Cold-water corals in a changing world: potential impacts of climate change across coral life history stages

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Maria Rakka

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Tese especialmente elaborada para obtenção do grau de Doutor em Ciências do Mar

“Trade-offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another” - S.C. Stearns, 1989

“When you light a candle, you also cast a shadow” - Ursula K. Le Guin

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Abstract

Corals are widely known for their remarkable capacity to build rich communities in tropical shallow ecosystems. However, corals can be encountered worldwide and many species thrive in deep waters where they create highly heterogenous habitats that support high biodiversity. The current thesis focuses on these organisms, known as cold-water corals or deep-sea corals. The studies presented herein were developed in the Azores Archipelago, where the most frequently encountered deep-sea corals belong to the subclass Octocorallia. Contrary to scleractinian corals which form reefs, octocorals create dense communities that are known as coral gardens. In the Azores, coral gardens are among the most frequently encountered deep-sea communities on seamounts and island slopes below 200 m of depth.

Deep-sea ecosystems are experiencing increasing pressure from several anthropogenic activities, including fishing, oil and gas extraction, prospective deep-sea mining and climate change. Model projections highlight that by the end of the century, deep-sea communities will have to face multiple changes, including warming, ocean acidification (OA), deoxygenation and decreased carbon input from the surface. Despite the importance of deep-sea octocorals as habitat-builders in the Azores, their potential response to climate change is currently unknown. The present dissertation aims at determining the potential impacts of climate change on deep-sea octocorals. The thesis focused on two common deep-sea octocorals in the Azores, the sea fan *Dentomuricea* aff. *meteor* Grasshoff 1977 and the whip coral *Viminella flagellum* (Johnson 1863), utilizing them as a case study.

In the first chapters, essential knowledge on the biology and ecophysiology of the two target species was generated, focusing on major processes throughout different life history stages, including reproduction (chapter 2), early life development (chapter 3) and metabolism (chapter 4). More specifically, in chapter 2, a histological study was performed to describe their reproductive characteristics, revealing that both species display an opportunistic reproductive strategy, with continuous presence of immature gametes, which likely go through the last steps of gametogenesis only when conditions are optimal. Chapter 3 provided the first detailed descriptions of embryo

and larval development for the target species and for deep-sea broadcast spawning octocorals in general. It also revealed that both target species produce lecithotrophic larvae with marked differences in larval characteristics, which are likely caused by different strategies of maternal investment and have major implications for their dispersal capacity. Chapter 4 is based on aquaria experiments to study the feeding biology of the two species and highlighted the importance of zooplankton for major physiological processes, such as respiration and excretion. It unveiled that both species are capable of exploiting different food sources, but are also adapted to exploit rapidly sporadic or seasonal inputs of high-quality food, such as zooplankton. More importantly, it underlined the different metabolic strategies of the two species, with *D*. aff. *meteor* displaying high metabolic rates accompanied by high losses, contrary to *V. flagellum* which displayed more reserved metabolic rates and higher efficiency. Subsequently, chapter 5 adopts an experimental approach to determine the physiological sensitivity of the species *V. flagellum* to OA and variable food supply. The species was able to withstand short-term conditions of OA projected to the end of the century by decreasing its metabolism, a response that was accentuated under fasting.

In the last chapter, the generated information was compiled along with available literature to discuss the potential fate of deep-sea octocorals under upcoming climate changes. Furthermore, a comparative approach was adopted to determine the vulnerability of the two target species to climate change stressors. This approach showcased that species responses to climate change depend on their biological and ecological traits. Based on the comparison between the two species, and the diversity that characterises the subclass Octocorallia, it is suggested that although deep-sea octocorals can be generally considered to be sensitive to climate change, opportunistic species might be able to persist, with potential consequences on ecosystem structure and function. The work presented herein constitutes a valuable contribution to our knowledge on the biology of deep-sea octocorals and highlights that basic biological knowledge is essential to understand potential future changes in deep-sea ecosystems.

Resumo

Os corais têm uma reconhecida capacidade de construir habitats em águas rasas tropicais. No entanto muitas espécies de corais habitam águas profundas em todos os oceanos do mundo, onde formam comunidades que sustentam grande biodiversidade. Estes organismos, conhecidos como corais de águas frias ou corais de profundidade, são o foco desta dissertação. Os estudos aqui apresentados foram desenvolvidos no Arquipélago dos Açores, onde as espécies de corais de águas frias mais comuns pertencem à subclasse Octocorallia. Ao contrário dos corais escleractínios que formam recifes de coral, os octocorais criam habitats conhecidos como jardins de corais. Nos Açores, são notáveis os jardins de corais formados por densas populações de octocorais que colonizam os montes submarinos e as encostas das ilhas abaixo dos 200 m de profundidade.

Os ecossistemas do mar profundo encontram-se actualmente ameaçados pelo impacto de diversas actividades humanas, tais como a pesca, a extração de petróleo e gás, a mineração do mar profundo e as alterações climáticas. Projeções com base em modelos, prevêm que até o final do século, os ecossistemas do mar profundo terão que enfrentar várias alterações nas propriedades das massas de águas, incluindo aquecimento das águas, acidificação dos oceanos (AO), desoxigenação e uma diminuição no fluxo de carbono orgânico da superfície para o oceano profundo. Apesar da importância das espécies de octocorais de profundidade na região dos Açores, o nosso conhecimento relativamente às estratégias utilizadas por estes organismos em resposta a diferentes cenários de stress ambiental é extremamente limitado. A presente dissertação visa determinar os impactos potenciais das alterações climáticas nos octocorais de profundidade, focando-se em dois octocorais de águas profundas comuns nos Açores, *Dentomuricea* aff. *meteor* Grasshoff 1977 e *Viminella flagellum* (Johnson 1863), utilizando-os como caso de estudo.

Nos primeiros capítulos da tese, foi produzida informação sobre a biologia e ecofisiologia das duas espécies-alvo durante várias fases da história de vida, incluindo a reprodução (capítulo 2), o desenvolvimento inicial (capítulo 3) e o metabolismo

(capítulo 4). No capítulo 2, foi realizado um estudo histológico para descrever as suas características reprodutivas, revelando que ambas as espécies apresentam uma estratégia reprodutiva oportunista, com presença contínua de gâmetas imaturos que provavelmente passam pelas últimas etapas da gametogénese apenas perante condições ótimas. O Capítulo 3 apresenta as primeiras descrições detalhadas das características do desenvolvimento embrionário e dos estágios larvais das espécies de estudo, e para octocorais de profundidade com reprodução sexuada por difusão na água ("broadcast spawning"), em geral. Resultados destes estudos revelaram também que ambas as espécies-alvo produzem larvas lecitotróficas, com diferenças significativas nas características das larvas, que são provavelmente causadas por diferentes estratégias de investimento parental e têm implicações importantes para a sua capacidade de dispersão. No Capítulo 4 foram utilizadas experiências em aquários para estudar a biologia alimentar das duas espécies, cujos resultados demonstraramo a importância do zooplâncton nos processos fisiológicos como a respiração, e a excreção de ambas as espécies. Para além disso, os resultados deste estudo revelaram que ambas as espécies são capazes de alimentar-se de diferentes tipos de alimento, mas também estão adaptadas para explorar episódios esporádicos ou sazonais de disponibilidade de alimento de elevada qualidade, como o zooplâncton. De maior relevância ainda, este estudo destacou as diferentes estratégias metabólicas das duas espécies, com *D.* aff. *meteor* apresentando elevadas taxas metabólicas acompanhadas de elevadas perdas, ao contrário de *V. flagellum* que apresentou menores taxas metabólicas e maior eficiência. Posteriormente, o capítulo 5 adotou uma abordagem experimental para determinar a resposta metabolica da espécie *V. flagellum* a condições de AO e diferentes quantidades de alimento. Os resultados demonstraram que a *V. flagellum* consegiu resistir a condições de curto prazo de AO projetadas para o final do século, diminuindo o seu metabolismo, uma resposta que se acentuou no tratamento sem alimento (jejum).

No último capítulo, as informações produzidas nos capítulos anteriores foram compiladas juntamente com a literatura disponível para discutir o potencial rumo dos octocorais do mar profundo face às alterações climáticas previstas para o futuro. Neste sentido, a tese adotou uma abordagem comparativa para determinar a

vulnerabilidade das duas espécies-alvo face às alterações climáticas. Essa abordagem mostrou que as respostas das espécies dependem das suas características biológicas e ecológicas. Com base na comparação das duas espécies, salientou também que, embora os octocorais de águas profundas sejam geralmente considerados sensíveis às alterações climáticas, as espécies de octocorais oportunistas podem ser capazes de persistir, com potenciais consequências na estrutura e função do ecossistema. Esta tese afigura-se como uma valiosa contribuição para o nosso conhecimento sobre os corais do mar profundo, e destaca o valor do conhecimento biológico para conseguirmos entender melhor as mudanças que os ecosistemas do mar profundo podem sofrer no futuro.

Chapter 1
General introduction

1.1. Overview

Corals are a very diverse group of animals that belong to the phylum Cnidaria. These organisms can have fundamental ecological roles and are considered ecosystem engineers (sensu Jones et al. 1994). They create habitat that can host a variety of other marine species and have an important role in carbon cycling and benthopelagic coupling (Watanabe & Nakamura 2019). Their structural simplicity, morphological plasticity and modularity resemble plant characteristics and their ecological role has been compared to the function of trees in a forest (Figure 1.1). This led to the inclusion of coral communities in the concept of marine animal forests (MAF), which are defined as living three-dimensional structures created by benthic marine species, such as cnidarians, sponges and mollusks (Rossi et al. 2017a).

The most famous representatives of coral communities are coral reefs that dominate in shallow tropical areas, however, corals can be encountered worldwide and approximately 65% of the known species can be encountered below 50 m (Cairns 2007a, Roberts et al. 2009). The existence of corals in deep waters was known since the 18th century, but technological advances during the 20th century allowed their systematic study, giving rise to the term “cold-water corals (CWCs)” (Roberts et al. 2006). The term has been used in different ways by several researchers (Altuna & Poliseno 2019, Chimienti et al. 2019) and many times interchangeably with the term deep-sea corals (Pérez et al. 2016). Its wide application has allowed the inclusion of a range of species, whether they occur in shallow depths in high latitudes (Mortensen & Buhl-Mortensen 2004), the continental shelf of the Mediterranean (Gori et al. 2017) or the bathyal zone of tropical regions (Cordes et al. 2008), highlighting that corals can be found in a variety of environmental settings, regardless of depth and climatic zone.

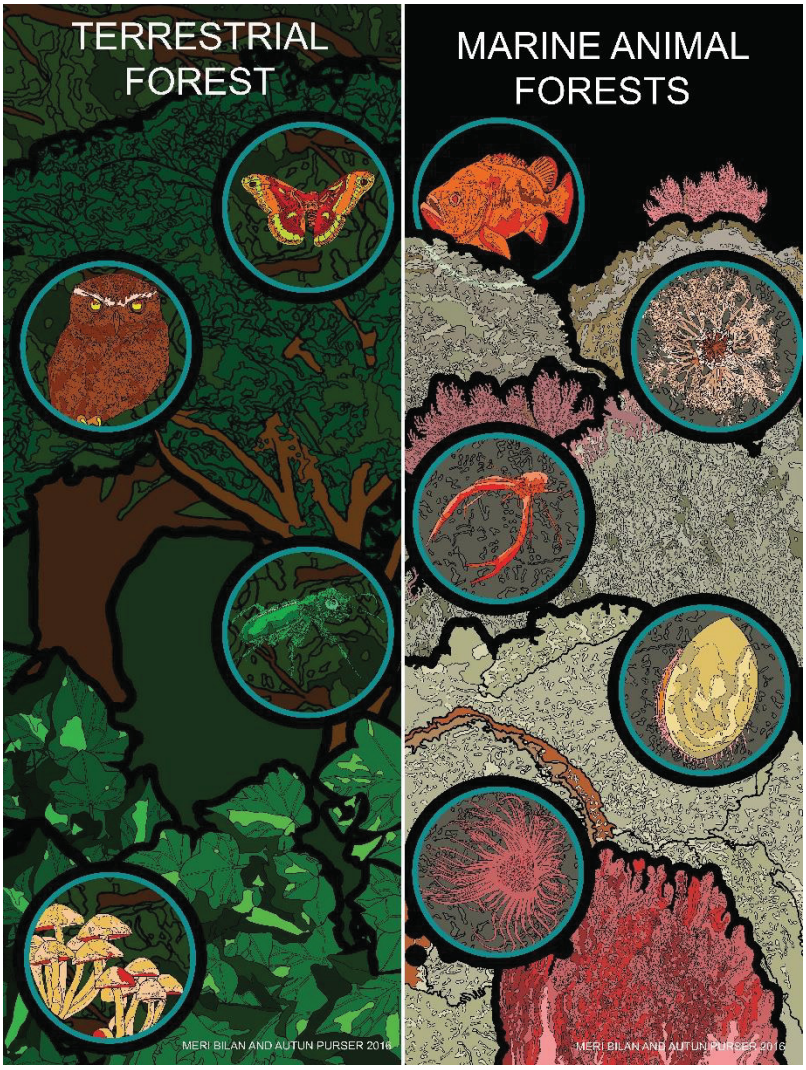

Figure 1.1: Corals are ecosystem engineers and create three-dimensional habitats similar to the ones created by trees, giving rise to the concept of marine animal forests. Image credits: Meri Bilan and Autun Purser.

Among the most conspicuous habitats created by CWCs are coral reefs and coral gardens/meadows. While reefs are mostly built by scleractinian species which deposit their massive calcium carbon skeletons, coral gardens can be formed by dense aggregation of species that present more erect growth forms, such as octocorals and antipatharians (Buhl-Mortensen et al. 2017). Both habitats have been recognized as important for a variety of marine species (Henry & Roberts 2017, Buhl-Mortensen et al. 2017), however attention very often focuses mainly on coral reefs and scleractinian species, while the rest of CWC taxa have received comparatively less attention. This limits our knowledge to the most common species and undermines our holistic

understanding of marine ecosystems, therefore studies on other CWC groups, like octocorals and antipatharians are essential.

The current thesis focuses on deep-sea octocorals, referring to octocoral species that may be encountered in shallow depths but form structural habitat mostly below 200 m (Chimienti et al. 2019). The studies presented within the thesis were developed in the Azores Archipelago, where dense and diverse coral gardens are frequently encountered on island slopes and seamounts (Tempera et al. 2012, Morato et al. 2020b).

Similarly to other ecosystems on earth, the deep-sea is expected to face multiple challenges due to anthropogenic pressure from fisheries, oil and gas exploration, prospective deep-sea mining and climate change (Ramirez-Llodra et al. 2011, Sweetman et al. 2017, Levin et al. 2020). The present dissertation aims at determining potential impacts of climate change on deep-sea octocorals in the Azores Archipelago. To fulfill its aim, the thesis consists of six chapters. The current chapter (Chapter 1) covers important aspects for the objectives of the study. It firstly reviews current knowledge on the biology and ecology of deep-water octocorals and subsequently describes the structure of the thesis in detail. The following chapters (Chapters 2-5) cover the main research questions of the thesis. Three of these chapters (Chapter 2, Section 3.1, Chapter 4) have been published in international peer-reviewed journals while two more are in preparation (Section 3.2, Chapter 5). Supplementary material of the published chapters are also provided as appendices. Lastly, the last chapter (Chapter 6) compiles the produced information, along with available knowledge from studies in the literature, to discuss the potential fate of deep-sea octocorals in a changing world.

1.2. Biology and ecology of deep-sea octocorals

1.2.1. Morphology and classification

Octocorallia is an extremely diverse group of anthozoans, characterized by eightfold symmetry. They are modular, colonial organisms, with the exception of one genus (*Taiaroa*, Bayer & Muzik 1976). Octocorals consist of multiple polyps which possess eight tentacles and are usually equipped with characteristic protrusions, called pinnules (Bayer 1973). Polyps are connected with tissue which grows around a skeletal axis (Figure 1.2). In addition, the skeletal system of octocorals contains calcium micro-skeletal elements called sclerites, which can be embedded in the coral tissue or cover it externally (Bayer et al. 1983). Sclerites may have several shapes and sizes, and their formations shape polyp architecture, constituting a major taxonomic character for species identification.

