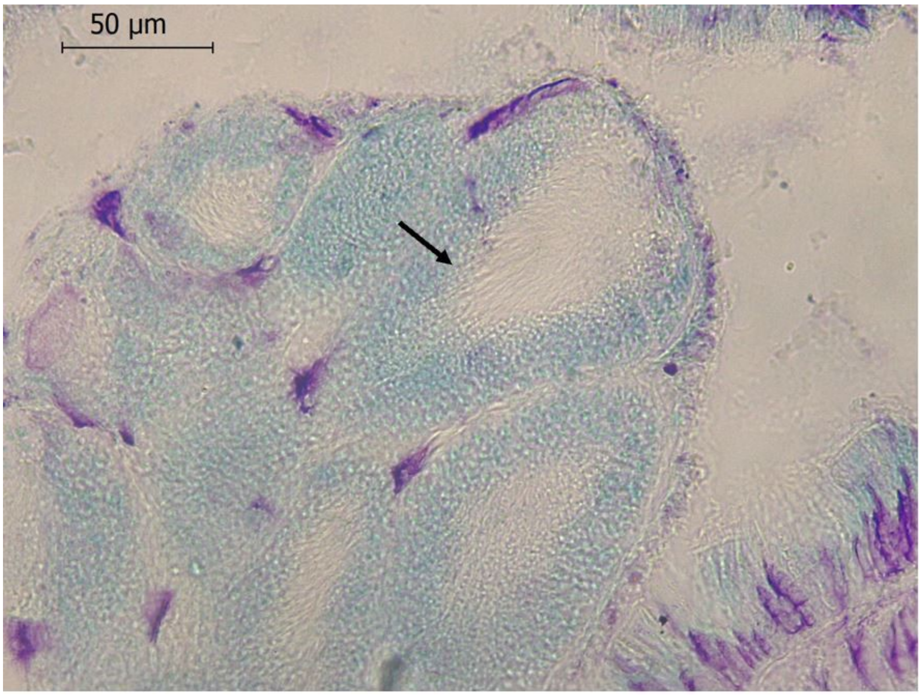

Fig. 26 Picture showing a mature spermatic cyst of a colony sampled in August with tails of spermatozoa visible. The arrows indicate tails of spermatozoa.

Both the number and the size of spermatic cysts were variable during the study period. The mean number of spermatic cysts per polyp followed a Gaussian distribution, starting from a small number in June and increasing by more than 5 times in August, followed by a gradual decrease in October (Fig. 27).

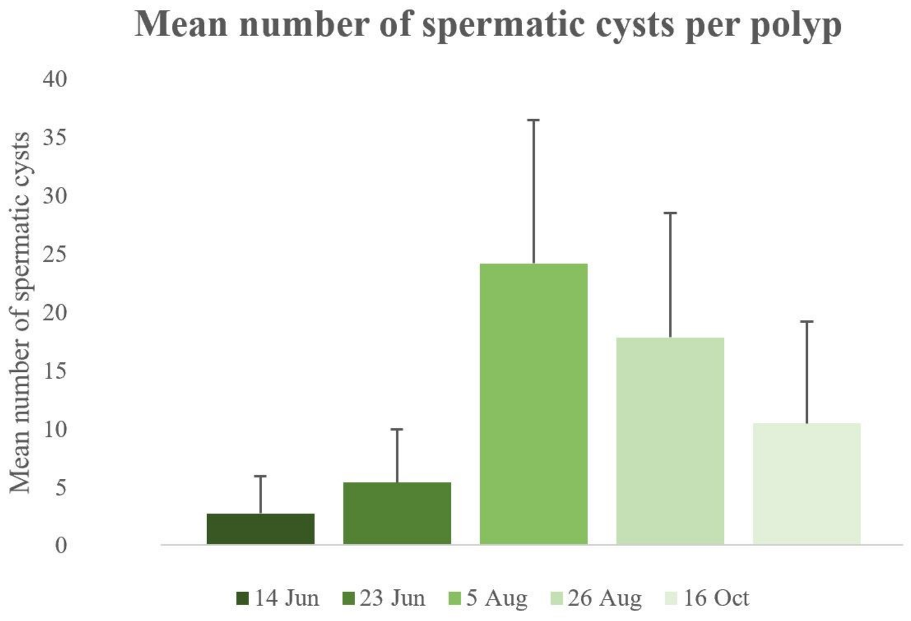

Fig. 27 Mean number of spermatic cysts per polyp ± SD during the study period.

The column chart below illustrates the mean size trend during the sampling period. An increase from June to October has been noticed; the minimum size (15 µm) was detected in June and August, and the maximum dimension (285 µm) on the 5th August, when concurrently a peak of germinal cells number was recorded. After 5th August, samples were characterized by a reduction both in number and in maximum size, while the mean size remained roughly stable up to October (Fig. 28).

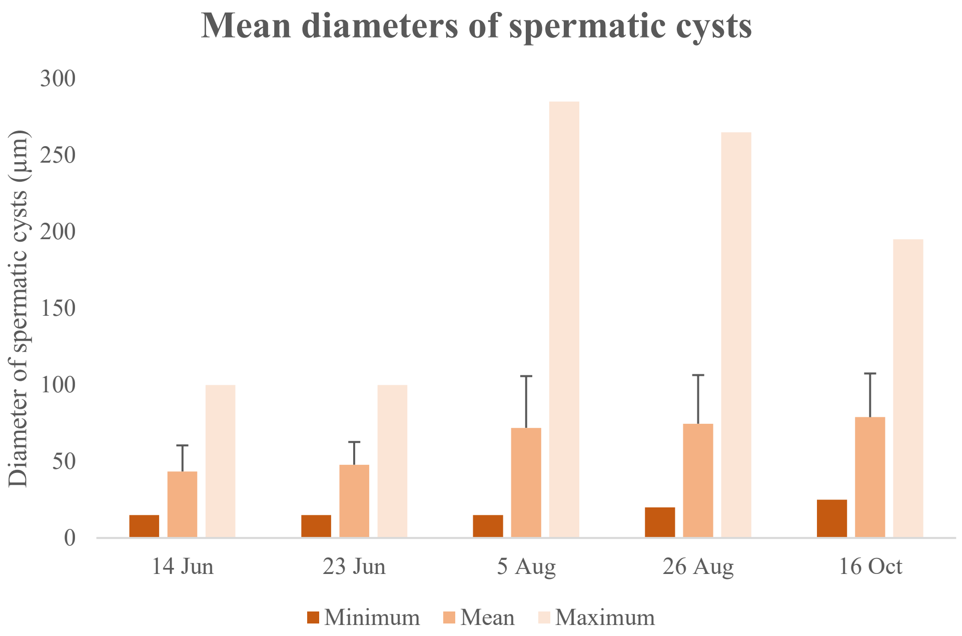

Fig. 28 Size trend of spermatic cyst (µm). Minimum, maximum, and mean diameter ± SD during the study period.

Mean diameter and standard deviation for the colonies from Punta Pennello and Mezzo Canale shoal are reported in the table below (Tab. 2).

Tab. 2 Mean diameter (µm) ± SD of *Antipathella subpinnata* colonies sampled in Mezzo Canale shoal and Punta Pennello.

		14th June	23th June	5th August	11th August	26th August	16th October
Geographical area	Mezzo Canale shoal				58,95±19,69♂		
	Punta Pennello	43,44±17,02♂	47,85±14,84♂	71,94±33,77♂		74,63±31,77♂	78,91±28,54♂

Frequency distribution of spermatic cysts size highlighted a progressive dimensional shift from June to August towards greater size classes (Fig. 29). June is characterized by a frequency concentrated in the size classes 21-60 µm with the greatest size in the class 81-100 µm. In August the % frequency among size classes is more evenly distributed, the greatest size classes reached up to 300 µm and are characterized by an extremely low % frequency. These greatest size classes were not detected in October, in which the % frequency is concentrated in the size classes 21-160 µm (Fig. 29).

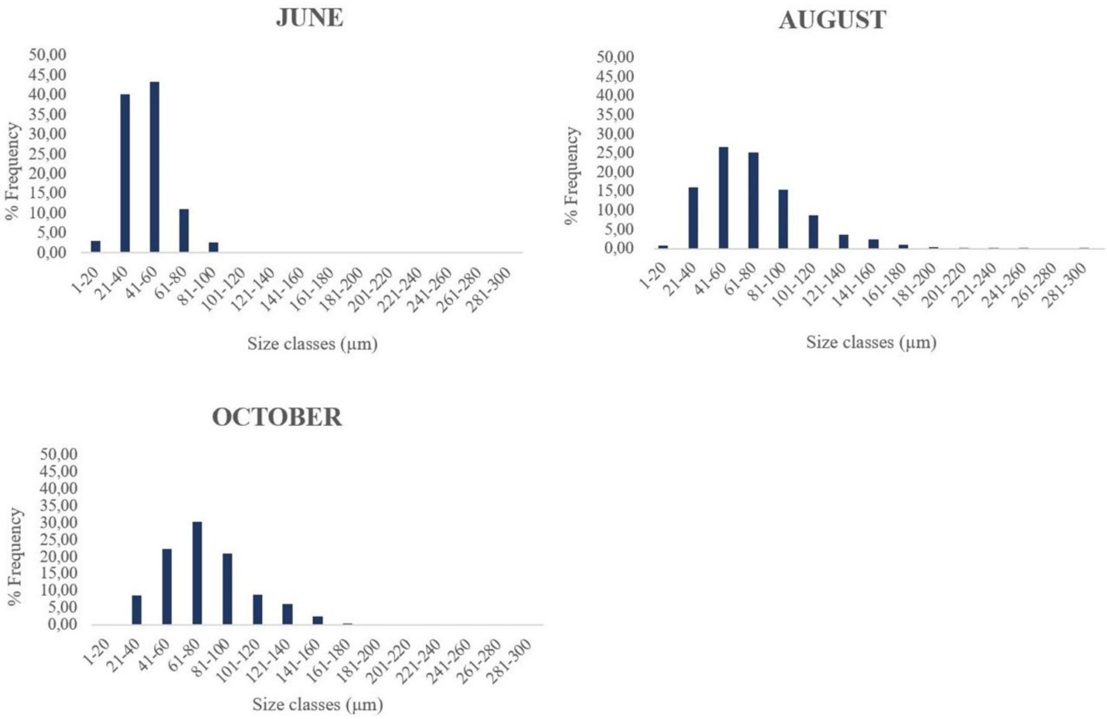

Fig. 29 Size- Frequency distribution of each size class of spermatic cysts in June, August and October.

Of the 12 total sampled colonies, three of them (colony 4, 5, 11) were monitored repeatedly during the study period, only colony 4 was not collected on the 26th August. The histogram below, describing the variation in the number of spermatic cysts, underlines the peak for the three colonies on the 5th of August (Fig. 30A). On the other hand, the size of germinal cells did not show a peak in August, with the mean diameter that was similar in August and October (Fig. 30B).

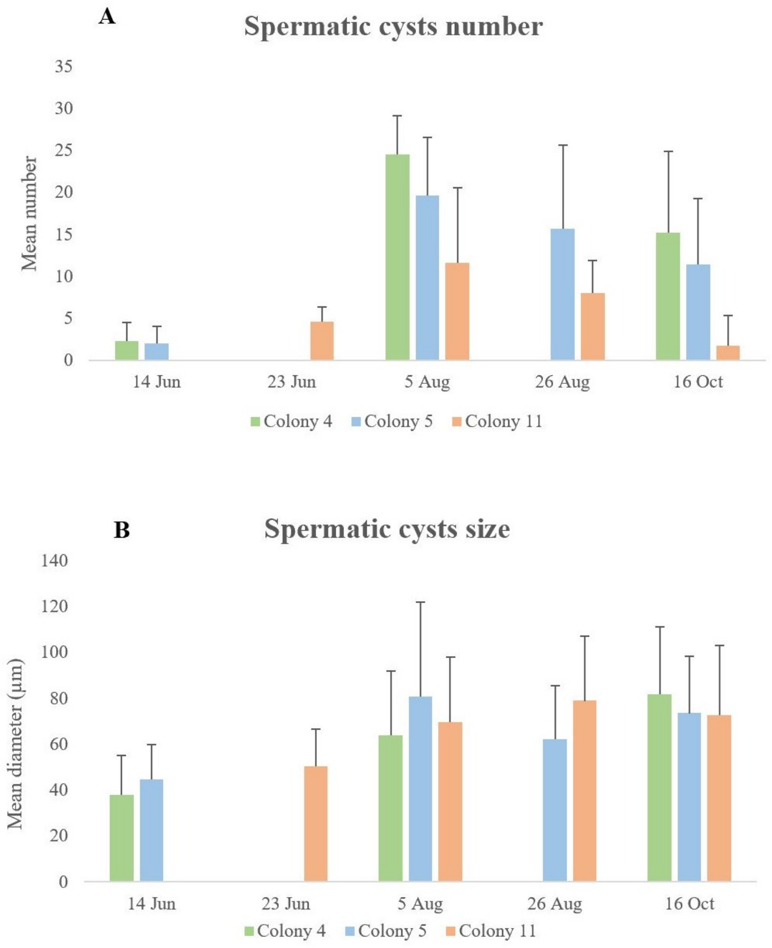

Fig. 30 (A) Mean number (±SD) and **(B)** Mean size (± SD) of spermatic cysts for the three repeatedly monitored colonies 4, 5, 11.

3.3 Statistical analysis

Kruskal-Wallis test showed a significant difference among diameter and number of spermatic cysts during the sampling months in Punta Pennello ($p<0.05$).

Kruskal-Wallis test revealed a strong significant difference ($p<0.01$) of spermatic cysts' size for the colonies sampled in August 2021 and 2022, in Mezzo Canale shoal and Punta Pennello, respectively.

3.4 Larval settlement devices

No larvae have been found on substrates collected both on August 26th and on October 16th. Consequently, our investigation on larval substrate's preference, ecology and histological analysis could not be performed.

Fourth chapter

4 DISCUSSION

4.1Literature analysis

The bibliographic research highlighted an increasing trend in the number of articles published through time, especially in the last 2 decades. This trend can be possibly explained by the improvement in the techniques allowing to reach more inaccessible environments and by a growing interest in the study of the reproduction of mesophotic and deep corals. In fact, the knowledge on this topic is crucial to assess and mitigate potential impacts of human activities together with climate change and to develop management and protection measures (Terzin et al., 2021).

The bibliographic research highlighted a worldwide distribution of the species investigated with a high number of studies focusing on the Mediterranean area and Tropical Northwestern Atlantic, probably due to the affiliation of the researchers studying the topic, which are mostly present in the countries facing the two ecoregions (Roveta et al., 2021). Additionally, the retrieved documents are limited to the upper mesophotic, especially for Hexacorallia, with only a low number of papers investigating depths below 3000 m. Until now, less than 0.1% of the deep-sea floor has been investigated (Taylor and Roterman, 2017), due to the complexity and inaccessibility of the bathyal and abyssal plain, still considered the final frontier on Earth (Ramirez-Llodra et al., 2010). As a point of fact, despite the use of low cost ROV and AUV are now available, deep-sea exploration still remains a challenging and quite expensive research (Liang et al., 2021).

Of the 87 articles included in the literature analysis, most of them dealt with the sexual reproduction, with very scarce data on the asexual one (only 10%), of these, one single record focused on Octocorallia, all the other regarded Hexacorallia. Among Hexacorallia, fragmentation is adopted as asexual strategy in Antipatharians as reported by Coppari et al. (2019) in *Antipathella subpinnata* reared in aquaria. Ruiz-Ramos et al. (2015) reported sign of self-fertilization in *Leiopathes glaberrima*, affirming that clonal geitonogamy (a type of self-pollination used by plants) is likely to occur in this black coral species. Regarding octocorals, parthenogenesis asexual strategy is reported to be adopted by *Alcyonium coralloides* in the Eastern Atlantic population (Groot and Weinberg, 1982), while, interestingly, the Mediterranean population reproduce sexually (Groot and Weinberg, 1982).

Concerning the sexual reproduction, most of documents focused on Hexacorallia (60%), and only a 30% on Octocorallia; this is because research on the reproductive biology in deep corals has mainly focused on deep scleractinians (Sun et al., 2010), while the reproductive study on soft corals and other octocorals has been predominantly focused on shallower species. The majority of mesophotic corals, whose reproduction is known, maintained similar type of reproduction and reproductive strategy of their shallow conspecific (Feldman et al., 2017). In fact, it is known that the broadcast strategy dominates in both mesophotic and deep cnidarians, although

hexacorals displayed a net predominance of broadcast (81%) compared to brooding (19%) strategy; conversely, octocorals showed almost an equilibrium among the two strategies (54.8% broadcast vs. 45.2% brooding). Among octocorals, broadcast species and brooders have been reported to be gonochoric, with only two brooder hermaphrodite species documented so far: *Drifa* sp. and *Drifa glomerata* (Sun et al., 2009b; Sun et al., 2010).

Among hexacorals, broadcast species were either gonochoric or hermaphrodite or gynodioecyis. The latter is a particular condition, in which the population consists of female and hermaphrodites, and recorded in one Scleractinia species, *Galaxea fascicularis* (Shlesinger et al., 2018). The majority of Antipatharians are known to be gonochoric, with a very limited number of polyps observed to present both oocytes and spermatic cysts (Andruccioli, unpublished). In this sense, our results are in line with literature data since the monitored colonies of *A. subpinnata* were only male with any evidence of hermaphrodite polyps. Wagner et al. (2011) sustained that sequential hermaphroditism is rare among black corals; in fact, the only record of sequential hermaphroditism is reported by Pesch (1914) for *Stichopathes saccula*.

Brooding strategy accounted only for 19% and has been detected among Scleractinia and Actinaria. In the Caribbean area, most of scleractinian species, exclusive to mesophotic depths, seemed to be brooders (Bongaerts et al., 2010). Lauretta et al. (2020) reported brooder as a strategy relevant in harsh condition, like Antarctic/ sub-Antarctic waters and deep sea, as it protects the offspring, maximizing their survival. Brooding is found in several scleractinian species of shallow waters, however, the occurrence of spawning in Scleractinia is dominant with the presence of some species such as *Balanophyllia malouinensis* presenting a different reproductive strategy depending on depth: gonochoric and brooder, hermaphrodite in mesophotic and shallow environment, respectively (Pendleton et al., 2021).

Sex ratio and timing of reproduction are crucial to understand population dynamics of marine organisms (Santangelo et al., 2013). Unbalanced sex ratio could determine low reproductive output, compromising the reproduction success (Bramanti et al., 2014). In the literature, a skewed sex ratio in favour of males or females, has been reported several times in Cnidarians, both in hexacorals (e.g., *Desmophyllum dianthus* had a male-biased sex ratio of 2:1;

Feehan et al., 2019) and octocorals (e.g., *Paramuricea clavata* with a skewed sex ratio in favour of males, 1:7; Gori et al., 2007). The sex ratio is also known to vary depending on the location and depth; as an example, Coma et al. (1995) reported a 1:1 male to female sex ratio for *Paramuricea clavata* in Spain, Medes Island, but the same species in Cape of Palos showed a sex ratio significantly male-biased (1:7) and in Portofino, Ligurian sea, Italy, Cerrano et al. (2005) reported a 3.3:1 male to female ratio relating it with the consequences of a mass-mortality event.