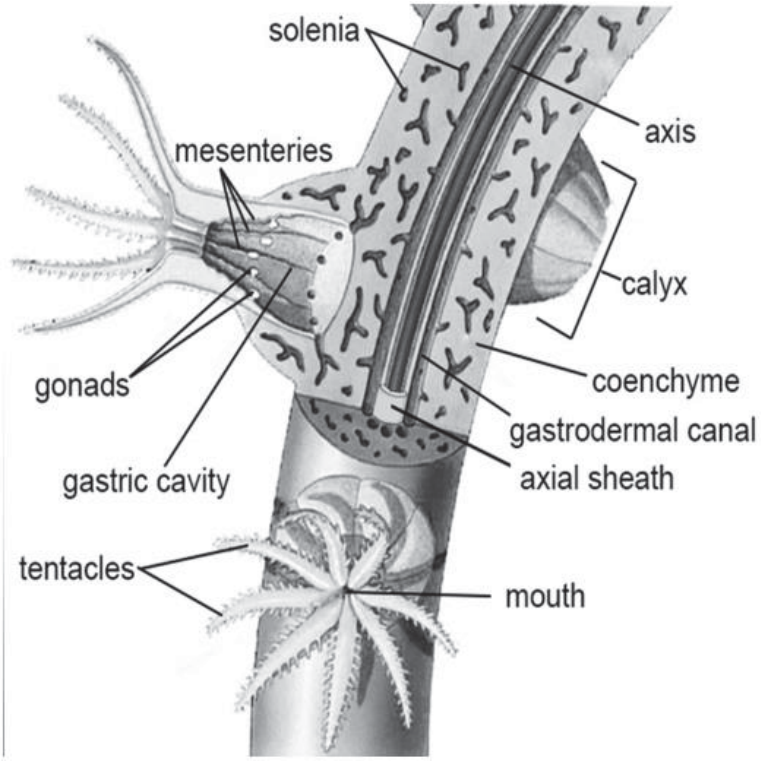

Figure 1.2: Morphology of an octocoral polyp. Source: Kupfner Johnson (2019), modified from Bayer, 1973.

To this date, the subclass Octocorallia counts more than 3400 species (Pérez et al. 2016). Their taxonomic classification has been proven a complicated task, due to the high plasticity of morphological characters, a high degree of intraspecific variation, numerous convergences, as well as discordances between molecular and traditional

taxonomic tools (McFadden et al. 2010, Pérez et al. 2016). The current classification, lastly modified by Bayer (1981), divides the subclass in three orders: Alcyonacea, Pennatulacea and Helioporacea.

Octocoral species are characterized by high morphological diversity (Figure 1.3). Their colony forms may vary, including monopodial, encrusting, or highly branched colonies with several possible growth forms, such as arborescent, fan-shaped, bushy and digitate (Bayer 1973). The exact shape of the colony may also vary within species (Fabricius & Alderslade 2001). Lastly, the general shape of octocoral polyps is similar among species, however polyp size, tentacle and pinnule size, as well as the skeletal architecture which is formed by sclerites may differ substantially (Figure 1.3). These attributes provide great morphological richness to the species of the subclass, allowing them to develop different life history strategies and occupy different niches.

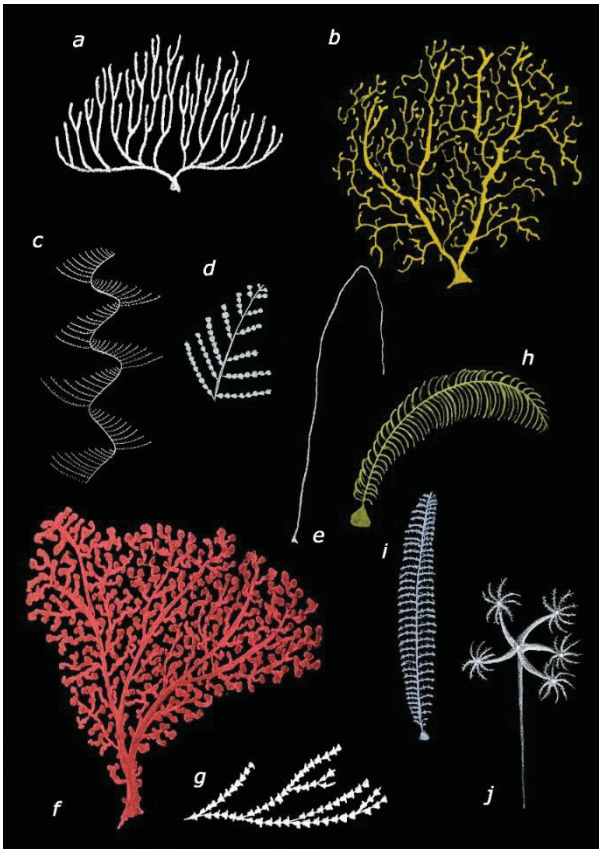

Figure 1.3: Examples of deep-sea octocoral species, displaying their high morphological diversity. Order Alcyonacea: (a) Narella sp., (b) Dentomuricea sp., (c) Iridogorgia sp., (d) Callogorgia sp., (e) *Viminella flagellum*; (f) Paragorgia sp.; (g) Narella sp., Order Pennatulacea: (h) Ptilosarcus sp.; (i) Stylatela sp.; (j) Umbellula sp.

1.2.2. Distribution

The geographic and bathymetric distribution of octocoral species is wide (Pérez et al. 2016). They can be encountered from polar to tropical zones (Figure 1.4), in depths ranging from the intertidal to abyssal depths (6400 m, Zapata Guardiola & López-González). The highest diversity of octocoral species per unit area worldwide is encountered in tropical, shallow reefs of the Indo-pacific (Fabricius & Alderslade 2001), however high species richness is also observed in the deep-sea (Watling et al. 2011, Pérez et al. 2016). More than 75% of the known octocoral species can be found below 50 m of depth (Cairns 2007a).

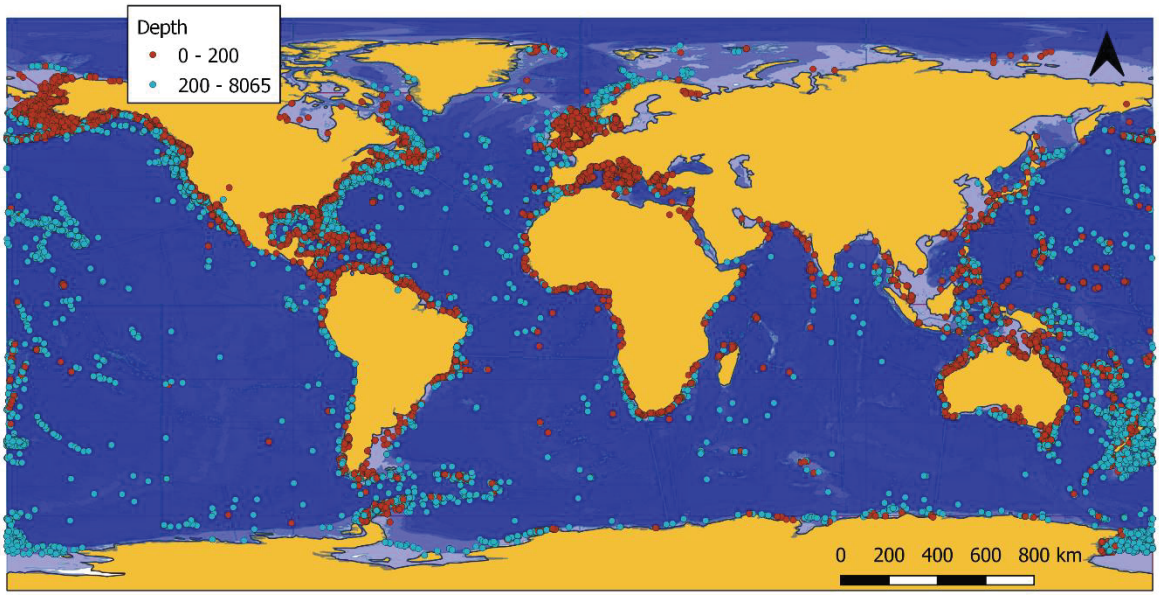

Figure 1.4: Occurrences of shallow (0-200) and deep-water (>200 m) octocorals. Data accessed through the Ocean Biodiversity Information System (OBIS, date of access: 27/5/2021)

1.2.3. Feeding biology

Octocorals are passive suspension feeders, catching prey and particles from the surrounding water. For these animals, a variety of food particles are available in the water column, such as phytoplankton, zooplankton, particulate organic matter (POM), detritus and dissolved elements. These resources are widely available in the euphotic and mesophotic zone, and they can be transferred in the deep-sea in different ways, such as sinking, transportation by downwelling currents and internal waves (Davies et al. 2009, Duineveld et al. 2012, Findlay et al. 2013), as well as daily migration of zooplankton (Gili et al. 2006, Guihen et al. 2018, Van Engeland et al. 2019).

Modular organisms consist of repeated units that can participate in food acquisition, thus food capture can be performed from the whole body, contrary to unitary organisms in which feeding is achieved through a single structure (Burgess et al. 2017). As a result, food capture increases proportionally to the available coral surface and depends on species-specific characteristics such as colony shape and polyp size (Sebens et al. 2017). At the same time, octocorals need to maintain their polyps open to capture food particles, a process that can be energetically costly, creating a trade-off between food capture and energy expenditure. Energy requirements depend on species-specific features, especially the ratio of surface to volume (Burgess et al. 2017) which also depends on colony architecture, polyp size and polyp morphology. Octocorals display great diversity in terms of morphological features and thus have developed a variety of feeding and metabolic strategies.

Great part of the existing literature on the dietary characteristics of octocoral species comes from Mediterranean and tropical species (Table 1.1). Overall, most octocorals have small polyps and tentacles with relatively weak nematocysts which are generally considered more efficient in capturing phytoplankton (Fabricius et al. 1995b, Orejas et al. 2003, Leal et al. 2013) and small zooplankton (<200 μm) with low swimming ability (Cocito et al. 2013, Coma et al. 2015). Octocorals are also capable of feeding on dissolved organic matter (Gori et al. 2014). In the case of deep-sea octocorals, information is scarcer and mainly comes from trophic markers (Table 1.1). Most studied deep-sea octocorals up to date base their diet on phytodetritus and particulate organic matter (Sherwood et al. 2008, Salvo et al. 2018), while only a few species feed mainly on microzooplankton (Sherwood et al. 2005, Imbs et al. 2016).

A number of studies have highlighted the ability of octocoral species to adapt to seasonal variability in food availability. For example, in temperate, oligotrophic ecosystems octocorals often switch their diet seasonally to the most abundant prey (Coma et al. 2000, Coma & Ribes 2003, Rossi et al. 2004). Moreover, some species display seasonal cycles in biochemical composition (Rossi et al. 2006, Gori et al. 2013) and have adjusted their major physiological processes such as growth and reproduction, which also follow seasonal cycles (Coma & Ribes 2003). In the deep-sea,

similar seasonality has been reported for Antarctic species (Gili et al. 2001, Orejas et al. 2001), but is yet to be demonstrated for other deep-sea octocorals.

1.2.1. Growth and longevity

In unitary multicellular animals, growth continues until maximum size or age is achieved. In contrast, modular invertebrates such as octocorals, grow by adding new modules to their structure, which allows them to grow indeterminately, reaching extremely large sizes and ages (Roark et al. 2009).

Octocoral growth does not only require the addition of new modules, but also the secretion of skeleton. In order to build their sclerites and main axis, most octocorals need to calcify. In contrast to scleractinian corals, which build exclusively massive skeletons by using a single calcium carbonate polymorph (aragonite), octocorals display a remarkable diversity of skeletal elements and biomineralization strategies (Conci et al. 2021). Their sclerites are mostly made of high magnesium-calcite, but their skeletal axis can be made of calcite (suborder Calcaxonia), scleroproteins (suborder Holaxonia) or fused, embedded sclerites (suborder Scleraxonia) (Bostock et al. 2015, Conci et al. 2021). Moreover, species of the order Helioporacea build massive aragonitic skeletons, similarly to scleractinians.

Regardless of the composition and calcifying strategy, secretion of skeleton and tissue growth are energetically demanding processes. Theoretically, there is no limit to octocoral growth but in practice colony maximum size and age are limited by a variety of internal and external factors (Sebens et al. 2017, Lartaud et al. 2017), such as energetic and metabolic constrains including food availability and allometric scaling (Kim & Lasker 1998), as well as water properties such as

Table 1.1: Studies comparing the use of different food sources by octocoral species, with indication of the methodological approach and main food sources.

Study	Approach	Species	Main food sources	Region	Depth
Cocito et al. 2013	Trophic markers	*Leptogorgia sarmentosa*	zooplankton/SOM	Mediterranean	10-200
		Paramuricea clavata	POM, SOM		10-110
		Eunicella verrucosa	zooplankton/SOM		10-110
		Eunicella singularis	zooplankton		5-60
Ribes et al. 1999	Aquaria experiments	*Paramuricea clavata*	detrital POM	Mediterranean	15
Leal et al. 2014b	trophic markers	*Heteroxenia fuscescens*	Algae	Mediterranean	
		Sinularia flexibilis	no herbivory		
Ribes et al. 2003	Aquaria experiments	*Leptogorgia sarmentosa*	microplankton, POC	Mediterranean	20-200
Coma et al., 2015	Gut content	*Eunicella singularis*	zooplankton, 40-920 µm	Mediterranean	4-35
Rossi et al. 2004	Gut content	*Leptogorgia sarmentosa*	low motile zooplankton (80-200 µm)	Mediterranean	20
Leal et al. 2014a	trophic markers	*Leptogorgia virgulata*	pico, nanoplankton (<10 µm), microplankton (10-64 µm)	Southeast US coast	7-10
Orejas et al., 2003	Gut contents/experimental	*Primnoisis antarctica*	diatoms, dinoflagellates, ciliates	Antarctic	200-500
		Primnoella sp.	dinoflagellates, ciliates, centric diatoms		200-500
Fabricius et al., 1995a	Aquaria experiments	*Dendronephthya hemprichi*	phytoplankton	Red sea	10-28
		Dendronephthya sinaiensis	phytoplankton		10-28
		Scleronephthya corymbosa	phytoplankton		10-28
		Acabaria sp.	phytoplankton		10-28
Orejas et al., 2001	Gut contents/aquaria experiments	*Anthomastus bathyproctus*	zooplankton	Antarctic	400-450
		Clavularia cf. frankliniana	diatoms		shallow
Sherwood et al., 2008	Trophic markers	*Paragorgia arborea*	fresh phytodetritus	Newfoundland/Labrador	370-1300
		Primnoa resedaeformis	fresh phytodetritus & microzooplankton		160-1100

Study	Approach	Species	Main food sources	Region	Depth
		Acanella arbuscula	degraded POM		150-1400
		Acanthogorgia armata	degraded POM		170-1400
		Anthomastus grandiflorus	degraded POM		170-1400
		Duva florida	degraded POM		50-1400
		Keratoisis ornata	degraded POM		200-1100
		Paramuricea sp.	degraded POM		150-1400
Imbs et al., 2016	Trophic markers	*Ampilaphis sp.*	Phytoplankton; herbivorous zooplankton	Northwest Pacific	120-620
		Plumarella sp.	Phytoplankton; herbivorous zooplankton		80
		Plumarella carinata	Phytoplankton; herbivorous zooplankton		200
		Primnoa pacifica	Phytoplankton; herbivorous zooplankton		130
		Thouarella sp.	Phytoplankton; herbivorous zooplankton		80-200
		Calyptrophora japonica	Phytoplankton; herbivorous zooplankton		460
		Gersemia rubiformis	Opportunistic		27
		Gersemia fruticosa	Opportunistic		85-360
		Acanthogorgia sp.	Opportunistic		140
		Calcigorgia spiculifera	Opportunistic		130
		Paragorgia arborea	Opportunistic		170
Sherwood et al., 2005	Trophic markers	*Primnoa spp.*	zooplankton, sinking POM	Northwest Atlantic	64-783

temperature, salinity, oxygen, pH and hydrodynamic conditions (Tracey et al. 2007, Lartaud et al. 2017). Deep-sea octocorals display low growth rates, which has been attributed mostly to the low temperatures and low food availability in large depths (Thresher 2009). Overall, radial growth, i.e. the thickening of the main axis, of deep-sea octocorals ranges between 0.05-0.4 mm/y while linear growth, i.e. the elongation of the branches and main stem, varies between 0.1-4 cm/y (Mortensen & Buhl-Mortensen 2005, Sherwood & Edinger 2009, Andrews et al. 2009, Bennecke & Metaxas 2017). Some deep-sea octocorals display high longevity, e.g. specimens of *Keratoisis* sp. have been shown to live for 300-420 years (Thresher 2009, Sinclair et al. 2011). In many cases, however, colony age is limited between 10-100 years (Watling et al. 2011), which might be a result of anthropogenic impacts, as fisheries remove larger and older colonies (Sherwood & Edinger 2009).