Differences in population sex ratio among depths have been also reported in several anthozoan species (Banayahu and Loya, 1983): *Montastrea cavernosa* showed a sex ratio 1:1 on shallow environments, but it became significantly male-biased (3.6:1) at mesophotic depths. The skewed sex ratio favouring males could be the result of asexual reproduction, with fragmentation creating aggregation of one sex; or a strategy adopted to enhance fertilization success in broadcast species, in which a higher proportion of males may mitigate the dilution effects of gametes. Another explanation could be the gender survivorship, the production of oocytes rich in lipid is more energetically expensive than producing male gametes (Bloomerang et al., 2021).

Conversely, among *Drifa* sp. colonies, no males were detected, so the sex ratio was in favour of females (1:0). Authors proposed three different explanations for this result, (i) freezing at -20°C after collection may have deteriorate male tissues, (ii) the sampling months may have missed the spermatogenesis, (iii) the specie is parthenogenetic, or female dominated hermaphrodite (Sun et al., 2009b). For this species the last hypothesis was confirmed by later studies (Sun et al., 2010). A skewed sex ratio in favour of females was also detected for a deep scleractinian species, *Flabellum angulare*, sampled at bathyal depths (with a sex ratio from 3:1 to 5:1 across collection sites; Mercier et al., 2010) and for the deep-sea anemones, *Actinostola crassicornis*, (Lauretta et al., 2020) for which many females population have been reported (Dunn, 1975 a; Ottaway, 1979). Also, the female skewed sex ratio of gorgonians has been shown to vary depending on both geographic location (Santangelo et al., 2013; Tsounis et al, 2006; Gori et al., 2007) and depth (Benayahu and Loya, 1983). As a point of fact, in the Mediterranean Sea, the sex ratio for *Corallium rubrum* is reported to be biased to females near Livorno (Santangelo et al., 2003) whilst it was balanced (1:1 male to female), in the Medes Islands (Tsounis et al. 2006). In the NW Atlantic *Anthomastus grandiflorus* also displayed a female-biased sex ratio (4:1) (Mercier and Hamel, 2011). Records of similar biased sex ratio exist for temperate octocorals; although the opposite is reported (Gori et al., 2007).

In general, a trend of decreasing fecundity with depth has been observed in deep-water solitary corals (Waller et a., 2002; Flint et al., 2007). Shlesinger et al. (2018) reported a decrease in reproduction performance with depth, through the measure of fecundity and oocytes size of seven scleractinian corals. In contrast, Holstein et al. (2015) found out higher fecundity for *Orbicella faveolata* in mesophotic environments, hypothesizing that the energy allocated for reproduction was decoupled from light saturation. Environmental factors play a key role in the energy budget available for reproduction (Harrison and Wallace, 1990), with corals in mesophotic environments exposed to energy limitation. Depth also influenced the duration of the reproductive cycle with *Seriatopora hystrix* showing shorter reproductive cycle in mesophotic environments compared to shallow populations (Prasetia et al. 2017). Also, the larval stages are found to be affected by depth with mesophotic planulae being smaller, most likely as a result of lower energy availability.

4.2 Sexual reproduction of **Antipathella subpinnata**

Our study on the sexual reproduction of *A. subpinnata* confirmed the occurrence of spawning in August, accompanied also by a brown-orange colour variation, according to Gaino and Scoccia (2010). Although, in general the colour variation before the spawning event has been mostly reported for female colonies, attributed to the yolk content (Parker et al., 1997; Lauretta and Penchaszadeh, 2017).

Moreover, in our study male colonies have been observed to be fertile in October, suggesting a prolonged spawning period. For this species a longer duration of the gametogenesis has been already observed in a female colony collected in the Ligurian sea (Mestice, unpublished) but also in other Italian regions such as Sicily in which fertile colonies of both sexes were recorded in October. The Atlantic congeneric *Antipathella wollastoni,* has been studied by Rakka et al. (2017) in Azores at 27-30 m depth and showed gametes of both sexes reached maturity of all the colonies monitored in October, and in November gametes started to disappear from the polyp, suggesting a spawning event in autumn. Among other Mediterranean black coral species, *Leiopathes glaberrima* thriving at 200-500 m depth, presented a wider gametogenic cycle, with fertile colonies recorded until December (Andruccioli, unpublished).

Spawning in multiple events is reported for tropical antipatharians, because of stable seawater temperature. As an example, *Antipathes griggi* exhibited a spawning in successive events, with the greatest intensity between November and December in Hawaii (Wagner et al., 2011). By contrast, in temperate ecosystems, the annual variation in seawater temperatures, limits the possibility of the same pattern described for tropical species (Rakka et al., 2017).

Water temperature is recognized as a trigger for reproductive processes for many anthozoans, including Antipatharians (Wagner et al., 2011). In fact, Gaino and Scoccia (2010) reported the spawning event concurrently with the peak of sea water temperature recorded in the study period (16°). This is confirmed by previous studies on the congeneric species *A. fiordensis,* which reproduces in mid- to late- summer, March in New Zealand (Parker et al., 1997). Also, Rakka et al. (2017) recorded a positive correlation between sea water temperature and gamete maturation, both for males and females, suggesting the temperature might be a cue for final gamete maturation rather than for spawning. In our study performed in mesophotic depth (70 m) a correlation between temperature and spawning, was not observed as the temperature remained constant to 14° throughout all the duration of the study. Therefore, other factors such as nutrients input, the duration of the day, moon cycle and even the tides might drive the spawning event although with the present evidence we can just hypothesize which might be the triggering factors. Indeed, previous studies already reported the spawning to occur in the period coinciding with the highest availability of phytoplankton (Mercier et al., 2010). Sun et al. (2010), reported a significant lunar pattern in planula release in a deep-sea octocoral, despite the bathymetrical range of distribution of the investigated species (350-1240 m).

Further studies will be needed to evaluate the role of nutrients inputs, tides and moon light to influence the reproductive cycle at mesophotic depth.

Regarding the mean size of spermatic cysts of *A. subpinnata*, when our data were compared with samples of the same species but collected in different geographic areas at a depth range of 50-100 m (Andruccioli, unpublished), it can be observed a smaller size of the germ size in Punta Pennello site compared with other localities. Again, the different environmental conditions might specifically influence the gametogenesis and the reproductive cycle in general (Table 3).

Table 3 The table reports mean size (µm) ± SD of germinal cells collected by Andruccioli (unpublished) in the first two rows and our data from Punta Pennello in the last line.

Depth	JUNE	AUGUST	OCTOBER
50-70 m	72±27♀	87±25♂	91±20♂
70-100 m		105+30♀	
70 m	46±16♂	73±33♂	79±26♂

The skewed sex ratio in favour of male observed in this study in Punta Pennello (12:0) and Mezzo Canale (9 males and 1 not sexed), confirmed the previous observation performed by Gaino and Scoccia (2010) in the same area. Even if in this previous work the result might be biased by a low sampling effort (only 5 colonies sampled and a skewed sex ratio (4:1) in favour of males) the same result obtained by the present study indicates more a characteristic of the species in this location. Also, the presence of only males in the samples from Mezzo Canale shoal strengthen the finding that this *A. subpinnata* present a patch distribution. As a point of fact, an unbalanced sex ratio has been reported also for two Ligurian sites (Bordighera and Portofino) for mesophotic populations of *A. subpinnata* (63-70 m depth). In this area, the sex ratio was strongly unbalanced in favour of females, with no records of males (35:0 and 10:0, respectively) (Andruccioli, unpublished). This unbalanced trend in favour of females has been detected as well among other Mediterranean black coral species (Andruccioli, unpublished), although with a more limited number of samples which may potentially bias the results.

Terzin et al. (2021) reported a shift of the sex ratio in colonies sampled in the upper (55-75 m) and lower mesophotic (152-210 m), with male individuals becoming more prevalent with increasing depth. But since the highest number of samples were collected at the same bathymetrical range (70 m) in two different regions (Liguria and Tuscany) the clear skewed ratio biased either in favour of females or males might be explained with the predominance of the asexual reproductive strategy for this species (as already observed in aquaria, Coppari et al. 2019) at a local scale and with the potential provision of sexual reproductive propagules from one site to the other: spermatic cysts might be released in the water column and following the dominant anticlockwise current circulation being able to fertilize the female colonies in the Ligurian area. A skewed sex ratio was already observed in other Antipatharian species collected in Hawaii: Wagner et al. (2011) reported a female-biased ratio sex 7:3 in *Cirrhipathes* cf. *anguina* and 9:5 in *Antipathes grandis*. On the other hand, *Antipathes griggi* displayed a sex ratio in favour of males with a sex ratio of 13:8; data differed depending on both sampling site and collection depth.

Terzin et al. (2021) investigated the genetic connectivity among *A. subpinnata* populations in Mediterranean Sea. Population from lower mesophotic (Liguria and Favignana) and upper mesophotic (Ligurian and Thyrrhenian Sea) were genetically different; these lasts were connected by gene flow, while lower mesophotic population were genetically distinct and less resilient to human impacts. The pattern of connectivity in shallow population can be explained by the strong current which flows westward along the shelf break (Astraldi, 1994; Betti, 2017). The linkage disequilibrium (LD) analysis suggested that the coastal population could be partially clonal, supporting both the asexual reproduction and the sexual reproduction in sustaining the connectivity among them. Results arose in our study highlighted that a population composed exclusively by males and that reproduces asexually, with a limited genetic variability, would not be resilient to face impacts. Indeed, an unbalanced sex ratio decreases the effective size of populations and compromise the resilience of population whenever an impact would occur (Dubreuil et al., 2010; Rosche et al., 2018).