1.2.2. Reproduction and early life history

Corals, due to their structural simplicity and flexibility, display a variety of reproductive strategies and can employ both sexual and asexual reproduction (Harrison & Wallace 1990, Kahng et al. 2011). Regarding sexual reproduction, coral colonies may develop exclusively male or female gametes (gonochoristic), gametes of both sexes (hermaphroditic), or may even occupy intermediate stages, depending on the species. Gametes develop in the polyps and fertilization may occur externally, after the release of gametes and fertilization in the water column (broadcast spawning), or internally (brooding). In the case of broadcast spawning, embryo development takes place in the water column, while in brooding the embryo stays with the mother colony, either internally or externally, until it reaches the planula stage.

Octocorals are mostly gonochoristic, but their reproductive mode varies with taxonomic order (Kahng et al. 2011). Up to date, all studies with species of the order Pennatulacea (sea pens) report broadcast spawning (Pires et al. 2009, Kahng et al. 2011). On the other hand, species of the order Alcyonacea display great flexibility and can be either broadcast spawning or brooding (Kahng et al. 2011, Watling et al. 2011). Octocorals do not possess specialized reproductive tissues but develop gametes from endodermal cells usually located in mesenteries (Fautin &

Mariscal 1991). Gametogenesis, i.e. the production of gametes, may be a continuous process, giving rise to continuous gamete presence, or seasonal with gametes developing only in certain times of the year (Kahng et al., 2011).

The number of studies on reproduction of deep-sea octocorals is increasing, however our knowledge is still limited to a few species (Figure 1.5). Up to date, most known deep-sea octocorals are gonochoristic, with only one exception, the species *Drifa* sp. (Sun et al. 2009). In terms of reproductive mode, both broadcast spawning and brooding are common strategies among deep-sea octocorals (Watling et al. 2011, Figure 1.5). Most deep-sea species display continuous gamete presence with gametogenic cycles that may last for more than one year and may be overlapping (Orejas et al. 2007). As a result, oocytes in different developmental stages are frequently encountered within the same polyp. In some cases, this leads to a constant pool of immature oocytes, which mature in specific seasons, a process that is sometimes coupled with environmental factors such as food availability and temperature (Orejas et al. 2002, Mercier et al. 2011). However, continuous gamete presence is not limited in deep-sea octocorals, suggesting that this might be a phylogenetically conserved strategy (Watling et al. 2011).

Oogenesis in most octocorals, including deep-sea species, results in relatively large oocytes (diameter >200 µm) (Mercier & Hamel 2011, Quintanilla et al. 2013, Rossin et al. 2017) that develop to lecithotrophic, i.e. non-feeding larvae (Watling et al. 2011). So far, our knowledge on the embryo and larval biology of deep-sea octocorals is limited to a few brooding species (Cordes et al. 2001, Sun et al. 2009, 2011). Because octocorals are sessile animals, the larval planktonic phase is the only stage in which dispersal may occur, rendering it extremely important for species dispersal and population connectivity (Metaxas & Saunders 2009, Cowen & Sponaugle 2009). Therefore, more studies on the larval biology of deep-sea octocorals are essential to understand their ecology.

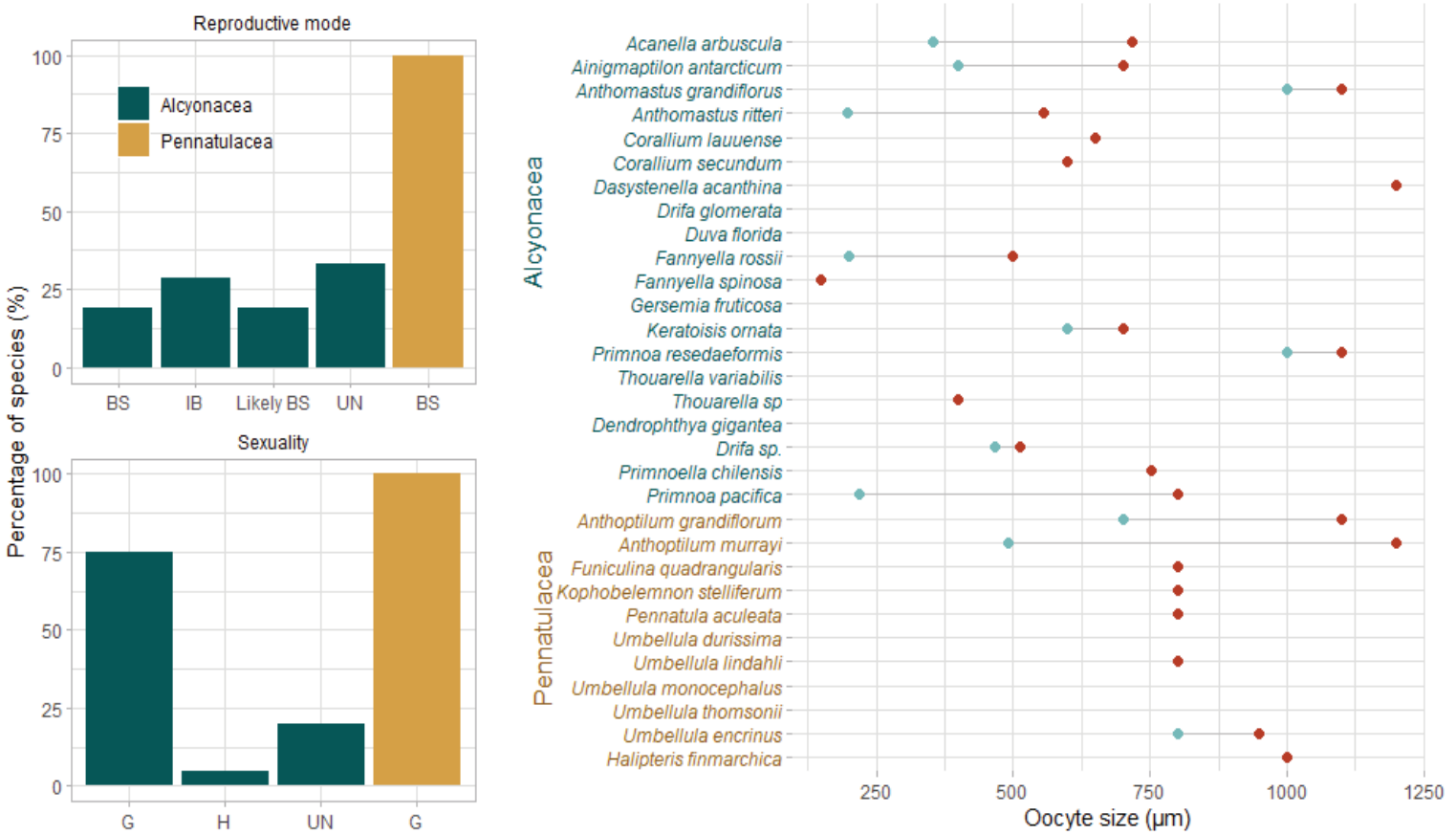

Figure 1.5: Knowledge on the reproductive mode, sexuality and oocyte size of some deep-sea octocorals studied up to date. Reproductive mode: broadcast spawning (BS), internal brooding (IB), likely broadcast spawning (Likely BS), unknown (UN); Sexuality: gonochoristic (G), hermaphroditic (H), unknown (UN). Oocyte size is provided as minimum and maximum size of vitellogenic oocytes, in blue and red respectively. Data compilation from: Baillon et al., 2015; Beazley et al., 2012; Brito et al., 1997; Cordes et al.,2001; Eckelbarger et al., 1998; Edwards & moore, 2009; Hamel et al., 2020; Hwank & Song, 2007; Lawson, 1991; Mercier and Hamel, 2011; Orejas et al., 2002; Orejas et al., 2007; Pires et al, 2009; Rice et al.,1992; Rossin et al., 2017; Sun et al., 2009; 2010; Sun et al.,2011; Tyler et al., 1995; Waller and Baco,2007; Waller et al., 2014.

1.2.3. Building deep-sea communities

Octocorals are distributed over a wide bathymetric range, however, in temperate areas they form dense populations mostly in the mesophotic zone (30-200 m) and the deep-sea (>200m, e.g. Gori et al., 2017). For example, dense octocoral populations can be encountered in the continental shelves of the temperate eastern (Mortensen & Buhl-Mortensen 2006, Sundahl et al. 2020) and western North Atlantic (Watling et al. 2011, Brooke et al. 2017), the Mediterranean Sea (Gori et al. 2017), as well as on several seamounts (Hall-spencer et al. 2007, Braga-Henriques et al. 2013).

The three-dimensional growth pattern and canopies of octocorals provide important habitat for a variety of invertebrates (Bourque & Demopoulos 2018, Rueda et al. 2019). Moreover, communities formed by octocorals have been shown to be important for several fish species (Pham et al. 2015, Mastrototaro et al. 2017, Buhl-Mortensen et al. 2017, D'Onghia 2019). These species do not only provide habitat, but can also shape the local environmental conditions, as ecosystem engineers: their dense aggregations can modify the local flow, retaining particles and increasing food availability within their canopies (Guizien & Ghisalberti 2017). They also have an essential role in the carbon cycle and benthopelagic coupling (Gili et al. 2006, Rossi et al. 2017b, Coppari et al. 2019). Since they feed on plankton and detritus, they capture carbon and other elements from the water column - a highly dynamic environment with high turnover, and incorporate it in the benthic community which is much more stable (Rossi et al. 2017b). Being long-lived organisms, they have the capacity to store energy and biomass for decades, centuries or even millennia. As a result, they constitute important paleoclimate archives, similarly to trees (McMahon et al. 2018, Williams 2020).

Communities formed by octocorals, often together with Antipatharia (black corals) and Stylasteridae (lace corals) are generally designated as "coral gardens" (OSPAR 2010). Because of their importance and life history traits (e.g. high longevity, slow growth, low reproductive output) which make them vulnerable to disturbance, coral gardens have been classified as Vulnerable Marine Ecosystems (VMEs) (FAO 2009) in need of protection under the OSPAR convention

(OSPAR 2010). Despite the high ecological importance of deep-sea octocoral species, our knowledge on their biology and ecology is still limited (Watling et al. 2011). This is primarily due to their remoteness, as access to the deep-sea involves complicated and expensive logistics. In addition, increasing demand for deep-sea resources such as fishing stocks, oil and gas, as well as rare metals, require urgent development of policies and management plans (Miller et al. 2018, Lodge & Verlaan 2018, Levin et al. 2020, Howell et al. 2021). Hence, priority is often given to studies on higher ecological levels and the use of big datasets which provide very important information but very often lack the necessary sensitivity to create knowledge on lower scales.

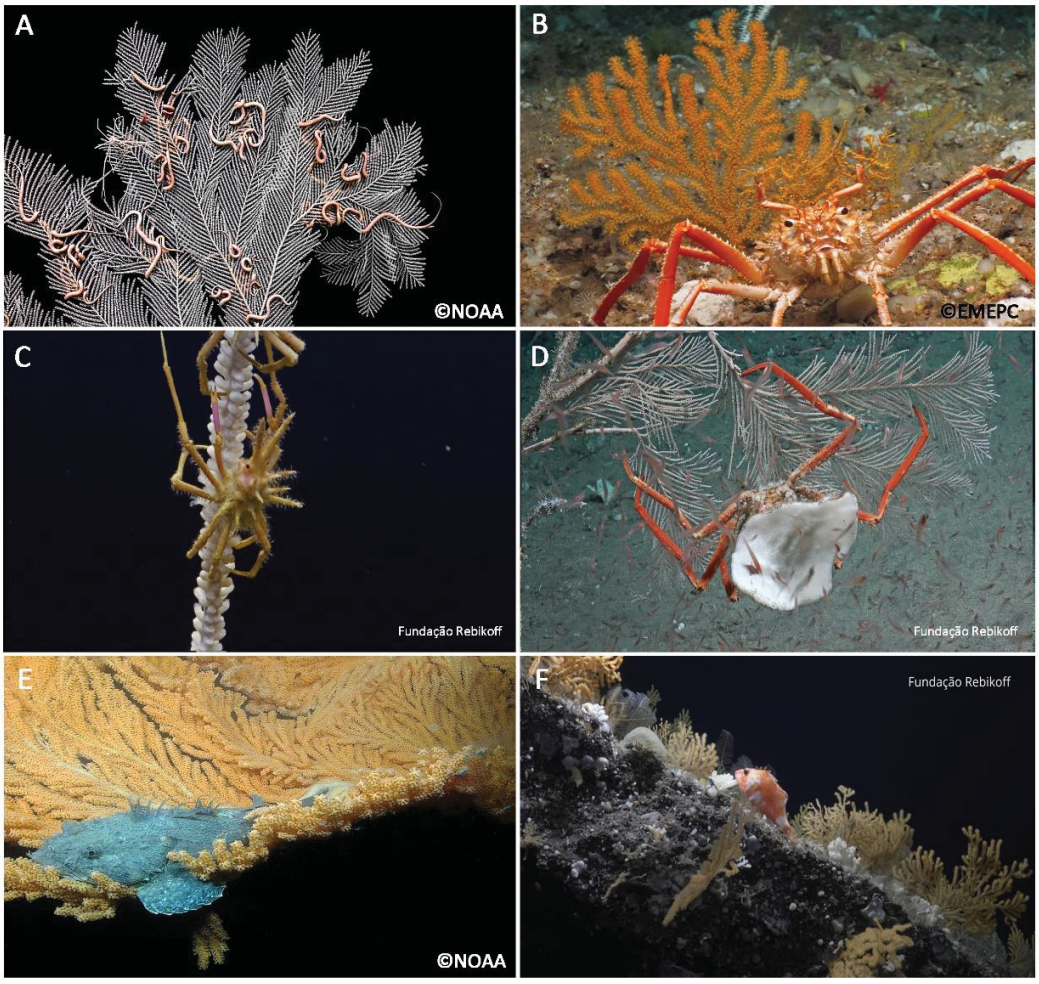

Figure 1.6: Deep-sea octocorals create important habitat for other invertebrate (A-D) and fish species (E-F). Image sources: A, E: NOAA Office of Ocean Exploration and Research; B: ©ROV LUSO/EMEPC, 2018, Expedição Oceano Azul organizada pela Fundação Oceano Azul e parceiros; C, D,F: Foundação Rebikoff-Niggeler.