We could hypothesize the deep refugia hypothesis, where deep refuges (i.e., black coral forests present in Santa Lucia Seamount) can aid shallower population, providing coral propagules. The genetic contribution of deeper population would help the shallower population to tackle human impacts and to be resilient. Future population genetic studies in the Punta Pennello area would be helpful to understand if the smallest colonies, present below the colonies sampled in our study, are fragments (clones) or generated by sexual reproduction, and if this population is shows genetic connectivity with the surrounding ones (both in the same bathymetric range of distribution or deeper).

Indeed, *A. subpinnata* displays a wide bathymetric range from 60 up to 600 m (Deidun et al., 2015). The samples collected in our area at 70 m, included colonies from the upper distribution limit of the species. Some behaviours could be variable when we consider samples close to their distribution limit.

Future studies, testing the sex ratio at lower distribution limit of *A. subpinnata* would be advisable, to evaluate if it maintains the same trend reported for the upper limit. At the intermediate distribution limit (Santa Lucia Seamounts at 150-200 m depth), the situation was more balanced, with still a sex ratio in favour of females but with the concomitant presence of some male colonies (Andruccioli, unpublished).

Our study has been possible thanks to the collaboration of technical divers through Citizen Science actions. The involvement of technical divers is essential, permitting to carry out studies at mesophotic depths, which are not always easy to reach for a researcher. In the future, we may engage technical divers from different areas both in Italy and in Europe, in order to replicate the study on a larger scale, providing temporal and spatial data. The involvement of technical divers, coupled with remote operated vehicle (ROV) or autonomous underwater vehicle (AUV) will allow to reach greater depth, providing a broadscale data collection.

4.3 Larvae

The larvae of *A. subpinnata* have never been observed, so the construction of our larval settlement devices was based on the known features for its congeneric specie, *A. fiordensis*. The larvae of this species observed in aquaria were negatively buoyant, negative phototaxic, lecithotrophic with low motility and survival time (Miller, 1996). The duration of larval pelagic phase in benthic organisms is in general positively correlated with dispersal potential. Planktotrophic larvae should be able to disperse over greatest distance than lecithotrophic larvae, however this pattern is not universally widespread for all species (Coelho and Lasker, 2016). In fact, *A. fiordensis* showed genetic similarity among population in different fjords, probably maintained by larvae exchange, despite being lecithotrophic (Miller, 1997). Related to this some authors suggested that lecithotrophic development is an adaptation for wide dispersal in deep environments, characterized by oligotrophy (Shilling and Manahan, 1994). Large scale dispersal is more common in broadcast species (Coelho and Lasker, 2016). The absence of larvae on our devices could be explained by several factors: a) larvae could be positively buoyant, as in many Scleractinia species, so the position of our substrates immediately below the parent colonies was not appropriate. Further studies could test different larval settlement devices, similar to the one adopted to collect larvae of scleractinians (Scucchia et al., 2020); b) if the population in Punta Pennello is truly composed by males only, the absence of larvae in this site becomes obvious. Male germinal cells could be transported by the currents of intermediate circulation, following an anticlockwise pattern and potentially fecundate populations of Ligurian Sea. Otherwise, deeper population in the surrounding areas, could aid shallower population, providing propagules. Further genetic studies may help in corroborating or not these hypotheses. A different approach to study larval ecology could include the collection of both male and female gametes during the spawning, followed to an in vitro fertilization to obtain the larvae.

BIBLIOGRAPHY

Airi V, Gizzi F, Marchini C, Goffredo S (2016) Sexual reproduction of mediterranean scleractinian corals. The Cnidaria, past, present and Future: The World of Medusa and her Sisters 269-278

Andruccioli A (2019) Studio preliminare sulla riproduzione sessuale nei coralli neri. Università degli studi di Genova

Astraldi M, Gasparini GP, Sparnocchia S (1994) The seasonal and interannual variability in the Ligurian-Provençal Basin. Coastal and Estuarine Studies 46:93–113. doi: 10.1029/ce046p0093

Avery and Liddell (1997) Sessile community recruitment patterns on western Atlantic shallow and deep-reef hard substrata. Proc. 8th Int. Coral Reef Symp., Panama.

Baillon S, Hamel J-F, Wareham VE, Mercier A (2014) Seasonality in reproduction of the deep-water pennatulacean coral *Anthoptilum grandiflorum*. Marine Biology 161:29–43. doi: 10.1007/s00227-013-2311-8

Baillon S, Hamel J-F, Mercier A (2015) Protracted oogenesis and annual reproductive periodicity in the deep-sea pennatulacean *Halipteris finmarchica* (Anthozoa, Octocorallia). Marine Ecology 36:1364–1378. doi: 10.1111/maec.12236

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annual Review of Ecology, Evolution, and Systematics 40:551–571. doi: 10.1146/annurev.ecolsys.110308.120220

Beazley LI, Kenchington EL (2012) Reproductive biology of the deep-water coral *Acanella arbuscula* (Phylum Cnidaria: Class Anthozoa: Order Alcyonacea), northwest Atlantic. Deep-Sea Research Part I: Oceanographic Research Papers 68:92–104. doi: 10.1016/j.dsr.2012.05.013

Beckmann A, Özbek S (2012) The nematocyst: A molecular map of the cnidarian stinging organelle. International Journal of Developmental Biology 56:577–582. doi: 10.1387/ijdb.113472ab

Berntson EA, France SC, Mullineaux LS (1999) Phylogenetic Relationships within the Class Anthozoa (Phylum Cnidaria) Based on Nuclear 18S rDNA Sequences. Mol Phylogenet Evol 13:417–433

Betti F, Bavestrello G, Bo M, Coppari M, Enrichetti F, Manuele M, Cattaneo-Vietti R (2017) Exceptional strandings of the purple snail *Janthina pallida* Thompson, 1840 (Gastropoda: Epitoniidae) and first record of an alien goose barnacle along the Ligurian coast (western Mediterranean Sea). European Zoological Journal 84:488–495. doi: 10.1080/24750263.2017.1379562

Bloomberg J, Holstein DM (2021) Mesophotic coral refuges following multiple disturbances. Coral Reefs 40:821–834. doi: 10.1007/s00338-021-02087-w

Bo M, Tazioli S, Spanò N, Bavestrello G (2008) *Antipathella subpinnata* (Antipatharia, Myriopathidae) in Italian seas. Italian Journal of Zoology 75:185–195. doi: 10.1080/11250000701882908

Bo M, Bavestrello G, Canese S, Giusti M, Salvati E, Angiolillo M, Greco S (2009a) Characteristics of a black coral meadow in the twilight zone of the central Mediterranean Sea. Mar Ecol Prog Ser 397:53–61. doi: 10.3354/meps08185

Bo M, Di Camillo CG di, Addamo AM, Valisano L, Bavestrello G (2009b) Growth strategies of whip black corals (Cnidaria: Antipatharia) in the Bunaken Marine Park (Celebes Sea, Indonesia)

Bo M, Di Camillo CG, Puce S, Canese S, Giusti M, Angiolillo M, Bavestrello G (2011a) A tubulariid hydroid associated with anthozoan corals in the Mediterranean Sea. Italian Journal of Zoology 78:487–496. doi: 10.1080/11250003.2011.568015

Bo M, Baker AC, Gaino E, Wirshing HH, Scoccia F, Bavestrello G (2011b) First description of algal mutualistic endosymbiosis in a black coral (Anthozoa: Antipatharia). Mar Ecol Prog Ser 435:1–11. doi: 10.3354/meps09228

Bo M, Bertolino M, Borghini M, Castellano M, Harriague AC, Di Camillo CG, Gasparini GP, Misic C, Povero P, Pusceddu A, Schroeder K, Bavestrello G (2011c) Characteristics of the mesophotic megabenthic assemblages of the Vercelli seamount (North Tyrrhenian Sea). PLoS One. doi: 10.1371/journal.pone.0016357

Bo M, Bavestrello G, Barucca M, Makapedua DM, Poliseno A, Forconi M, Olmo E, Canapa A (2012) Morphological and molecular characterization of the problematic whip black coral genus *stichopathes* (hexacorallia: Antipatharia) from indonesia (north sulawesi, celebes sea). Zool J Linn Soc 166:1–13. doi: 10.1111/j.1096-3642.2012.00834.x

Bo M, Bava S, Canese S, Angiolillo M, Cattaneo-Vietti R, Bavestrello G (2014) Fishing impact on deep Mediterranean rocky habitats as revealed by ROV investigation. Biol Conserv 171:167–176. doi: 10.1016/j.biocon.2014.01.011

Bo M, Barucca M, Biscotti MA, Brugler MR, Canapa A, Canese S, lo Iacono C, Bavestrello G (2018) Phylogenetic relationships of Mediterranean black corals (Cnidaria: Anthozoa: Hexacorallia) and implications for classification within the order Antipatharia. Invertebr Syst 32:1102–1110. doi: 10.1071/IS17043

Bongaerts P, Frade PR, Ogier JJ, Hay KB, van Bleijswijk J, Englebert N, Vermeij MJ, Pm Bak R, Visser PM, Hoegh-Guldberg O (2013) Sharing the slope: depth partitioning of agariciid corals and associated Symbiodinium across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. BMC Evol Biol 13:205.