1.3. Deep-sea octocorals in a changing world

Since the preindustrial era, anthropogenic CO_2 emissions have increased dramatically, raising the concentration of CO_2 in the atmosphere and causing an intensification of the greenhouse effect (Gattuso et al. 2015, Hansen & Stone 2015). The ocean is a key player in driving and regulating climate, constituting one of the most important sinks of heat and CO_2 (Sabine et al. 2004, Purkey & Johnson 2010, Gruber et al. 2019). It is estimated that during the last decades, the ocean has absorbed approximately 90% of the excess heat and 30% of the emitted CO_2 (IPCC 2019), which has caused changes in its biogeochemical properties (Figure 1.7). Ocean warming is already detected in observations (Purkey & Johnson 2010) and based on climate models, it is expected to spread to larger depths and ocean basins through ocean circulation until the end of the century (Sweetman et al. 2017, IPCC 2019). Excess CO_2 absorption has led to a decrease in water pH, a phenomenon known as ocean acidification (OA). Seawater carbonate chemistry is well constrained, so increasing CO_2 in the water will cause predictable changes in the availability of carbonate and bicarbonate ions that are necessary for calcification (Gattuso & Hansson 2011, Doney et al. 2012). Superficial waters rich in CO_2 are subducted to larger depths, already causing acidification of intermediate layers above 500 m (Byrne et al. 2010), a phenomenon predicted to reach deeper water layers by 2100 (Gehlen et al. 2014, Sweetman et al. 2017, Perez et al. 2018). At the same time, warming is expected to cause a decrease in oxygen concentration and enhance stratification, bringing major shifts in circulation patterns, expanding the existing zones of low oxygen (oxygen minimum zones) and causing widespread deoxygenation (Levin & Le Bris 2015, Oschlies et al. 2018). Enhanced stratification will also restrict nutrient input to surface waters with concomitant decreases in surface production and export of organic carbon to the deep-sea, depriving deep-sea ecosystems from food supply (Yool et al. 2013, Levin & Le Bris 2015).

These shifts are expected to pose substantial challenges to the physiology of deep-sea species. Temperature is a key factor for the physiology of marine invertebrates, which do not possess thermal homeostasis (Whiteley & Mackenzie 2016), and warming can interfere with physiological functioning at the molecular, cellular and organismal level (Pörtner 2002). Ocean acidification is

extremely challenging for calcifying organisms, as it alters carbon chemistry and especially the availability of $CaCO_3$ for calcification and skeleton formation (Fabry et al. 2008, Hofmann et al. 2010, Gattuso & Hansson 2011). Moreover, hypercapnia, the excess CO_2 concentration, may disrupt osmotic regulation and suppress metabolism (Pörtner 2008). Although our knowledge on the potential impacts of climate stressors on the physiology of marine invertebrates is increasing (e.g. Clements & Darrow 2018, Melzner et al. 2020), much less is known about the combined effects of multiple stressors (Figure 1.7).

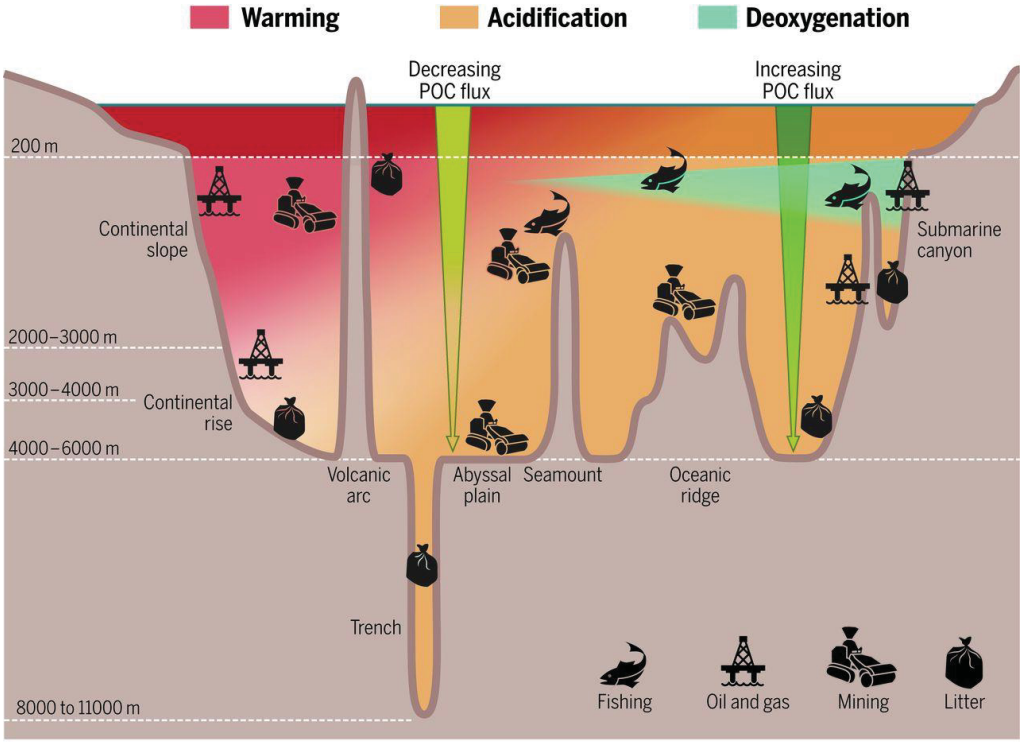

Figure 1.7: Climate change and other anthropogenic stressors in the deep-sea. The figure displays the effect of multiple anthropogenic activities and CO_2-induced changes in temperature, pH, oxygen and particulate organic carbon (POC) in the deep-sea. Source: (Levin & Le Bris 2015)

In general, organisms cope with physiological stress by using one of three strategies: avoidance, conformity and regulation (Wilmer et al. 2004, Sokolova et al. 2012). Organism responses depend on the stress intensity and duration, the availability of resources, as well as species phylogeny (Wilmer et al. 2004). Avoidance is not possible for sessile species, which do not have the capacity

to move to avoid adverse conditions. Conformity, which is the function of an organism under non-optimum conditions, can only be sustained under low level of stress or short-term perturbations (Guderley & Pörtner 2010, Sokolova et al. 2012, Sokolova 2013). Lastly, regulation is an energetically demanding process that may have negative consequences on organism performance (Pörtner 2002, Sokolova et al. 2012) and may not be sustainable in the long term. Many deep-sea calcifying invertebrates are sessile, have a low metabolism and a relatively weak ability to regulate intracellular pH when compared to vertebrates (Sokolova et al. 2012). Thus, increased concern has been raised on the capacity of these deep-sea organisms to withstand the multiple stressors brought by climate change and other anthropogenic activities, such as fisheries, oil and gas extraction, and prospective deep-sea mining (Levin et al. 2019b, 2020).

The paleoclimatic history of the Earth encompasses several climatic shifts, with dramatic impacts on biodiversity and evolution of current taxa both in the shallow (Svenning et al. 2015) and deep-sea (Yasuhara et al. 2008). Several periods of ocean warming and acidification have been linked with mass species extinctions and decrease in marine calcifiers, widely known as reef crises (Veron 2008, Kiessling & Simpson 2011). Reef crises developed especially in periods when ocean chemistry was characterized by a low Mg/Ca ratio, which favors the precipitation of calcite instead of aragonite. The diversity of skeletal formation strategies of octocorals, in combination with the niche availability brought by the extinction of aragonite calcifiers, especially reef-building scleractinian corals, have allowed them to persist and diversify during these periods (Quattrini et al. 2020, Conci et al. 2021). Moreover, octocoral species appear to increase in present reefs where scleractinian corals are declining due to environmental stressors such as warming, acidification and storms (Inoue et al. 2013, Lenz et al. 2015, Tsounis & Edmunds 2017, Lasker et al. 2020). This has been partly attributed to the high plasticity and diversity of Octocorallia, which display different forms of feeding, growth and reproduction that allow them to occupy a wide variety of niches and respond differently to stress (Lasker et al. 2020, Conci et al. 2021). The internal position of the octocoral skeleton and sclerites has been also suggested as a potential advantage, as tissue protects them from dissolution under conditions of low pH (Gabay et al. 2013). However, the actual factors that have constituted members of the subclass

Octocorallia more resilient to environmental change, both in past and current conditions, are still unclear due to our relatively limited knowledge on octocoral biology.

Octocorals and their physiological responses to climate change stressors have drawn less attention compared to scleractinian corals, and current information is mostly restricted to shallow and mesophotic species (Linares & Doak 2010, Pivotto et al. 2015, Gómez et al. 2015). As in all taxa, physiological responses to stressors are variable and species specific (Kroeker et al. 2010). In tropical regions, many octocorals have been found to be resilient to changes of temperature (Lopes et al. 2018, Pelosi et al. 2021) and pH (Gabay et al. 2013, Gómez et al. 2015) projected for 2100. On the other hand, other species appear to be more vulnerable to OA and warming (Tracy et al. 2020). In the Mediterranean, a large number of studies have reported extended mortality in response to acute warming events (Linares et al. 2008b, Crisci et al. 2017), while OA has been shown to have negative effects on calcification of the species *Corallium rubrum* (Bramanti et al. 2013, Cerrano et al. 2013).

In the deep-sea, experimental studies have focused largely on reef-building coral species and the impacts of OA on calcification. This is because many of these species thrive close to the aragonite saturation horizon, and based on current projections they will be largely exposed to undersaturated waters, posing a major challenge to their ability to calcify (Perez et al. 2018). Although many deep-sea scleractinian species have the capacity to maintain skeletal growth under acidified conditions by upregulating the pH at the site of calcification (McCulloch et al. 2012, Wall et al. 2015), this capacity protects only the skeleton that is covered by tissue. Bare skeleton that is very frequently encountered at the colony base beneath living polyps, is thus exposed to potential dissolution (Hennige et al. 2015, 2020, Murray Roberts et al. 2016). In the case of octocorals, studies on *C. rubrum* revealed that the species has a low capacity for pH regulation at the site of calcification (Le Goff et al. 2017), but its tissue protects all skeletal elements against the effects of OA (Gabay et al. 2014). Nevertheless, the effects of OA on octocoral calcification have only been studied on the species *C. rubrum*, and it is unknown if other octocorals possess similar mechanisms. Similarly, studies on the potential effects of other climate

change stressors on deep-sea octocorals are extremely limited (Gómez 2018, Rossin et al. 2019, Morato et al. 2020c).

1.4. Deep-sea octocorals in the Azores and their fate upon climate change

The current thesis was developed in the Azores, an Archipelago located above the Mid-Atlantic ridge (Figure 1.8). Because of its position, the archipelago is characterized by rich topography and a remarkable diversity of deep-sea ecosystems (Morato et al. 2020a). Deep-sea octocorals are the most common deep-sea corals encountered regionally (Braga-Henriques et al. 2013), counting a total of 101 octocoral species (Sampaio et al. 2019). Moreover, coral gardens are among the most prominent deep-sea communities on island slopes and seamounts (Tempera et al. 2012, Morato et al. 2020b), hosting a rich diversity of fish and invertebrate species (Pham et al. 2015, Carreiro-Silva et al. 2017, Gomes-Pereira et al. 2017).

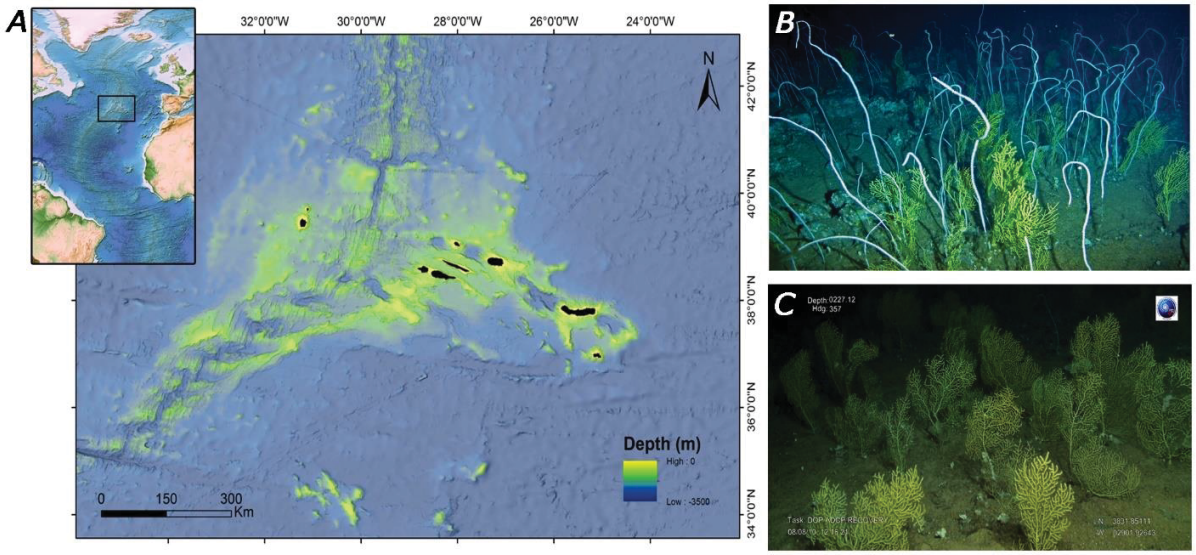

Figure 1.8: Map of the Azores Archipelago (A), and examples of octocoral populations of the two target species included in the current dissertation, *Dentomuricea* aff. *meteor* and *Viminella flagellum*: (B) mixed population formed by the two species. © Gavin Newman, Greenpeace (C) dense population of *D.* aff. *meteor.* ©EMEPC.

Deep-sea octocorals in the Azores are frequently landed as bycatch of long-line fisheries (Sampaio et al. 2012). Among the species that are frequently caught as bycatch, are the whip coral *Viminella flagellum* (Johnson 1863) and the fan-shaped gorgonian *Dentomuricea* aff. *meteor* Grasshoff 1977. Both species form dense coral gardens on seamounts of the Archipelago (Tempera et al. 2012) and are frequently encountered together in mixed populations (Figure 1.8), constituting ideal candidates for the focus of the current thesis.

These octocoral species, similarly to other deep-sea organisms, are expected to face several challenges due to climate change. Projections for 2100 (scenario RCP8.5) highlight that acidification and a decrease in organic carbon will be the critical changes that the local deep-sea ecosystems will have to cope with (Puerta et al. 2020). More specifically, pH may decrease up to 0.3, causing major decreases in the aragonite saturation state in some areas, while POC input to the seafloor is expected to decrease by more than 50% (Puerta et al. 2020). Based on these projections, habitat modelling approaches foresee a decrease in the available habitat for some deep-sea octocoral species (Morato et al. 2020c). However, the actual physiological impacts of climate change stressors on deep-sea octocorals and the potential fate of the local coral garden communities are so far unknown.

1.5. Objectives and thesis outline

The main aim of this thesis is to assess the potential impact of climate change on deep-sea octocoral species in the Azores. Due to the extended lack of knowledge on these organisms, the first research objective is to develop a strong knowledge background on three fundamental processes throughout the life history of the target species: reproduction (Chapter 2), early life development (Chapter 3), and metabolism (Chapter 4). The produced knowledge background is subsequently used in combination with experimental studies (Chapter 5), to assess the vulnerability of the two target species to climate change (Chapter 6).

More specifically:

Chapter 2 consists of a comprehensive study of the reproductive biology of the two target species. Based on histological analysis of specimens that were collected as fisheries by-catch, it firstly unveils their main reproductive characteristics, including sexuality and reproductive mode. Subsequently, their gametogenic cycles are described in detail, providing further insights to their reproductive seasonality.

Chapter 3 includes two sections that focus on the embryo and larval biology of the two target species. Here, the embryo and larval development as well as swimming behaviour of the two species are described, based on observations of live coral larvae that were obtained after successful fertilization in aquaria. Up to date, these are the first detailed descriptions of early life stages in deep-sea spawning octocorals, providing important contributions to our current knowledge on deep-sea octocorals. Moreover, in the case of *D.* aff. *meteor*, a higher quantity of obtained larvae allowed the further assessment of the effects of natural temperature variability on embryo development, larval survival, larval duration and behaviour. Throughout the two sections, differences in larval characteristics between the two species are revealed, providing useful insights to their dispersal capacity.

Chapter 4 focuses on the feeding biology and metabolism of the two target species. By using an experimental approach with isotopically labelled food prey, this study provides insights to the ability of the two species to utilize different food sources. The results showcase the importance of zooplankton for deep-sea octocorlas, and highlight differences in the metabolic strategies between the two target species, with relevant implications for their ecology.

Chapter 5 contains an experimental study on the potential impacts of climate change to the physiology of *V. flagellum*. The study is based on aquaria experiments which simulated ocean acidification and food availability scenarios. Combined with the utilization of isotopically labelled prey, this approach allowed to determine the metabolic response of the species under the experimental conditions, constituting the first experimental study on the impacts of multiple climate stressors on a deep-sea octocoral.

Lastly, in chapter 6 the generated knowledge is utilized to discuss the differential capacity of deep-sea octocoral species to cope with climate change stressors. The two target species are used as a case study to highlight how in-depth biological knowledge can be used to reach a more profound and holistic understanding of climate change impacts on deep-sea corals and ecosystems.

The chapters of the current thesis were based on the following manuscripts:

Chapter 2
Rakka M, Sampaio Í, Colaço A, Carreiro-Silva M. 2021. Reproductive biology of two deep-sea octocorals in the Azores Archipelago. Deep Sea Research Part I: Oceanographic Research Papers 175:103587.
DOI: 10.1016/J.DSR.2021.103587.

Chapter 3
Section 3.1
Rakka M., Godinho A., Orejas C., Carreiro-Silva M. 2022. Embryo and larval biology of the deep-sea octocoral *Dentomurice*a aff. *meteor* under different temperature regimes. Peer J , 9, e11604
DOI: 10.7717/peerj.11604

Section 3.2
Rakka M., Bilan M., Godinho A., Carreiro-Silva M. Larval biology of the deep-sea octocoral *Viminella flagellum.* In preparation

Chapter 4
Rakka M, Maier SR, Van Oevelen D, Godinho A, Bilan M, Orejas C, Carreiro-Silva M. 2021. Contrasting metabolic strategies of two co-occurring deep-sea octocorals. Scientific Reports 11:10633.
DOI: 10.1038/s41598-021-90134-5.

Chapter 5
Rakka M, van Oevelen D, Maier S, Puerta P, Godinho A, Bilan M, Martins I, Orejas C, Hennige S, Wolff G, Carreiro-Silva M. Metabolism of the deep-sea octocoral *Viminella flagellum* under acidification and variable food availability. In preparation

Chapter 2
Reproductive biology of two deep-sea octocorals in the Azores Archipelago[1]

2.1. Abstract

Octocorals are prominent habitat builders in deep-sea ecosystems. The octocorals *Dentomuricea* aff. *meteor* and *Viminella flagellum* are common deep-sea octocoral species in the Azores Archipelago, where they form dense, structurally complex and diverse communities between 150 and 600 meters of depth. The objective of this study was to determine the reproductive biology of the studied species, including basic reproductive traits, gametogenic cycle and reproductive timing. Specimens were collected during 2010 and 2011 as by-catch from deep-sea long-line fisheries and scientific cruises and were histologically processed. Both species were found to be gonochoric and most likely broadcast spawners. Gamete presence was observed throughout the study period, indicating continuous or quasi-continuous gametogenesis. In some sampling sites gametogenic peaks were found in October for *D.* aff*. meteor* and May for *V. flagellum*, however oocyte size distributions and fecundity did not display any marked seasonality throughout the region. Reproductive knowledge is rarely available for deep-sea octocorals, despite its fundamental importance, and relevance in management and conservation.

2.2. Introduction

The Azores Archipelago (North Atlantic) hosts a remarkable diversity of octocorals (Sampaio et al. 2019), which form dense aggregations in seamounts and island slopes at depths ranging from 200 to more than 1500 m, together with Antipatharia (black corals), and Stylasteridae (lace corals) (Braga-Henriques et al. 2013). Octocoral populations provide complex three-dimensional structural habitats comparable in form and function to terrestrial forests, leading to their

[1] Rakka M, Sampaio Í, Colaço A, Carreiro-Silva M (2021) Reproductive biology of two deep-sea octocorals in the Azores Archipelago. Deep Sea Res Part I Oceanogr Res Pap 175:103587.

inclusion in the concept of “marine animal forests” (Rossi et al. 2017a). These communities support high levels of biodiversity by providing refuge, nursery and feeding opportunities for associated fauna (Pham et al. 2015, Guizien & Ghisalberti 2017, Carreiro-Silva et al. 2017, Gomes-Pereira et al. 2017).

Deep-sea octocorals face a number of threats from commercial bottom fisheries, oil and gas extraction, bioprospecting and the potential development of deep-sea mining, as well as global ocean change (Van Dover et al. 2017, Morato et al. 2020c). Many octocoral species are slow growing and long-lived (e.g. Sherwood et al. 2005, Sherwood & Edinger 2009), with low recruitment success (Girard et al. 2016). This makes them extremely vulnerable to disturbance, with recovery times potentially requiring decades to centuries (Bennecke et al. 2016, Girard et al. 2019). Concerns raised on the impacts of fishing activities and long recovery times of these animals have resulted in the consideration of octocoral gardens as Vulnerable Marine Ecosystems (VMEs) (FAO 2009) and as priority habitats in need of protection (OSPAR 2010). However, knowledge on the basic biology and ecology of deep-sea octocorals is still limited, restricting our ability to effectively manage and protect them (Watling et al. 2011, Wangensteen et al. 2016).

Knowledge on reproduction, one of the most fundamental biological processes, is essential to understand how coral populations can be maintained and replenished, as well as to allow the assessment and mitigation of potential impacts of human activities on deep-sea ecosystems. Sexuality, reproductive mode and reproductive seasonality are essential characteristics of a species reproductive biology, with sexuality referring to the degree and manner of sexual allocation within the colony (Kerr et al. 2010) and reproductive mode describing the way mating occurs. Most studies on deep-water octocoral species to date revealed them to be gonochoric (Orejas et al. 2007, Waller et al. 2014, Rossin et al. 2017), with only one hermaphroditic species reported so far (*Drifa* sp.; Sun et al. 2010). Two main modes of reproduction have been described for deep-water octocorals: broadcast spawning (e.g. Baillon et al. 2013) and internal brooding (Cordes et al. 2001, Sun et al. 2011). Broadcast spawning includes the release of gametes in the water column where fertilization occurs externally, while internal brooding involves fertilization

and embryogenesis within the polyp (Kahng et al. 2011). In deep-sea corals, gamete presence is often continuous and gametes of different developmental stages might be present in a single mesentery or polyp (Cordes et al. 2001, Rossin et al. 2017), which may indicate continuous gametogenesis and aperiodic or overlapping gametogenic cycles (Orejas et al. 2007).

Despite increasing efforts to study the reproductive biology of deep-sea corals, most studies focus on basic reproductive characteristics, while more in-depth information on the reproductive periodicity and demographic features of cold-water coral populations is scarcer (Rossin et al. 2017). This is mostly due to the remoteness of these communities and the complicated logistics associated to the repetitive sampling required to study temporal reproductive patterns (Pires et al. 2009). In this study we focused on the reproduction of two important habitat-forming alcyonaceans in the Azores, *Dentomuricea* aff. *meteor* Grasshoff 1977 and *Viminella flagellum* (Johnson 1863). The two species form dense populations between 150 and 600 meters (Braga-Henriques et al. 2013), often co-occurring in the same community (e.g. Condor Seamount, (Tempera et al. 2012) and are frequently captured as by-catch during deep-water longline fishing operations (Sampaio et al. 2012). By establishing a collaboration with local fishermen and fisheries observers, we had the opportunity to collect samples caught as fisheries bycatch, covering a wide geographic and temporal scale. The objectives of the study were to describe the species: (1) basic reproductive traits i.e. sexuality and reproductive mode, (2) gametogenic cycle and (3) reproductive seasonality. Such knowledge is extremely useful to evaluate the population status of marine habitat formers and create a strong background for impact assessment and ecosystem management (Wangensteen et al. 2016).

2.3. Materials and Methods

2.3.1. Target species

The octocoral *Dentomuricea* aff. *meteor* Grasshoff 1977 is a branching species of the family Plexauridae, with distribution restricted to seamounts of the North Mid-Atlantic ridge. It is commonly encountered between 200-400 m (Braga-Henriques et al. 2013) where it forms dense

coral gardens, often in conjunction with other octocoral species (Figure 2.1A). Its polyps are small (Figure 2.1B) in comparison to *Viminella flagellum*, and are densely distributed around a thin skeletal axis (Figure 2.1B).

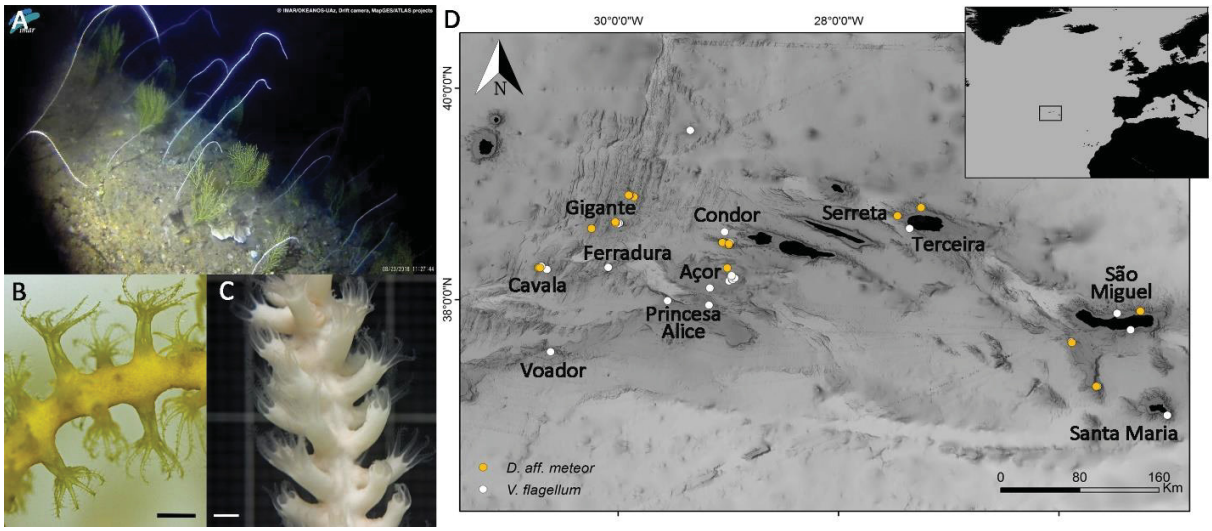

Figure 2.1: Target species and sampling. (A) Mixed coral garden of the fan shaped octocoral *Dentomuricea* aff. *meteor* and the whip coral *Viminella flagellum*; (B) Polyp close-up of the species *D.* aff. *meteor* (Scale bar: 1mm); (C) Polyp close-up of the species *V. flagellum* (Scale bar: 1mm) and (D) Sampling locations for the two target octocoral species in the Azores region.

The species *V. flagellum* (Johnson 1863) is common in the east and central North Atlantic and the Mediterranean Sea (Carpine & Grasshoff 1975, Brito & Ocaña 2004, Giusti et al. 2012). In the Azores, it is commonly encountered between 120-500 m and it frequently forms communities with branching octocoral species such as *D. aff. meteor* (Tempera et al. 2012). It is a whip coral of the family Ellisellidae that creates mostly monopodial colonies with relatively bigger polyps than *D. aff. meteor* (Figure 2.1C), arranged in two sides of a thick skeletal axis (Figure 2.1C).

2.3.2. Sampling

Specimens of *D.* aff. *meteor* and *V. flagellum* were obtained between 2010-2011, as by-catch from commercial deep-sea bottom longline fisheries and a few scientific expeditions in the Azores. A total of 66 specimens of *Dentomuricea aff. meteor* and 42 specimens of *Viminella*

flagellum were collected (Table 2.1). Most specimens (90%) were collected at depths between 150-500 meters and covered 11 sites (Figure 2.1D, Table 2.1). Specimens were mainly caught in active fishing grounds throughout the Azores Archipelago, herein referred to as sampling sites (Figure 2.1D). Due to the low number of vessels involved in the sampling scheme, it was not always possible to obtain specimens from more than one sampling site in each month, leading to a sampling design containing sites nested in time in most cases (Table 2.1). Upon collection, specimens were photographed and branchlets of 5-10 cm length were dissected and fixed either in 10% seawater formalin or preserved directly in 70% ethanol without prior fixation.

2.3.3. Histological processing

Three to five polyps were dissected from each specimen. After careful removal of the skeletal axis, polyps were decalcified in a solution of 10% formic acid. Decalcification time varied between samples, being usually completed within 20-40 minutes. Subsequently, a standard histological procedure was used, consisting of dehydration by subsequent immersions in ethanol solutions (70%, 80% for 30 minutes, 90%, 95% for 15 minutes and 100% for 60 minutes), followed by clearing in xylene for 20 minutes and infiltration in paraffin (Merck Histosec, 56-58^{o}C) at 58 °C for one hour. Dehydration and clearing were performed under a vacuum hood. Samples were embedded in paraffin blocks which were then sectioned in serial 5 µm longitudinal sections using a Leica 2035 microtome. Sections were subsequently stained using a standard Hematoxylin-Eosin protocol.

Table 2.1: Collected specimens of the target octocoral species: *Dentomuricea* aff. *meteor* and *Viminella flagellum* in the Azores region. Information includes collection year, month and location, number of encountered specimens for each of them (N_{yml}) and total number of specimens for each month (N_m). Depth is approximate.

Species	Year	Month	Location	Depth (m)	Specimens (N_{yml})	Total N_m
Dentomuricea aff. meteor	2010	May	Condor seamount	157	2	2
		June	Terceira island slope	304	1	1
		July	Ferradura Seamount	251	3	3
		September	Condor seamount	228	13	15
			Gigante seamount	397-415	1	
			Ferradura Seamount	280	1	
		October	Gigante seamount	208-324	2	2
	2011	February	Gigante seamount	280-287	4	4
		May	São Miguel island slope	369	1	5
			Serreta ridge	350	4	
		June	Serreta ridge	350	6	6
		July	Serreta ridge	350	6	6
		August	Serreta ridge	360	1	1
		September	Condor seamount	180	8	9
			Serreta ridge	351	1	
		October	Condor seamount	237	12	12
Viminella flagellum	2010	April	Condor seamount	215	1	1
		May	Mar da Prata seamount	383	1	2
			Santa Maria island slope	211	1	
		June	Açores seamount	165-188	3	5
			Princesa Alice Seamount	256-348	2	
		July	Ferradura Seamount	289	2	3
			Voador seamount	400	1	
		August	Condor seamount	230	1	4
			Açores seamount	480	3	
		September	Ferradura seamount	420	1	1
		October	Gigante seamount	208-324	1	3
			Voador seamount	560-570	1	
			Açores seamount	187-269	1	

Species	Year	Month	Location	Depth (m)	Specimens (N_{yml})	Total N_m
	2011	February	Gigante seamount	280-287	3	4
			Princesa Alice seamount	256-348	1	
		May	Mar da Prata	200	3	6
			Terceira island slope	430	2	
			São Miguel island slope	160	1	
		July	Açores seamount	283	1	2
			Cavala seamount	424	1	
		September	Condor seamount	198	1	2
			Açores seamount	180	1	
		October	Condor seamount	208	3	3

In order to achieve better results with specimens that were stored directly in 70% ethanol without prior fixation, the following procedure was followed before processing: dissected polyps were firstly rehydrated, using serial submersions in ethanol solutions of decreasing concentration (45%, 30% and 15%) for 30, 45 and 60 minutes respectively, followed by submersion in distilled water for 2 hours. After rehydration, specimens were transferred into 10% formalin for at least 12 hours. A posteriori submersion in formalin solution caused hardening of the tissues which facilitated the histological process. A preliminary study was performed to confirm that rehydration of the tissues did not cause overly increase of oocyte size, by comparing gametes obtained with this protocol with oocytes of the same specimen that were fixed in formalin immediately after specimen collection (Figure S 0.1, Table S 0.1). Histological slides were photographed with a Leica DFC420 camera attached to a LEICA DM6000 microscope (x10).