Bongaerts P, Cooke IR, Ying H, Wels D, Den Haan S, Hernandez-Agreda A, Brunner CA, Dove S, Englebert N, Eyal G, van Oppen MJH, Hoegh-Guldberg O (2021) Morphological stasis masks ecologically divergent coral species on tropical reefs. Current Biology 31:2286-2298. doi: 10.1016/j.cub.2021.03.028

Bramanti L, Vielmini I, Rossi S, Tsounis G, Iannelli M, Cattaneo-Vietti R, Priori C, Santangelo G (2014) Demographic parameters of two populations of red coral (*Corallium rubrum* L. 1758) in the North Western Mediterranean. Mar Biol 161:1015–1026. doi: 10.1007/s00227-013-2383-5

Bramanti L, Benedetti MC, Cupido R, Cocito S, Priori C, Erra F, Iannelli M, Santangelo G (2017) Demography of animal forests: The example of mediterranean gorgonians. Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots 529–548. doi: 10.1007/978-3-319-21012-4_13

Brandtneris VW, Brandt ME, Glynn PW, Gyory J, Smith TB (2016) Seasonal variability in calorimetric energy content of two Caribbean mesophotic corals. PLoS One. doi: 10.1371/journal.pone.0151953

Bronsdon SK, Tyler PA, Rice AL, Gage JD (1993) Reproductive biology of two epizoic anemones from the deep north-eastern atlantic ocean. Journal of the Marine Biological Association of the United Kingdom 73:531–542. doi: 10.1017/S0025315400033087

Brook G (1889) Report on the Antipatharia Collected by H.M.S. Challenger During the Years 1873-76. Johnson Reprint Corporation

Brooke S, Young CM (2003) Reproductive ecology of a deep-water scleractinian coral, *Oculina varicosa*, from the southeast Florida shelf. Cont Shelf Res 23:847–858. doi: 10.1016/S0278-4343(03)00080-3

Brooke S, Järnegren J (2013) Reproductive periodicity of the scleractinian coral *Lophelia pertusa* from the Trondheim Fjord, Norway. Mar Biol 160:139–153. doi: 10.1007/s00227-012-2071-x

Capel KCC, Migotto AE, Zilberberg C, Kitahara M v. (2014) Another tool towards invasion? Polyp “bail-out” in *Tubastraea coccinea*. Coral Reefs 33:1165. doi: 10.1007/s00338-014-1200-z

Cárdenas P, Rapp HT, Klitgaard AB, Best M, Thollesson M, Tendal OS (2013) Taxonomy, biogeography and DNA barcodes of *Geodia* species (Porifera, Demospongiae, Tetractinellida) in the atlantic boreo-arctic region. Zool J Linn Soc 169:351–311. doi: 10.1111/zoj.12060

Cerrano C, Danovaro R, Gambi C, Pusceddu A, Riva A, Schiaparelli S (2010) Gold coral (*Savalia savaglia*) and gorgonian forests enhance benthic biodiversity and ecosystem functioning in the mesophotic zone. Biodivers Conserv 19:153–167. doi: 10.1007/s10531-009-9712-5

Cerrano C, Arillo A, Azzini A, Calcinai B, Castellano L, Muti C, Valisano L, Zega G, Bavestrello G (2015) Gorgonian population recovery after a mass mortality event. Acquatic conservation: marine and freshwater ecosystems 15:147–157

Cerrano C, Bastari A, Calcinai B, Di Camillo C, Pica D, Puce S, Valisano L, Torsani F (2019) Temperate mesophotic ecosystems: gaps and perspectives of an emerging conservation challenge for the Mediterranean Sea. European Zoological Journal 86:370–388

Chimienti G, De Padova D, Mossa M, Mastrototaro F (2020) A mesophotic black coral forest in the Adriatic Sea. Sci Rep. doi: 10.1038/s41598-020-65266-9

Coelho MAG, Lasker HR (2016) Larval dispersal and population connectivity in anthozoans. The Cnidaria, past, present and Future: The World of Medusa and her Sisters. Springer International Publishing, pp 291–315

Coma R, Zabala M, Gili J-M (1995) Sexual reproductive effort in the Mediterranean gorgonian *Paramuricea clavata.* Mar Ecol Prog Ser 117:185–192

Conci N, Vargas S, Wörheide G (2021) The Biology and Evolution of Calcite and Aragonite Mineralization in Octocorallia. Front Ecol Evol 9

Coppari M, Mestice F, Betti F, Bavestrello G, Castellano L, Bo M (2019) Fragmentation, re-attachment ability and growth rate of the Mediterranean black coral *Antipathella subpinnata*. Coral Reefs 38:1–14. doi: 10.1007/s00338-018-01764-7

Coppari M, Fumarola L, Bramanti L, Romans P, Pillot R, Bavestrello G, Bo M (2020) Unveiling asexual reproductive traits in black corals: polyp bail-out in *Antipathella subpinnata*. Coral Reefs 39:1517–1523. doi: 10.1007/s00338-020-02018-1

Cordes EE, Nybakken JW, VanDykhuizen G (2001) Reproduction and growth of *Anthomastus ritteri* (Octocorallia: Alcyonacea) from Monterey Bay, California, USA. Mar Biol 138:491–501. doi: 10.1007/s002270000470

Costantini F, Addamo AM, Machordom A, Abbiati M (2017) Genetic connectivity and conservation of temperate and cold-water habitat-forming corals. Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots 1061–1082. doi: 10.1007/978-3-319-21012-4_32

Dahl MP, Pereyra RT, Lundälv T, André C (2012) Fine-scale spatial genetic structure and clonal distribution of the cold-water coral *Lophelia pertusa*. Coral Reefs 31:1135–1148. doi: 10.1007/s00338-012-0937-5

Daly M, Gusmão L (2007a) The first sea anemone (Cnidaria: Anthozoa: Actiniaria) from a whale fall. J Nat Hist 41:1–11. doi: 10.1080/00222930601121775

Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, Mcfadden CS, Opresko DM, Rodriguez E, Romano SL, Stake JL (2007b) The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668:127–182

De Meeûs T, Prugnolle F, Agnew P (2007) Asexual reproduction: Genetics and evolutionary aspects. Cellular and Molecular Life Sciences 64:1355–1372. doi: 10.1007/s00018-007-6515-2

Deidun A, Andaloro F, Bavestrello G, Canese S, Consoli P, Micallef A, Romeo T, Bo M (2015) First characterisation of a *Leiopathes glaberrima* (Cnidaria: Anthozoa: Antipatharia) forest in Maltese exploited fishing grounds. Italian Journal of Zoology 82:271–280. doi: 10.1080/11250003.2014.986544

Dunn CW, Wagner GP (2006) The evolution of colony-level development in the *Siphonophora* (Cnidaria:Hydrozoa). Dev Genes Evol 216:743–754. doi: 10.1007/s00427-006-0101-8

Dunn DF (1975) Gynodioecy in an animal. Nature 253:528–529.

Eckelbarger KJ, Tyler PA, Langton RW (1998) Gonadal morphology and gametogenesis in the sea pen *Pennatula aculeata* (Anthozoa: Pennatulacea) from the Gulf of Maine. Mar Biol 132:677–690.

Ehrlich H (2019) Marine Biological Materials of Invertebrate Origin. Springer

Eyal G, Eyal-Shaham L, Loya Y (2021) Symbiodiniaceae conduct under natural bleaching stress during advanced gametogenesis stages of a mesophotic coral. Coral Reefs 40:959–964. doi: 10.1007/s00338-021-02082-1

Eyal-Shaham L, Eyal G, Tamir R, Loya Y (2016) Reproduction, abundance and survivorship of two *Alveopora* spp. in the mesophotic reefs of Eilat, Red Sea. Sci Rep. doi: 10.1038/srep20964

Fautin DG (2002) Reproduction of Cnidaria. Can J Zool 80:1735–1754. doi: 10.1139/z02-133

Fautin DG (2009) Structural diversity, systematics, and evolution of cnidae. Toxicon 54:1054–1064. doi: 10.1016/j.toxicon.2009.02.024

Feehan KA, Waller RG (2015) Notes on reproduction of eight species of Eastern Pacific cold-water octocorals. Journal of the Marine Biological Association of the United Kingdom 95:691–696. doi: 10.1017/S0025315415000053

Feldman B, Shlesinger T, Loya Y (2018) Mesophotic coral-reef environments depress the reproduction of the coral *Paramontastraea peresi* in the Red Sea. Coral Reefs 37:201–214. doi: 10.1007/s00338-017-1648-8

Flint HC, Waller RG, Tyler PA (2007) Reproductive ecology of *Fungiacyathus marenzelleri* from 4100 m depth in the northeast Pacific Ocean. Mar Biol 151:843–849. doi: 10.1007/s00227-006-0539-2

Foster NL, Baums IB, Mumby PJ (2007) Sexual vs. asexual reproduction in an ecosystem engineer: The massive coral *Montastraea annularis*. Journal of Animal Ecology 76:384–391. doi: 10.1111/j.1365-2656.2006.01207.x

Fountain CT, Waller RG, Auster PJ (2019) Individual and population level variation in the reproductive potential of deep-sea corals from different regions within the Gulf of Maine. Front Mar Sci. doi: 10.3389/fmars.2019.00172

Frade PR, Reyes-Nivia MC, Faria J, Kaandorp JA, Luttikhuizen PC, Bak RPM (2010) Semi-permeable species boundaries in the coral genus *Madracis*: Introgression in a brooding coral system. Mol Phylogenet Evol 57:1072–1090. doi: 10.1016/j.ympev.2010.09.010