2.3.4. Image processing

Images were analysed using the freeware software Image J (Schneider et al. 2012) to locate gametes, describe the reproductive tissues and measure gamete area to subsequently calculate feret diameter, i.e. gamete diameter assuming gametes were perfect circles (Waller et al. 2002, Fountain et al. 2019). Size was measured as close as possible to the gamete center, which was

located by comparing sequential sections of each gamete. To describe reproductive tissues, whenever needed, histological slides were observed directly at higher magnifications (x20, x40). Collected information on gamete presence for each specimen was firstly used to define the reproductive mode and sexuality of the two target species and subsequently each specimen was classified as female, male or infertile. To describe the reproductive tissues and gametogenic cycle, we used anatomical and morphological terms provided by Bayer et al. (1983) and adapted previous terms on octocoral gametogenesis (Mercier et al. 2011, Baillon et al. 2013). All collected data are available at the platform PANGAEA: https://doi.org/10.1594/PANGAEA.925801.

To analyse polyp fecundity in female specimens, because of the existence of multiple oocyte cohorts within one polyp, we adapted the term Effective Relative Fecundity (ERF), used by Mercier & Hamel (2011), referring to the total number of late vitellogenic oocytes per polyp and also considered the total number of immature oocytes per polyp (stages 1-3), referred herein as Immature Oocyte Fecundity (IOF).

2.3.5. Statistical analysis

Preliminary graphical exploration (Zuur et al. 2010) was conducted with all collected data to determine the characteristics of the dataset. Because of the low number of specimens and included gametes, data were pooled together across sampling depths and sampling sites after confirming that these factors did not have any significant effects on the dependent variables. Although not used in the analysis, sampling site was retained as a factor in visualizations of oocyte size and fecundity, to aid data interpretation. Chi-square tests were used to compare sex ratios within each gorgonian species. Generalized Linear models (GLMs) were employed to analyse the rest of the variables, as a function of month (categorical variable) and year (categorical variable). In the case of fecundity (IOF and ERF) a GLM with a Poisson distribution was utilized, typically used for count data (Crawley 2007, Zuur et al. 2009a). Lastly, Anderson Darling k-sample tests were used to compare oocyte size distributions among colonies and test for synchronicity among colonies collected in the same sampling month.

In the case of GLMs, independent variables were gradually added in the models and the Akaike Information Criterion (AIC) along with Likelihood Ratio Tests (LRT) were used to test the effects of each variable on the constructed models and dependent variables in question (Crawley 2007). Model diagnostics of all created models were inspected to confirm there were no violations of the modelling assumptions. Statistical analysis was performed with the software R (R Development Core Team, 2015, http://www.R-project.org).

2.4. Results

2.4.1. Reproductive traits

Both species were gonochoric at the polyp and colony level. We found 27 female and 14 male colonies of *Dentomuricea* aff. *meteor*, as well as 16 female and 5 male colonies of *Viminella flagellum*. A total of 25 colonies of *D.* aff. *meteor* and 15 colonies of *V. flagellum* were infertile. Sex ratio was mostly female-skewed and significantly different from a 1:1 ratio, with *D.* aff. *meteor* exhibiting 1.92:1 female to male ratio (Chi square test: X^2=4.12, df=1, p=0.04), and *V. flagellum* presenting a 3.2:1 female to male ratio (Chi square test: X^2=5.76, df=1, p=0.01). There were no morphological differences among colonies of the two sexes thus there was no indication of sexual dimorphism. Lastly, no larvae were encountered, as a result it is very likely that the study species are broadcast spawners.

2.4.2. Gametogenic cycle

The two species displayed gamete presence throughout the duration of the study (Figure 2.2). In fertile colonies, gametes were encountered in all examined polyps except for a few smaller, immature polyps. Gametes of both species were encountered at the ventral side of the polyps, with smaller oocytes being embedded within the mesenterial walls or attached to their ends (Figure 2.3) and larger oocytes being mostly free at the base of the gastrovascular cavity.

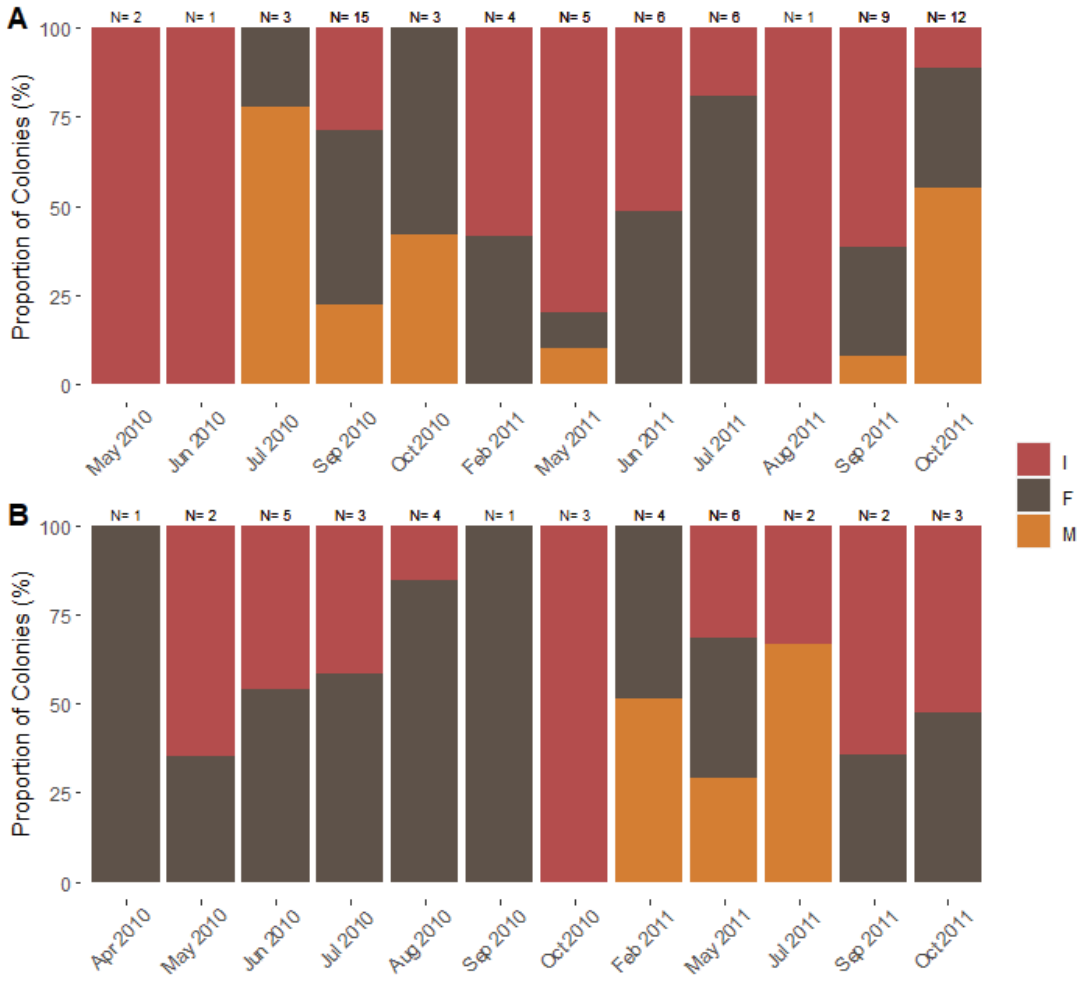

Figure 2.2: Percentage of infertile (I), female (F) and male (M) colonies of the octocorals *Dentomuricea* aff. *meteor* (A) and *Viminella flagellum* (B) during the sampling period (2010-2011). Numbers (N) on top of bars correspond to the total number of available specimens.

Premature oocytes (stage 1) were characterized by basophilic translucent ooplasm and a big nucleus-ooplasm ratio. They were small (Table 2.2) and mostly embedded in the mesenteries, in aggregations with more developed oocytes. Pre-vitellogenic oocytes (stage 2) were slightly larger (Table 2.2) with basophilic translucent ooplasm. Early vitellogenic oocytes (stage 3) were characterized by vacuolated ooplasm and were slightly eosinophilic (Figure 2.3). In *D. aff. meteor*, some of the oocytes on this stage were encountered free in the gastrovascular cavity (Figure 2.3).

Table 2.2: Feret diameter (μm) of oocytes and spermatocysts of the species *Dentomuricea* aff. *meteor* and *Viminella flagellum* throughout gametogenesis. Stages: 1) Premature; 2) pre-vitellogenic; 3) early vitellogenic; 4) late vitellogenic oocyte; and spermatogenesis: 1) early; 2) developing; 3) mature spermatocyst

	Dentomuricea* aff. *meteor		***Viminella flagellum***	
Stage	♀	♂	♀	♂
1	30.38 ± 9.41	81.047 ± 20.55	32.73 ± 9.38	84.34 ± 37.09
2	56.21 ± 15.45	163.67 ± 34.29	65.70 ± 16.65	169.51 ± 23.26
3	90.95 ± 27.65	227.97 ± 30.64	114.47 ± 24.61	220.91 ± 13.92
4	166.74 ± 46.72		234.12 ± 45.89	

Late vitellogenic oocytes (Stage 4, Figure 2.3) had eosinophilic and highly vacuolated ooplasm and were mostly surrounded by a thick follicle cell layer constituted by squamous epithelium. In polyps of the species *D. aff. meteor*, late vitellogenic oocytes were always free in the gastrovascular cavity, whereas in *V. flagellum* they were mostly attached to mesenteries, very often along with oocytes of earlier stages (Figure 2.3). Oocyte size increased gradually but significantly with stage (*D. aff. meteor*: F=418, df=3, p<0.01; *V. flagellum*: F=1059, df=3, p<0.01), with the last transition to the late vitellogenic stage exhibiting a greater increase in size, especially for *V. flagellum* (Table 2.2).

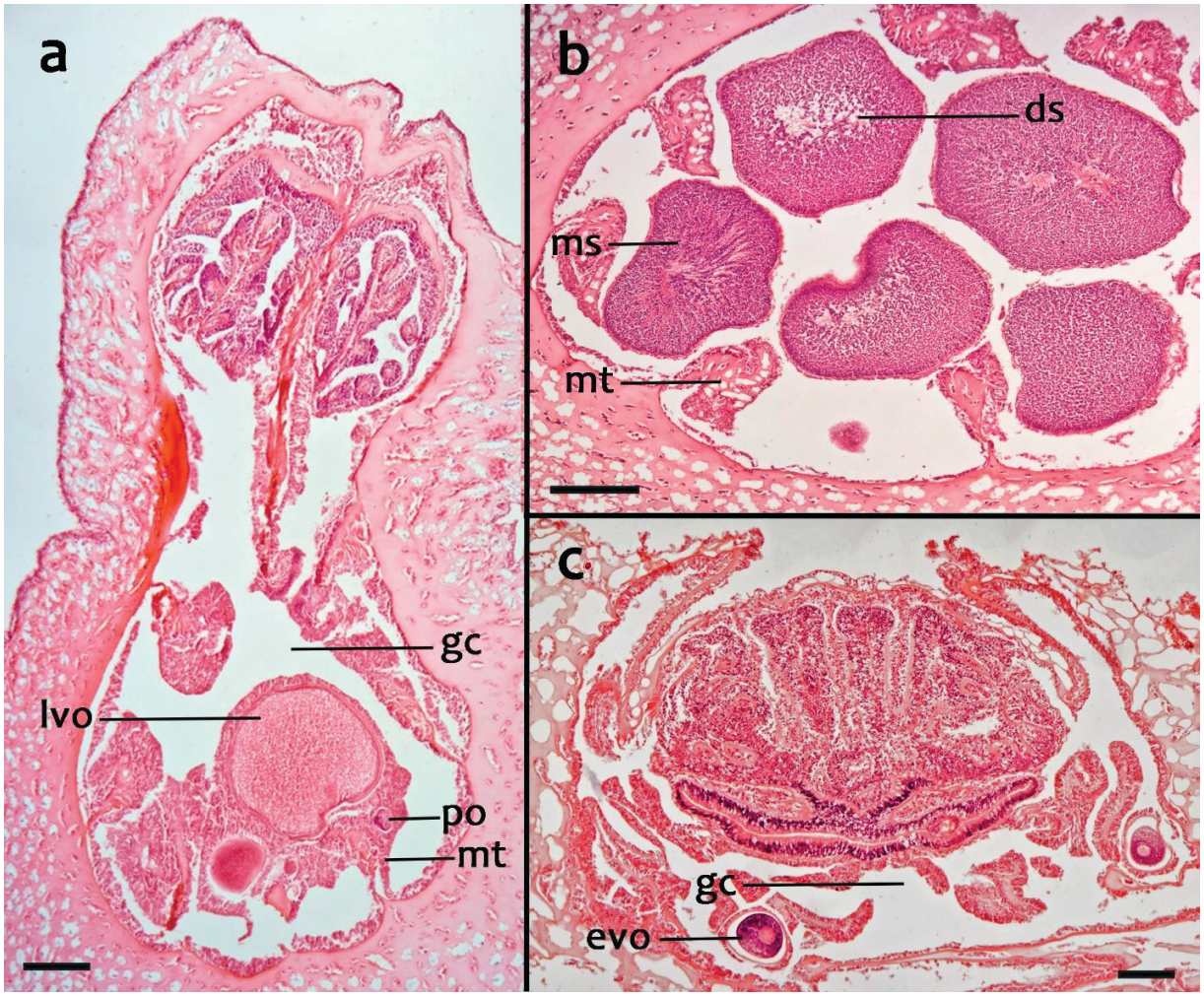

Figure 2.3: Microphotographs of histological sections of fertile polyps of the octocorals *Dentomuricea* aff. *meteor* and *Viminella flagellum*. (a) Fertile female polyp of *V. flagellum*. The gastrovascular cavity (gc) is occupied by a late vitellogenic oocyte (lvo) along with oocytes of earlier stages including a previtellogenic oocyte (po), all attached to mesenteries (mt). (b) Gastrovascular cavity of fertile male polyp of *V. flagellum*, occupied by spermatocysts in various stages. Developing spermatocytes (ds) display a distinct empty lumen while mature spermatocysts (ms) are packed with spermatocytes. (c) Female fertile polyp of D. aff. meteor with early vitellogenic oocytes (evo) occupying the gastrovascular cavity.

Early spermatocysts were small in both species (Table 2.2), eosinophilic, with a few small rounded spermatocytes and early spermatids arranged close to the spermatocyst walls. Developing spermatocysts were larger (Table 2.2), with a distinct and empty lumen (Figure 2.3). Spermatocytes and spermatids were arranged at the spermatocyst periphery, while early spermatozoa with conical shape and tails were developing towards the lumen (Figure 2.3). Only

a few mature spermatocysts were encountered. These were large (Table 2.2) with lumen packed with early and late spermatozoa (Figure 2.3). Spermatocyst size increased significantly with stage for both species (*D. aff. meteor*: F=41.6, df=2, p<0.01*; V. flagellum*: F=91.05, df=2, p<0.01).

Late vitellogenic oocytes of *D.* aff. *meteor* had an average feret diameter of 166.74 ± 46.72 µm while late vitellogenic oocytes of *V. flagellum* were significantly larger (F=110, df=1, p<0.001) with an average feret diameter of 227.97 ± 42.61 µm. Late spermatocysts did not display any significant difference (F=170.7, df=1, p=0.6) with average sizes of 220.91 ± 13.92 µm and 250.53 ± 16.49 µm respectively.