Fujiwara S, Kezuka D, Ishimori H, Saito T, Okamoto M (2016) A New Approach to Coral Reef Restoration:The Coral Settlement Device. The News Journal of the International Society for Reef Studies 31: 32-28

Furla P, Allemand D, Malcolm Shick J, Ferrier-Page`s C, Page`s P, Richier S, Plantivaux A, Merle P-L, Tambutté S (2005) The Symbiotic Anthozoan: A Physiological Chimera between Alga and Animal 1. Integr Comp Biol 45:595–604

Gaino E, Bo M, Boyer M, Scoccia F (2008) Sperm morphology in the black coral *Cirrhipathes* sp. (Anthozoa, Antipatharia). Invertebrate Biology 127:249–258. doi: 10.1111/j.1744-7410.2008.00132.x

Gaino and Scoccia (2010) Gamete spawning in *Antipathella subpinnata* (Anthozoa, Antipatharia): a structural and ultrastructural investigation. Zoomorphology 129:213–219

Genikhovich G, Technau U (2017) On the evolution of bilaterality. Development (Cambridge) 144:3392–3404. doi: 10.1242/dev.141507

Gilmour J, Speed CW, Babcock R (2016) Coral reproduction in Western Australia. PeerJ. doi: 10.7717/peerj.2010

Gleason DF, Hofmann DK (2011) Coral larvae: From gametes to recruits. J Exp Mar Biol Ecol 408:42–57. doi: 10.1016/j.jembe.2011.07.025

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Chang Biol 2:495–509

Godefroid M, Zeimes T, Bramanti L, Romans P, Bo M, Toma M, Danis B, Dubois P, Guillaumont C (2023) Low vulnerability of the Mediterranean antipatharian *Antipathella subpinnata* (Ellis and Solander, 1786) to ocean warming. Ecological Modelling 475

Goldson AJ, Hughes RN, Gliddon CJ (2001) Population genetic consequences of larval dispersal mode and hydrography: a case study with bryozoans. Mar Biol 138:1037–1042.

Gori A, Viladrich N, Gili J, Kotta M, Cucio C, Magni L, Bramanti L, Rossi S (2012) Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). Coral Reefs 31:823–837. doi: 10.1007/s00338-012-0904-1

Grinyó J, Viladrich N, Díaz D, Muñoz A, Mallol S, Salazar J, Castillo R, Gili J-M, Gori A (2018) Reproduction, energy storage and metabolic requirements in a mesophotic population of the gorgonian *Paramuricea macrospina.* PLoS One. doi: 10.1371/journal.pone.0203308

Groot S, Weinberg S (1982) Biogeography, Taxonomical Status and Ecology of *Alcyonium (Parerythropodium) coralloides* (PALLAS, 1766). Marine Ecology 3:293–312.

Guest JR, Heyward AJ, Omori M, Iwao K, Morse ANC, Boch C (2010) Reef rehabilitation manual. Coral Reef Targeted Research and Capacity Building for Management Program

Guest JR, Baria M v., Gomez ED, Heyward AJ, Edwards AJ (2014) Closing the circle: Is it feasible to rehabilitate reefs with sexually propagated corals? Coral Reefs 33:45–55. doi: 10.1007/s00338-013-1114-1

Hamel J-F, Wareham-Hayes VE, Mercier A (2020) Reproduction of a bathyal pennatulacean coral in the Canadian Arctic. Deep Sea Res 1 Oceanogr Res Pap. doi: 10.1016/j.dsr.2020.103321

Harrison P, Wallace C (1990) Reproduction, dispersal and recruitment of scleractinian corals. Coral Reefs

Harrison PL (2011) Sexual reproduction of scleractinian corals. In: Coral Reefs: An Ecosystem in Transition. Springer Netherlands, pp 59–85

Hickman C, Roberts L, Keen S, Eisenhour D, Larson A (2016) Zoologia. 16th ed. Milano: McGraw-Hill

Highsmith RC (1982) Reproduction by Fragmentation in Corals. Mar Ecol Prog Ser 7:207–226.

Holstein DM, Smith TB, Gyory J, Paris CB (2015) Fertile fathoms: Deep reproductive refugia for threatened shallow corals. Sci Rep 5. doi: 10.1038/srep12407

Horowitz J, Opresko D, Molodstova N, Molodstova T, Beaman R, Cowman P, Bridge T (2023) Five new species of black coral (Anthozoa; Antipatharia) from the Great Barrier Reef and Coral Sea, Australia.

Hughes TP, Jackson JBC (1985) Population dynamics and life histories of foliaceous corals’. Ecological Monograph 55:141–166.

Iacono R, Napolitano E, Palma M, Sannino G (2021) The tyrrhenian sea circulation: A review of recent work. Sustainability 13. doi: 10.3390/su13116371

Ingrassia M, di Bella L (2021) Black coral distribution in the italian seas: A review. Diversity (Basel). doi: 10.3390/d13070334

Karako-Lampert S, Katcoff DJ, Achituv Y, Dubinsky Z, Stambler N (2004) Do clades of symbiotic dinoflagellates in scleractinian corals of the Gulf of Eilat (Red Sea) differ from those of other coral reefs? J Exp Mar Biol Ecol 311:301–314. doi: 10.1016/j.jembe.2004.05.015

Kuz'micheva EI (1983) Deep-water corals in the Mediterranean Sea and the central part of the Atlantic Ocean. Vestnik - Moskovskogo Universiteta, Seriya Geologiya 1:23–31.

Lacharité M, Metaxas A (2013) Early Life History of Deep-Water Gorgonian Corals May Limit Their Abundance. PLoS One. doi: 10.1371/journal.pone.0065394

Lapian HFN, Barucca M, Bavestrello G, Biscotti MA, Bo M, Canapa A, Tazioli S, Olmo E (2007) A systematic study of some Black Corals species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. Mar Biol 151:785–792. doi: 10.1007/s00227-006-0525-8

Larsson AI, Järnegren J, Strömberg SM, Dahl MP, Lundälv T, Brooke S (2014) Embryogenesis and larval biology of the cold-water coral *Lophelia pertusa*. PLoS One. doi: 10.1371/journal.pone.0102222

Lauretta D, Penchaszadeh PE (2017) Gigantic oocytes in the deep sea black coral *Dendrobathypathes grandis* (Antipatharia) from the Mar del Plata submarine canyon area (southwestern Atlantic). Deep Sea Res 1 Oceanogr Res Pap 128:109–114. doi: 10.1016/j.dsr.2017.08.011

Lauretta D, Vidos C, Martinez MI, Penchaszadeh PE (2020) Brooding in the deep-sea sea anemone *Actinostola crassicornis* (Hertwig, 1882) (Cnidaria: Anthozoa: Actiniaria) from the southwestern Atlantic Ocean. Polar Biol 43:1353–1361. doi: 10.1007/s00300-020-02713-3

Liang J, Feng JC, Zhang S, Cai Y, Yang Z, Ni T, Yong Yan H (2021) Role of deep-sea equipment in promoting the forefront of studies on life in extreme environments. iScience 24: 1-21

Liberman R, Shlesinger T, Loya Y, Benayahu Y (2018) Octocoral sexual reproduction: Temporal disparity between mesophotic and shallow-reef populations. Front Mar Sci. doi: 10.3389/fmars.2018.00445

Liberman R, Shlesinger T, Loya Y, Benayahu Y (2022) Soft coral reproductive phenology along a depth gradient: Can "going deeper" provide a viable refuge? Ecology 103(9). doi: 10.1002/ecy.3760

Linares C, Coma R, Mariani S, Díaz D, Hereu B, Zabala M (2008) Early life history of the Mediterranean gorgonian *Paramuricea clavata*: Implications for population dynamics. Invertebrate Biology 127:1–11. doi: 10.1111/j.1744-7410.2007.00109.x

Maier SR, Bannister RJ, van Oevelen D, Kutti T (2020) Seasonal controls on the diet, metabolic activity, tissue reserves and growth of the cold-water coral *Lophelia pertusa*. Coral Reefs 39:173–187. doi: 10.1007/s00338-019-01886-6

Mass T, Giuffre AJ, Sun CY, Stifler CA, Frazier MJ, Neder M, Tamura N, Stan CV, Marcus MA, Gilbert P (2017) Amorphous calcium carbonate particles form coral skeletons. Proc Natl Acad Sci USA 114:E7670–E7678. doi: 10.1073/pnas.1707890114

Mcfadden CS, Donahue R, Hadland BK, Weston R (2001) A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus *Alcyonium*. Evolution 55(1):54–67.

McFadden CS, Quattrini AM, Brugler MR, Cowman PF, Dueñas LF, Kitahara M v., Paz-García DA, Reimer JD, Rodríguez E (2021) Phylogenomics, Origin, and Diversification of Anthozoans (Phylum Cnidaria). Syst Biol 70:635–647. doi: 10.1093/sysbio/syaa103

Mercier A, Hamel JF (2009) Reproductive periodicity and host-specific settlement and growth of a deep-water symbiotic sea anemone. Can J Zool 87:967–980. doi: 10.1139/Z09-085

Mercier A, Hamel JF (2011) Contrasting reproductive strategies in three deep-sea octocorals from eastern Canada: *Primnoa resedaeformis, Keratoisis ornata,* and *Anthomastus grandiflorus*. Coral Reefs 30:337–350. doi: 10.1007/s00338-011-0724-8

Mercier A, Sun Z, Hamel J-F (2011) Reproductive periodicity, spawning and development of the deep-sea scleractinian coral *Flabellum angulare*. Mar Biol 158:371–380. doi: 10.1007/s00227-010-1565-7

Mercier A, Baillon S, Daly M, Macrander J, Hamel JF (2017) Biology of a deep-water sea anemone (Anthozoa: Actiniidae) from eastern Canada: Spawning, development, and growth. Deep Sea Res 2 Top Stud Oceanogr 137:359–367. doi: 10.1016/j.dsr2.2016.01.006

Mestice F (2018) Sexual, asexual reproduction and growth rate of the black coral *Antipathella subpinnata* (Ellis and Solander, 1786). Università degli Studi di Milano

Miller K, Grange K (1995) Population genetic studies of antipatharian black corals from Doubtful and Nancy Sounds, Fiordland, New Zealand. In: Proceedings of the 6th International Conference on Coelenterate Biology. pp 353–363

Miller K (1996) Piecing together the reproductive habits of New Zealand’s endemic black corals. Water and atmosphere 4(2)

Miller KJ (1997) Genetic structure of black coral populations in New Zealand’s fiords. Marine Ecology Progress Series 161:123–132.