2.4.3. Reproductive timing and seasonality

Oocyte size distributions were either unimodal or bimodal, for both species (Figure 2.4), depending on the existence of late vitellogenic oocytes in the polyps and suggesting the possible development of two cohorts within the same polyp: one cohort consisting of smaller immature oocytes (stages 1-3) and a second mainly consisting of larger, late-vitellogenic (stage 4) oocytes. In some cases only the earliest cohort was present, e.g. specimens of *D.* aff. *meteor* did not bear a second cohort in February and June 2011 and specimens of *V. flagellum* did not have a second cohort in September 2011 (Figure 2.4). During the rest of the sampling months, both species had a second cohort with at least a few late-vitellogenic oocytes. In some cases, peaks in oocyte size were recorded in both years. For *D.* aff*. meteor*, this occurred in September and October, although in the October each year contained specimens from a different site (Figure 2.4). For *V. flagellum*, peaks in May were recorded in both years (Figure 2.4), with the peak in 2011 including samples from more than one site (Figure 2.4). Analysis of oocyte size frequencies of different specimens for the same sampling month was only feasible for specimens of *D.* aff. *meteor* collected in September 2010, June and July 2011, for which more than three female fertile colonies where encountered. In June 2011, all colonies had similar oocyte size distributions, which was also confirmed by the Anderson-Darling test (N=3, AD=2.79, p=0.17). In September 2010 and July 2011 oocyte size distributions were not similar across colonies (N=6, AD=71.02, $p=1.7 \times 10^{-5}$ for September 2010, N=5 AD= 9.28, p= 0.005 for July 2011) but this was due to one

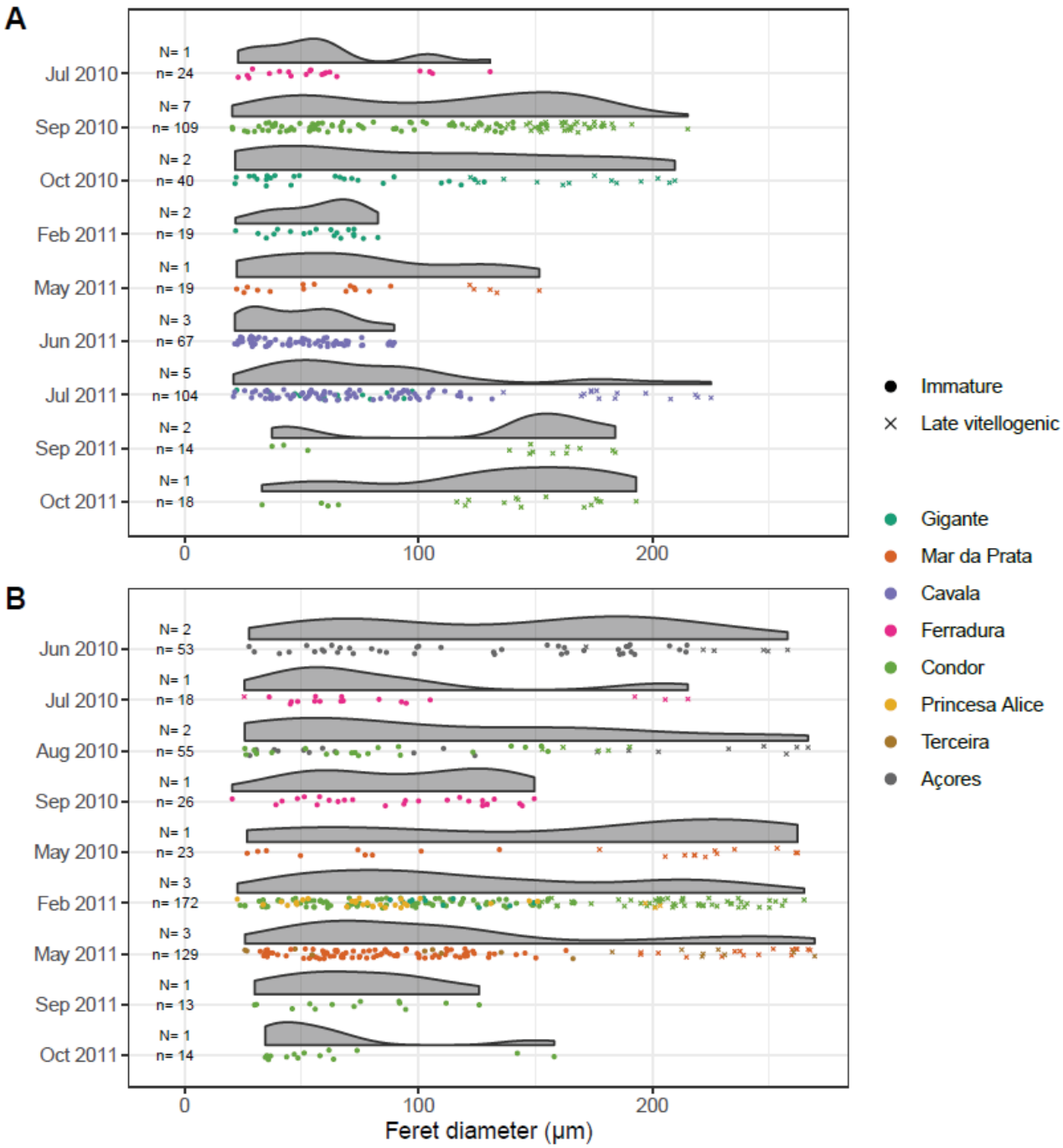

Figure 2.4: Oocyte size distributions of the species *Dentomuricea* aff. *meteor* (A) and *Viminella flagellum* (B) during the sampling period. Grey areas represent probability density estimate of the oocyte size for all oocytes encountered during a sampling month, while points below each density area provide additional information of the distribution of the available data and, their origin (sampling site, demonstrated with colour) and maturity (demonstrated with different shapes). Sample sizes are also provided: N the number of available fertile specimens and n the total number of encountered oocytes.

colony in September 2010 and two colonies in July 2011 which displayed completely different dynamics from the rest (Figure 2.5).

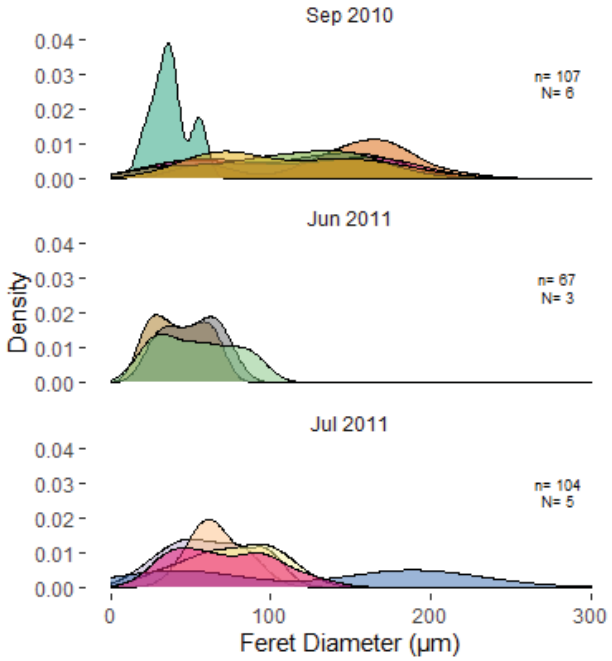

Figure 2.5: Oocyte size distributions (kernel density estimates) from the same sampling location, showing relative synchronicity among individual colonies of *Dentomuricea* aff. *meteor*. Only months in which at least three fertile female colonies were encountered were included in the analysis. Individual colonies are represented by different colours.

Due to the low number of encountered male colonies, our samples did not cover the whole cycle of spermatogenesis and did not allow for an in-depth analysis of male reproductive timing. Presence of mature spermatocysts was detected in September 2010 and October 2011 for *D.* aff. *meteor* and in May 2011 for *V. flagellum*. In these months, spermatocyst size was also higher and coincided with some of the peaks in oocyte size (October for *D.* aff. *meteor*, May for *V. flagellum*).

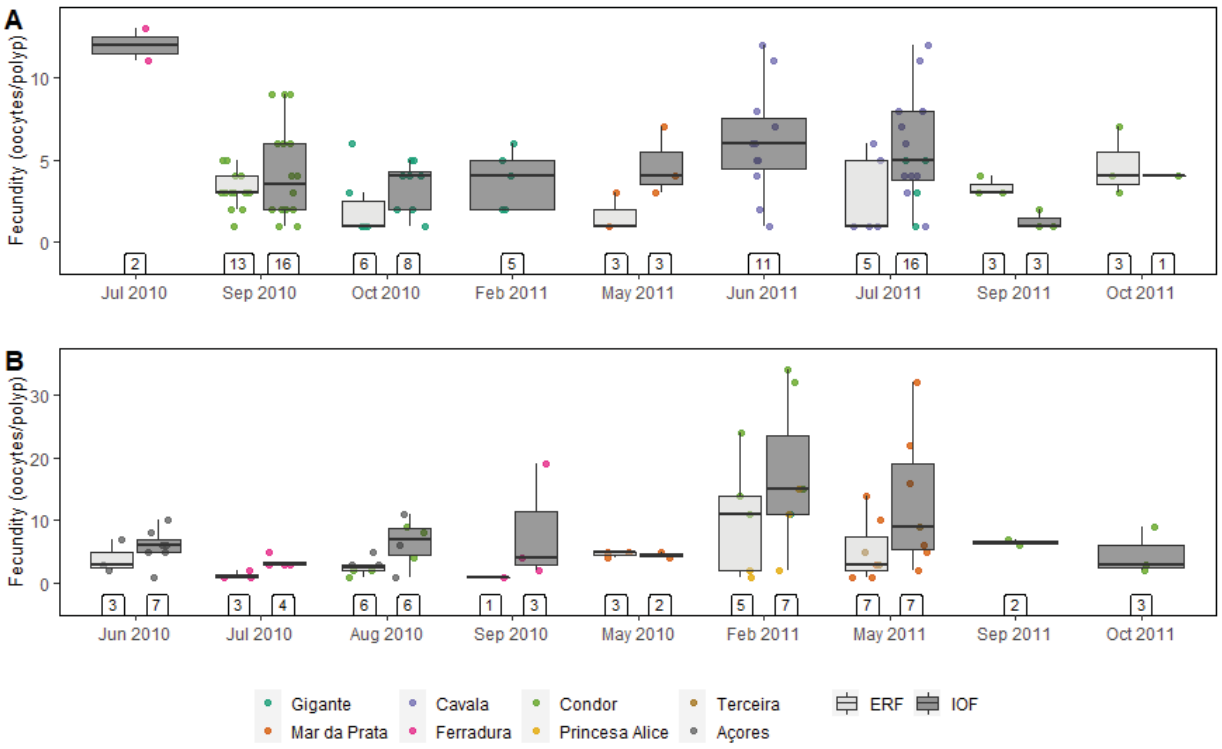

Figure 2.6: Polyp fecundity of *Dentomuricea* aff. *meteor* (A) and *Viminella flagellum* (B) including the number of immature (IOF) and vitellogenic oocytes (ERF) during the sampling period. Boxplots are accompanied by points which provide additional information of the distribution of the available data points and their origin (sampling site, demonstrated by colour). Sample sizes (number of specimens) are also provided in boxes attached to the x axis.

Fecundity varied strongly among samples and sampling months. *Dentomuricea* aff. *meteor* exhibited an average polyp fecundity of 5.80 ± 3.5 oocytes/polyp and an IOF of 4.92 ± 3.13 oocytes/polyp. In 2010 IOF displayed a significant ($D=19.7$, $df=2$, $p<0.001$) descending pattern with the highest recorded in July (Figure 2.6) while in 2011 it remained mostly stable, with the exception of a significant decrease in September (significant effect of month: $D=16.8$, $df=5$, $p=0.004$; Table B1). When late vitellogenic oocytes were detected, ERF was on average 2.93 ± 1.7 oocytes/polyp and did not display any significant changes (2010: $D=1.47$, $df=1$, $p=0.22$; 2011: $D=4.64$, $df=3$, $p=0.29$). In *V. flagellum* polyp fecundity was on average 7.75 ± 4.72 oocytes/polyp and IOF was on average 2.84 ± 1.84 oocytes/polyp. During 2010 IOF did not change significantly ($D=8.8$, $df=4$, $p=0.06$), however during 2011 it followed a statistically decreasing pattern ($D=37.4$,

df=3, p<0.01; Figure 2.6). The number of late vitellogenic oocytes (ERF) were on average 5.62 ± 4.02 oocytes/polyp and did not differ significantly among months in 2010 (D=8.5, df=4, p=0.07). In 2011 ERF was higher in February than in May (D=10, df=1, p=0.001; Table S 0.2) but in both months a high standard deviation was observed (Figure 2.6).

2.5. Discussion

The current study used coral specimens obtained as fisheries by-catch to study the reproductive biology of two of the most common deep-sea octocoral species in the Azores Archipelago. The collected data covered two years and a wide geographic area, allowing a detailed description of the basic reproductive traits and gametogenic cycle of the target species and providing insights to the reproductive seasonality that they exhibit in the region.

The basic reproductive traits of the two species were similar to the ones reported for other octocoral species. Both species were gonochoric and sex ratios were female skewed, both common features among octocorals (Kahng et al. 2011, Watling et al. 2011). However, similarly to other studies, the sex ratio estimates presented herein might be biased towards females which display gamete presence during most of the year, in contrast to males which appear to have shorter gametogenic cycles (Kahng et al. 2011). Both octocoral species are most likely broadcast spawners, as no larvae were found internally or externally. Although a variety of reproductive modes are exhibited among deep-sea Alcyonacea (Table 2.3), most Plexaurid species, like *Dentomuricea* aff. *meteor,* are also broadcast spawners (Kapela & Lasker 1999, Chang 2007). Oogenesis and spermatogenesis of *D.* aff. *meteor* and *Viminella flagellum* exhibited similar stages to the ones reported for most deep octocoral species (Mercier et al. 2011, Watling et al. 2011, Beazley & Kenchington 2012, Quintanilla et al. 2013). Nevertheless, in the present study only a few male colonies were examined and few mature spermatocysts were encountered. As a result, further studies on the spermatogenesis of the two species are essential.