Miller KJ, Gunasekera RM (2017) A comparison of genetic connectivity in two deep sea corals to examine whether seamounts are isolated islands or stepping stones for dispersal. Sci Rep. doi: 10.1038/srep46103

Molodtsova TN, Pasternak FA (2005) Redescription of *Parantipathes euantha* (Pasternak, 1958) (Anthozoa: Antipatharia) from Kurile-Kamchatka Trench. Invertebrate Zoology 2(2):169–179

Molodstova TN (2006) New species of *Hexapathes Kinoshita*, 1910 (Anthozoa, Antipatharia, Cladopathidae) from the South-West Pacific. Zoosystema 28(3): 597-606

Mortensen PB, Mortenses L-B, Gordon D (2005) Evidence of fisheries damage to deep-water gorgonians in the Northeast Channel, Nova Scotia. American Fisheries Society Symposium 41:369–382.

Opresko DM and Genin A (1990) A New Species of Antipatharian (Cnidaria: Anthozoa) from Seamounts in the Eastern North Pacific. Bull Mar Sci 46:301–310.

Opresko DM (2001) Revision of the Antipatharia (Cnidaria: Anthozoa). Part I. Establishment of a new family, Myriopathidae. Zool Med Leiden 75:1–18.

Opresko DM (2003) Revision of the Antipatharia (Cnidaria: Anthozoa). Part III. Cladopathidae. Zool. Med. Leiden 77:495-536

Opresko DM (2005) A new species of antipatharian coral (Cnidaria: Anthozoa: Antipatharia) from the southern California Bight. Zootaxa 852:1–10.

Orejas C, Lopez-Gonzalesz PJ, Gili JM, Teixidò N, Gutt J, Arntz WE (2002) Distribution and reproductive ecology of the Antarctic octocoral *Ainigmaptilon antarcticum* in the Weddell Sea. Mar Ecol Prog Ser 231:101–114.

Orejas C, Gili JM, López-González PJ, Hasemann C, Arntz WE (2007) Reproduction patterns of four Antarctic octocorals in the Weddell Sea: An inter-specific, shape, and latitudinal comparison. Mar Biol 150:551–563. doi: 10.1007/s00227-006-0370-9

Orejas C, Gori A, Gili JM (2008) Growth rates of live *Lophelia pertusa* and *Madrepora oculata* from the Mediterranean Sea maintained in aquaria. Coral Reefs 27:255. doi: 10.1007/s00338-007-0350-7

Otero M, Numa C, Bo M, Orejas C, Garrabou J, Cerrano C, Kružic P, Antoniadou C, Aguilar R, Kipson S, Linares C, Terrón-Sigler A, Brossard J, Kersting D, Casado-Amezúa P, García S, Goffredo S, Ocaña O, Caroselli E, Maldonado M, Bavestrello G, Cattaneo-Vietti R, Özalp B (2017) Overview of the conservation status of Mediterranean anthozoa. IUCN International Union for Conservation of Nature

Ottaway JR (1979) Population Ecology of the Intertidal Anemone *Actinia tenebrosa.* III. Dynamics and Environmental Factors. Aust J Mar Freshwater Res 30:41–62.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P. and Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst. Rev. The BMJ, 71:1-11. doi: 0.1136/bmj.n71

Parker NR, Mladenov P v., Grange KR (1997) Reproductive biology of the antipatharian black coral *Antipathes fiordensis* in Doubtful Sound, Fiordland, New Zealand. Mar Biol 130:11–22.

Pax F, Van Praet M, Doumenc D (1987) Ordre des antipathaires. Traite de zoologie-anatomie, systematique, biologie Cnidaires Antozhoaries 189–210

Pendleton A, Hartill E, Waller R (2021) Notes on reproduction in the deep-sea cup coral *Balanophyllia malouinensis* (Squires 1961) from the Southern Ocean. Polar Biol 44:977–986. doi: 10.1007/s00300-021-02854-z

Pesch AJV (1914) The Antipatharia of the Siboga Expedition. Ed: Leiden, EJ Brill. doi: 10.5962/t.173163

Pires DO, Castro CB, Silva JC (2009) Reproductive biology of the deep-sea pennatulacean *Anthoptilum murrayi* (Cnidaria, Octocorallia). Mar Ecol Prog Ser 397:103–112. doi: 10.3354/meps08322

Pires DO, Silva JC, Bastos ND (2014) Reproduction of deep-sea reef-building corals from the southwestern Atlantic. Deep Sea Res II Top Stud Oceanogr 99:51–63. doi: 10.1016/j.dsr2.2013.07.008

Prasetia R, Sinniger F, Harii S (2016) Gametogenesis and fecundity of *Acropora tenella* (Brook 1892) in a mesophotic coral ecosystem in Okinawa, Japan. Coral Reefs 35:53–62. doi: 10.1007/s00338-015-1348-1

Prasetia R, Sinniger F, Hashizume K, Harii S (2017) Reproductive biology of the deep brooding coral *Seriatopora hystrix*: Implications for shallow reef recovery. PLoS One 12(5). doi: 10.1371/journal.pone.0177034

Prata KE, Riginos C, Gutenkunst RN, Latijnhouwers KRW, Sánchez JA, Englebert N, Hay KB, Bongaerts P (2022) Deep connections: Divergence histories with gene flow in mesophotic Agaricia corals. Mol Ecol 31:2511–2527. doi: 10.1111/mec.16391

Priori C, Mastascusa V, Erra F, Angiolillo M, Canese S, Santangelo G (2013) Demography of deep-dwelling red coral populations: Age and reproductive structure of a highly valued marine species. Estuar Coast Shelf Sci 118:43–49. doi: 10.1016/j.ecss.2012.12.011

Quattrini AM, Rodríguez E, Faircloth BC, Cowman PF, Brugler MR, Farfan GA, Hellberg ME, Kitahara M v., Morrison CL, Paz-García DA, Reimer JD, McFadden CS (2020) Palaeoclimate ocean conditions shaped the evolution of corals and their skeletons through deep time. Nat Ecol Evol 4:1531–1538. doi: 10.1038/s41559-020-01291-1

Ramirez-Llora E, Brandt A, Danovaro R, De Mol B, Escobar E, German CR, Levin LA, Arbizu PM, Menot L, Buhl Mortenses (2010) Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences, 7: 2851-2899. doi:10.5194/bg-7-2851-2010

Rakka M, Orejas C, Sampaio I, Monteiro J, Parra H, Carreiro-Silva M (2017) Reproductive biology of the black coral *Antipathella wollastoni* (Cnidaria: Antipatharia) in the Azores (NE Atlantic). Deep Sea Res 2 Top Stud Oceanogr 145:131–141. doi: 10.1016/j.dsr2.2016.05.011

Rakka M, Godinho A, Orejas C, Carreiro-Silva M (2021a) Embryo and larval biology of the deep-sea octocoral *Dentomuricea* aff. *meteor* under different temperature regimes. PeerJ. doi: 10.7717/peerj.11604

Rakka M, Sampaio Í, Colaço A, Carreiro-Silva M (2021b) Reproductive biology of two deep-sea octocorals in the Azores Archipelago. Deep Sea Res 1 Oceanogr Res Pap. doi: 10.1016/j.dsr.2021.103587

Rice AL, Tyler PA, Paterson GJL (1992) The Pennatulid *Kophobelemnon stelliferum* (Cnidaria: Octocorallia) in the Porcupine Seabight (north-east Atlantic Ocean). J mar biol Ass UK 72:417–434.

Richmond R (1997) Reproduction and Recruitment in Corals: Critical Links in the Persistence of Reefs. doi: 10.1007/978-1-4615-5995-5_8

Rinkevich B, Loya Y (1987) Variability in the pattern of sexual reproduction of the coral *Stylophora pistillata* at Eilat, red sea: A long-term study. Biological Bulletin 173:335–344. doi: 10.2307/1541546

Roark B, Guilderson T, Dunbar R, Ingram L (2006) Radiocarbon-based ages and growth rates of Hawaiian deep-sea corals. Mar Ecol Prog Ser 327:1–14.

Rogers AD (2009) Cnidarians (Cnidaria). The timetree of life 233–238.

Rosche C, Schrieber K, Lachmuth S, Durka W, Hirsch H, Wagner V, Schleuning M, Hensen I (2018) Sex ratio rather than population size affects genetic diversity in *Antennaria dioica*. Plant Biol 20:789–796. doi: 10.1111/plb.12716

Rossi S (2013) The destruction of the “animal forests” in the oceans: Towards an over-simplification of the benthic ecosystems. Ocean Coast Manag 84:77–85. doi: 10.1016/j.ocecoaman.2013.07.004

Rossi S, Bramanti L, Gori A, Orejas C (2017) An overview of the Animal Forests of the World. Marine Animal Forests. doi: 10.1007/978-3-319-17001-5_1-11

Rossi S, Bramanti L, Gori A, Orejas C (2020) Perspectives on the Marine Animal Forests of the World. Springer Cham

Roveta C, Annibaldi A, Afghan A, Calcinai B, Di Camillo CG, Gregorin C, Illuminati S, Pulido Mantas T, Truzzi C, Puce S. (2021) Biomonitoring of heavy metals: the unexplored role of marine sessile species. Appl. Sci. 11, 580: 1-16. doi: 10.3390/app11020580

Ruiz-Ramos D v., Saunders M, Fisher CR, Baums IB (2015) Home bodies and wanderers: Sympatric lineages of the deep-sea black coral *Leiopathes glaberrima*. PLoS One. doi: 10.1371/journal.pone.0138989

Salvini-Plawen LV (2009) On the origin and evolution of the lower Metazoa. Journal of Zoological Systematics and Evolutionary Research 16:40–87. doi: 10.1111/j.1439-0469.1978.tb00919.x

Sammarco PW (1982) Polyp Bail-Out: An Escape Response to Environmental Stress and a New Means of Reproduction in Corals. Mar Ecol Prog Ser 10:57–65.

Santangelo G, Maggi E, Bramanti L, Bongiorni L (2004) Demography of the over-exploited Mediterranean redcoral (*Corallium rubrum* L. 1758). Sci Mar 68:199–204

Scucchia F, Nativ H, Neder M, Goodbody-Gringley G, Mass T (2020) Physiological Characteristics of *Stylophora pistillata* Larvae Across a Depth Gradient. Front Mar Sci 7(13). doi: 10.3389/fmars.2020.00013

Shilling FM, Manahan DT (1994) Energy Metabolism and Amino Acid Transport During Early Development of Antarctic and Temperate Echinoderms. Biology Bulletin 187:398–407.

Shlesinger T, Grinblat M, Rapuano H, Amit T, Loya Y (2018) Can mesophotic reefs replenish shallow reefs? Reduced coral reproductive performance casts a doubt. Ecology 99:421–437. doi: 10.1002/ecy.2098

Soares M de O, Araújo JT de, Ferreira SMC, Santos BA, Boavida JRH, Costantini F, Rossi S (2020) Why do mesophotic coral ecosystems have to be protected? Science of the Total Environment. doi: 10.1016/j.scitotenv.2020.138456

Sun Z, Hamel J-F, Mercier A (2009a) Planulation of deep-sea octocorals in the NW Atlantic. Coral Reefs 28(3) 781. doi: 10.1007/s00338-009-0505-9

Sun Z, Hamel J-F, Edinger E, Mercier A (2009b) Reproductive biology of the deep-sea octocoral *Drifa glomerata* in the Northwest Atlantic. Mar Biol 157:863–873. doi: 10.1007/s00227-009-1369-9

Sun Z, Hamel J-F, Mercier A (2010) Planulation periodicity, settlement preferences and growth of two deep-sea octocorals from the northwest Atlantic. Mar Ecol Prog Ser 410:71–87. doi: 10.3354/meps08637

Sun Z, Hamel J-F, Mercier A (2011) Planulation, larval biology, and early growth of the deep-sea soft corals *Gersemia fruticosa* and *Duva florida* (Octocorallia: Alcyonacea). Invertebrate Biology 130:91–99. doi: 10.1111/j.1744-7410.2011.00229.x

Taylor ML, Roterman MN (2017) Invertebrate population genetics across Earth's largest habitat: the deep-sea floor. Mol. Ecol., 26: 4872-4896. doi: 10.1111/mec.14237

Terrana L, Eeckhaut I (2019) Sexual reproduction of the shallow-water black coral *Cirrhipathes anguina* (Dana, 1846) from Madagascar. Marine Biology Research 15:410–423. doi: 10.1080/17451000.2019.1662444

Terzin M, Paletta MG, Matterson Kenan, Coppari M, Bavestrello G, Abbiati M, Bo M, Costantini F (2021) Population genomic structure of the black coral *Antipathella subpinnata* in Mediterranean Vulnerable Marine Ecosystems. Coral Reefs. doi: 10.1007/s00338

Torrents O, Garrabou J, Marschal C, Harmelin JG (2005) Age and size at first reproduction in the commercially exploited red coral *Corallium rubrum* (L.) in the Marseilles area (France, NW Mediterranean). Biol Conserv 121:391–397. doi: 10.1016/j.biocon.2004.05.010

Tsounis G, Rossi S, Gili J-M, Arntz W (2006) Population structure of an exploited benthic cnidarian: the case study of red coral (*Corallium rubrum* L.). Mar Biol 149:1059–1070. doi: 10.1007/s00227-006-0302-8

Tyler PA, Bronsdon SK, Young CM, Rice AL (1995) Ecology and Gametogenic Biology of the Genus *Umbellula* (Pennatulacea) in the North Atlantic Ocean. Internationale Revue der gesamten Hydrobiologie und Hydrographie 80:187–199. doi: 10.1002/iroh.19950800207

Van-Praet M (1990) Gametogenesis and the reproductive cycle in the deep-sea anemone *Paracalliactis stephensoni* (cnidaria: Actiniaria). Journal of the Marine Biological Association of the United Kingdom 70:163–172. doi: 10.1017/S0025315400034287

Vize PD (2006) Deepwater broadcast spawning by *Montastraea cavernosa*, *Montastraea franksi*, and *Diploria strigosa* at the Flower Garden Banks, Gulf of Mexico. Coral Reefs 25:169–171. doi: 10.1007/s00338-005-0082-5

Waggoner B, Collins AG (2004) Reductio ad absurdum: testing the evolutionary relationships of ediacaran and paleozoic problematic fossils using molecular divergence dates. J Paleontol 78:51–61. doi: 10.1666/0022-3360(2004)078<0051:raatte>2.0.co;2

Wagner D, Waller RG, Toonen RJ (2011) Sexual reproduction of Hawaiian black corals, with a review of the reproduction of antipatharians (Cnidaria:

Anthozoa: Hexacorallia). Invertebrate Biology 130:211–225. doi: 10.1111/j.1744-7410.2011.00233.x

Wagner D, Waller RG, Montgomery AD, Kelley CD, Toonen RJ (2012) Sexual reproduction of the Hawaiian black coral *Antipathes griggi* (Cnidaria: Antipatharia). Coral Reefs 31:795–806. doi: 10.1007/s00338-012-0882-3

Waller RG, Tyler PA, Gage JD (2002) Reproductive ecology of the deep-sea scleractinian coral *Fungiacyathus marenzelleri* (Vaughan, 1906) in the northeast Atlantic Ocean. Coral Reefs 21:325–331. doi: 10.1007/s00338-002-0252-7

Waller RG, Tyler PA (2005) The reproductive biology of two deep-water, reef-building scleractinians from the NE Atlantic Ocean. Coral Reefs 24:514–522. doi: 10.1007/s00338-005-0501-7

Waller RG, Tyler PA, Gage JD (2005) Sexual reproduction in three hermaphroditic deep-sea *Caryophyllia* species (Anthozoa: Scleractinia) from the NE Atlantic Ocean. Coral Reefs 24:594–602. doi: 10.1007/s00338-005-0031-3

Waller RG, Baco AR (2007) Reproductive morphology of three species of deep-water precious corals from the Hawaiian archipelago: *Gerardia* sp., *Corallium secundum*, and *Corallium lauuense*. Bull Mar Sci 81:533–542

Waller RG, Tyler PA, Smith CR (2008) Fecundity and embryo development of three Antarctic deep-water scleractinians: *Flabellum thouarsii, F. curvatum* and *F. impensum*. Deep Sea Res 2 Top Stud Oceanogr 55:2527–2534. doi: 10.1016/j.dsr2.2008.07.001

Waller RG, Tyler PA (2011) Reproductive patterns in two deep-water solitary corals from the north-east Atlantic-*Flabellum alabastrum* and *F. angulare* (Cnidaria: Anthozoa: Scleractinia). Journal of the Marine Biological Association of the United Kingdom 91:669–675. doi: 10.1017/S0025315410000822

Waller RG, Feehan KA (2013) Reproductive ecology of a polar deep-sea scleractinian*, Fungiacyathus marenzelleri* (Vaughan, 1906). Deep Sea Res 2 Top Stud Oceanogr 92:201–206. doi: 10.1016/j.dsr2.2013.03.006

Waller RG, Stone RP, Rice LN, Johnstone J, Rossin AM, Hartill E, Feehan K, Morrison CL (2019) Phenotypic Plasticity or a Reproductive Dead End? *Primnoa pacifica* (Cnidaria: Alcyonacea) in the Southeastern Alaska Region. Front Mar Sci. doi: 10.3389/fmars.2019.00709

Wells JW, Hill D (1956) Anthozoa: general features. Paleontology s. In: Moore RC (ed) Treatise on invertebrate paleontology. Part F: Coelenterata. The University of Kansas Press, Lawrence, KS, p F161–F166

Won JH, Rho BJ, Song JI (2001) A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. Coral Reefs 20:39–50. doi: 10.1007/s003380000132

SITOGRAPHY

Argentario Divers: https://www.argentariodivers.com/

Bank of biology https://www.bankofbiology.com/2012/03/

Google Earth: https://www.google.com/intl/it/earth/

Google Maps: https://www.google.it/maps.com

Scopus: https://www.scopus.com

Taxateca.com: http://www.taxateca.com/claseanthozoa.html

WoRMS Editorial Board (2022). World Register of Marine Species: https://www.marinespecies.org/. Last Access: 2022-12-31.