Table 2.3: Reproductive characteristics of deep-sea, temperate and/or cold-water alcyonaceans. Variables are given as average, average ± sd or maximum (max). GC: gonochoristic, HP: hermaphroditic, EB: External brooder, IB: Internal brooder, BS: Broadcast spawning

Species	Suborder	Sexuality	Repr. mode	Gametog. cycle	Late vitellogenic oocyte diameter (µm)	Polyp fecundity	Depth range [1]	Reference
Alcyonium acaule	Alcyoniina	GC	EB	Annual	473 ± 37	14 max	Shallow	Garrabou 1999, Fiorillo et al. 2013, Teixidó et al. 2016
Alcyonium coralloides		GC	IB	Annual	632 ± 125	7 ± 4	Shallow	Quintanilla et al. 2013
Alcyonium siderium		HP	IB				Shallow	Sebens 1983
Anthomastus grandiflorus		GC	IB	Annual	1.000–1.100		Deep	Mercier and Hamel, 2011
Heteropolypus ritteri		GC	IB	Continuous	376 ± 178.8	5.3 ± 2.7	Deep	Cordes et al., 2001
Capnella gaboensis		GC	EB	Annual	492 ± 69	5-10	Shallow	(Farrant 1986)
Dendronephthya gigantea		GC	IB	Annual	300-480	9.8 ± 2.0	Shallow	(Hwang & Song 2007)
Dendronephthya suensoni		GC	BS	Annual	249.29 ± 36.24	10-11 max	Shallow	(Hwang & Song 2007)
Duva florida)		GC	IB				Deep	Sun *et al.*, 2011
Drifa sp.		H	IB	Continuous	490.1 ± 23.8		Deep	Sun *et al.*, 2010
Drifa glomerata		GC	IB	Continuous			Deep	Sun et al., 2010
Gersemia fruticosa		GC	IB				Deep	Sun *et al.*, 2011
Ainigmaptilon antarcticum	Calcaxonia	GC	BS	Continuous/ overlapping	700 max	3 ± 2	Deep	Orejas et al., 2002
Dasystenella acanthine		GC		Continuous/ overlapping	394.2 ± 26.6	1.2 ± 0.08	Deep	Orejas et al., 2007
Fannyella rossii		GC	IB	Annual	264.7 ± 32.2	1.5 ± 0.06	Deep	Orejas et al., 2007
Fannyella spinosa		GC	IB	Annual	183.1 ± 9.82	1.4 ± 0.08	Deep	Orejas et al., 2007
Primnoa pacifica		GC		Continuous/ overlapping	50-200	86 ± 12	Shallow	Waller et al., 2014

Species	Suborder	Sexuality	Repr. mode	Gametog. cycle	Late vitellogenic oocyte diameter (µm)	Polyp fecundity	Depth range [1]	Reference
Primnoa resedaeformis		GC	BS	Continuous/ overlapping	1000^{max}	84.3 ± 3.1	Deep	Mercier and Hamel, 2011
Thouarella sp		GC	IB	Continuous/ overlapping	261.3 ± 15.6	1.1 ± 0.1	Deep	Orejas et al., 2007
Acanella arbuscula		GC	BS	Continuous/ overlapping	717^{max}	21.07 ± 17.5	Deep	Beazley and Kenchington., 2012
Keratoisis grayi		GC	BS	Annual	700^{max}	10–60	Deep	Mercier and Hamel, 2011
Metallogorgia melanotrichos		GC					Deep	Simpson et al. 2005
Viminella flagellum		GC	BS	Annual	227.97 ± 42.61	7.75 ± 4.72	Deep	This study
Eunicella singularis	Holaxonia	GC	IB	Annual	450–860	13 ^{max}	Shallow	Weinberg & Weinberg 1979, Ribes et al. 2007
Anthoplexaura dimorpha		GC	BS	Continuous/ overlapping	359 ± 62		Shallow	Seo et al. 2008
Dentomuricea aff. meteor		GC	BS	Annual	166.74 ± 46.72	5.80 ± 3.5	Deep	This study
Paramuricea clavata		GC	EB	Annual	400-500	2.1 ± 0.3-27.9±2	Shallow	Coma et al.,1995; Gori et al., 2007
Paramuricea macrospina		GC	IB	Annual	373 ± 91	10.5 ± 5.9 ^{max}	Shallow	Grinyó et al. 2018
Paramuricea placomus		GC			64 ± 46	23.4 ± 4.3	Deep	Simpson et al. 2005, Fountain et al. 2019
Spinimuricea klavereni		GC	BS	Continuous/ overlapping	150-500	87 ± 27	Shallow	Topçu & Öztürk 2016
Corallium laauense	Scleraxonia	GC		Continuous/ overlapping	660^{max}		Deep	Waller & Baco 2007
Corallium rubrum		GC	IB	Annual	500-900	0.66 ± 0.024	Shallow	Tsounis et al. 2006

Species	Suborder	Sexuality	Repr. mode	Gametog. cycle	Late vitellogenic oocyte diameter (µm)	Polyp fecundity	Depth range [1]	Reference
Corallium secundum		GC		Continuous/ overlapping	600^{max}		Deep	Waller and Baco,2007
Tripalea clavaria		GC	IB	Annual	700^{max}	6-8	Shallow	Excoffon et al. 2004

[1] Shallow: 0-200m; Deep: >200m

During the study period, a substantial number of infertile colonies were encountered but did not exhibit any temporal pattern. Fertile colonies bore oocytes in different developmental stages, suggesting continuous or quasi-continuous gametogenesis, which is common among shallow and deep-sea octocorals (Kahng et al. 2011, Watling et al. 2011). The simultaneous presence of late vitellogenic oocytes and mature spermatocysts, as well as the peak in the respective oocyte size at the same sampling month in both years indicate a gametogenic peak in autumn for *D.* aff. *meteor* and spring for *V. flagellum*. However, these peaks appear in specific sampling sites. The oocyte size distributions and variable fecundity for the rest of the study period do not point towards a clear reproductive cycle. This might be an artifact of the sampling design, as reproductive seasonality might differ among sampling locations. Nevertheless, the continuous presence of a first cohort of immature oocytes and frequent presence of late vitellogenic oocytes throughout the study period suggest either overlapping cycles (Orejas et al. 2007), or the availability of a constant pool of immature oocytes which develop further only under optimum conditions (Waller et al. 2014). Although peaks of gametogenesis might provide indications of spawning activity, completion of a gametogenic cycle and spawning might not always be consecutive (Eckelbarger & Watling 1995). In the case of *D.* aff. *meteor*, spawning in autumn was confirmed by a subsequent study in which coral colonies collected from Condor seamount in October 2019 and maintained in aquaria spawned in November 2019 (Rakka M, in press). However, further studies are needed to clarify the reproductive seasonality of the two species.

Some studies on deep-sea corals report asynchronicity among colonies of the same population, e.g. colonies of *Primnoa pacifica* exhibit different reproductive seasonality with maturation happening once a year for some colonies and twice for others (Waller et al. 2014). Similarly, female colonies of *Paramuricea clavata* have been found to be asynchronized until reaching the last maturation state (Coma et al. 1995). In the case of *D.* aff. *meteor*, only some colonies were found to be synchronized. Synchronicity requires either the existence of intrinsic reproductive timing or the simultaneous response of coral colonies to a general environmental cue. The presence of some asynchronized colonies might indicate differences in access to resources or health status. Octocoral oocytes are typically large and require high energy investment (Watling

et al. 2011), therefore it is likely that only a fraction of the colonies complete oogenesis during suitable conditions.

The present study included specimens from a wider geographic region, but the sampling design (sampling site nested in time) and low number of fertile specimens did not allow a thorough analysis of reproductive seasonality and a comparison of reproductive parameters among sampling sites and depths. Similarly, it was not possible to provide further insights to potential environmental factors driving differences in oocyte size and number. Nevertheless, reproductive patterns of *D.* aff. *meteor* and *V. flagellum* did not seem to be related to the regional temperature seasonality (Amorim et al. 2017, Figure S 0.2), but some gametogenic peaks coincided with periods marked by high surface primary productivity, in spring and autumn (Santos et al. 2013). A number of deep-sea species display similar behaviour, with potential spawning linked to organic matter input during seasonal phytoplankton blooms (Mercier et al. 2011, Sun et al. 2011, Baillon et al. 2013). During these events, the high amounts of phytodetritus and zooplankton densities can provide an important food resource for physiological processes of deep-sea corals, (Carlier et al. 2009), including reproduction. Reproductive patterns are not only subject to effects of environmental factors but also to phylogenetic constraints, since species biological and physiological characteristics might limit a range of mechanisms, including the ability to process and store organic matter and nutrients, the rate of converting energy to oocyte production and internal rate of vitellogenesis and gametogenesis (Eckelbarger & Watling 1995). Thus, the distinct phylogenetic history and ecophysiology of *D. aff. meteor* and *V. flagellum* are likely to define their potential responses to specific environmental cues, leading to differences in their reproductive strategies.

Oocyte size of *V. flagellum* was similar to the size reported for other species in the same family (Ellisellidae, Chang 2007, Tsai et al. 2014), but late vitellogenic oocytes of *D.* aff. *meteor* were relatively smaller compared to other plexaurids (Kapela & Lasker 1999, Beiring & Lasker 2000) and most deep-sea octocorals (Table 2.3) reported so far. Oocytes of *V. flagellum* were also larger than oocytes of *D.* aff. *meteor*. This may reflect anatomical differences between the two species,

with the large polyps of *V. flagellum* (average diameter: 1.4 ± 0.10 mm) providing more available space for reproductive cells than polyps of *D.* aff. *meteor* (average diameter: 0.91 ± 0.06 mm). Large oocytes, which are thought to develop into lecithotrophic larvae (Edwards & Moore 2009), are provisioned with high amounts of yolk enabling long larval duration times under favourable conditions (Rice et al. 1992). Moreover, larger oocytes in broadcast spawning species may have higher fertilization success due to their higher available surface (Levitan 2006) which is crucial especially in cases of high female to male sex ratios. As a result, the smaller oocyte size of *D.* aff. *meteor* might have implications on the larval biology and dispersal capacity of the species. Moreover, although increased gamete size may have advantages, the limited available space in coral polyps frequently establishes a trade-off between gamete size and the number of available gametes (Hall & Hughes 1996, Levitan 2006). Overall, the two target octocorals displayed similar levels of fecundity, but the large variation associated with fecundity estimates for both species did not allow further comparisons.

Reproductive traits such as mode of reproduction, oocyte size and reproductive seasonality can influence fundamental ecological processes including recruitment, larval dispersal, population connectivity and genetic diversity (Wangensteen et al. 2016, Hock et al. 2019). Reproductive traits can also determine the capacity of a species to recover from environmental and anthropogenic disturbance (Lytle 2001). Both target species in this study displayed characteristics that can render them vulnerable to future disturbance. Continuous gametogenesis requires constant energy investment which can be impaired if energy has to be allocated towards physiological recovery (Henry & Hart 2005, Waller et al. 2019). Broadcast spawning has often advantages for dispersal and population structure (Ayre & Hughes 2000, Nishikawa et al. 2003), but it leaves both gametes, as well as the embryo exposed to potential changes in water chemistry which may happen due to ocean acidification (Albright & Mason 2013, Lucey et al. 2015) or pollution (Reichelt-Brushett & Harrison 1999, Hudspith et al. 2017). Coral gametes and embryos may also be vulnerable to sediment plumes created by prospective deep-sea mining activities, due to particle binding, injuries and ecotoxicological effects (Jones et al. 2015, Miller et al. 2018). At the population level, reproductive success of broadcast spawning species can be

influenced by population density (Babcock 1991, Levitan 2005) and thus even low levels of adult mortality can have cascading effects for reproductive success (Linares et al. 2008b). This is especially true in populations with skewed sex ratios, where sperm limitation can pose additional pressure to fertilization (Levitan & Petersen 1995). Lastly, in many octocoral species reproductive maturity and fecundity are linked to colony size, with larger colonies contributing disproportionally more reproductive material to the population (Cordes et al. 2001, Santangelo et al. 2003, Mercier et al. 2011). In the Azores, selective removal of larger individuals due to bycatch may eventually lead to lower recruitment, making population resilience low and recovery highly unlikely (Pham et al. 2014), rendering both species vulnerable to potential disturbance.

The reproductive strategies of habitat-building species not only shape their ecology and evolution but are also key to the structure and function of the communities they form (Wangensteen et al. 2016). Increasing our knowledge on fundamental biological processes such as reproduction can allow a better understanding of the dynamics and potential resilience of deep-sea ecosystems to human and environmental impacts. Linking reproduction to population variables, including density, colony size, age and growth should be priority areas of research (e.g. Fountain et al. 2019) to better assess the condition of coral populations, an essential element to inform management and conservation efforts.

2.6. Conclusions

The present study provides useful insights to the reproductive characteristics of two deep-sea octocoral species, contributing to our limited knowledge on deep-sea octocorals and the communities they form. Both target octocoral species were found to be gonochoric, broadcast spawning, with continuous or quasi-continuous gametogenesis. The two species appeared to have different reproductive strategies, with *Dentomuricea* aff. *meteor* investing in smaller oocytes compared to *V. flagellum*. These differences might result from other species-specific life history traits and might have consequences on larval characteristics with further implications for species dispersal, survival and vulnerability to disturbance. Future work should provide further

insights to demographic characteristics such as height at first reproduction, the relationship between height and reproductive output and the environmental factors that affect reproductive parameters, especially reproductive timing.

Chapter 3
Early life history stages of deep-sea octocoral species in the Azores Archipelago

3.1. Embryo and larval biology of the deep-sea octocoral *Dentomuricea* aff. *meteor* under different temperature regimes[2]

3.1.1. Abstract

Deep-sea octocorals are common habitat-formers in deep-sea ecosystems, however, our knowledge on their early life history stages is extremely limited. The present study focuses on the early life history of the species *Dentomuricea* aff. *meteor*, a common deep-sea octocoral in the Azores. The objective was to describe the embryo and larval biology of the target species under two temperature regimes, corresponding to the minimum and maximum temperatures in its natural environment during the spawning season. At temperature of 13 ± 0.5°C, embryos of the species reached the planula stage after 96h and displayed a median survival of 11 days. Planulae displayed swimming only after stimulation, swimming speed was 0.24 ± 0.16 mm s^{-1} and increased slightly but significantly with time. Under a higher temperature (15°C ± 0.5°C) embryos reached the planula stage 24h earlier (after 72h), displayed a median survival of 16 days and had significantly higher swimming speed (0.3 ± 0.27 mm s^{-1}). Although the differences in survival were not statistically significant, our results highlight how small changes in temperature can affect embryo and larval characteristics with potential cascading effects in larval dispersal and success. In both temperatures, settlement rates were low and metamorphosis occurred even without settlement. Such information is rarely available for deep-sea corals, although essential to achieve a better understanding of dispersal, connectivity and biogeographical patterns of benthic species.

[2] Rakka M., Godinho A., Orejas C., Carreiro-Silva M. 2021. Embryo and larval biology of the deep-sea octocoral *Dentomurice*a aff. *meteor* under different temperature regimes. Peer J 9, e11604.

3.1.2. Introduction

Species persistence requires the successful completion of a life cycle against biotic and abiotic odds, in most cases starting with survival at early life history stages. For benthic marine invertebrates, larval stages constitute the only pelagic phase that ensures dispersal and connectivity among populations (Cowen & Sponaugle 2009). Moreover, early life events such as larval survival and settlement determine the fate of the sessile, adult phase and are extremely important (Marshall & Morgan 2011, Byrne 2012). In deep-sea communities, which are dominated by benthic marine invertebrates, knowledge on early life stages is therefore key in understanding species distributions, biogeographical patterns and metapopulation dynamics (Treml et al. 2015), constituting an essential tool for management (Hilário et al. 2015).

Deep-sea octocorals are major habitat-formers in the deep-sea, usually occurring in complex geological settings such as continental shelves and margins (Yesson et al. 2012, Taylor et al. 2013), underwater canyons (Brooke et al. 2017) and seamounts (Tempera et al. 2012, Braga-Henriques et al. 2013). Due to the habitat requirements of some octocoral species, including hard substrates for settlement and strong currents which optimize food delivery, their distribution can be quite patchy (Bryan & Metaxas 2006, Tong et al. 2012), as observed for other deep-sea benthic species (Miller & Gunasekera 2017). Anthropogenic disturbance and global climate change are likely to cause habitat fragmentation by altering its characteristics (Sweetman et al. 2017, Levin et al. 2019a) and causing a decrease in the available suitable habitat of some species (Morato et al. 2020c). Under these circumstances, obtaining a solid understanding of larval biology and population connectivity is essential to understand community dynamics and the potential of deep-sea octocoral populations to recover from disturbance (Cowen et al. 2007, Levin et al. 2020).

So far, our knowledge on larval biology of deep-sea octocorals is limited to a few brooding species (Cordes et al. 2001, Sun et al. 2010b, 2011, Mercier & Hamel 2011). In most of these cases, larvae displayed short competency periods with limited swimming behaviour (Sun et al. 2010b), settlement within 2-5 days after release and rapid metamorphosis into primary polyps (Cordes

et al. 2001, Sun et al. 2011). However, many deep-sea octocorals are broadcast-spawners and are therefore expected to display different larval characteristics and dispersal capabilities (Harrison & Wallace 1990, Nishikawa et al. 2003). To our knowledge, up to date there is no detailed description of embryo and larval development of broadcast spawning deep-sea octocorals. Larvae from broadcast spawning species undergo early development in the water column, where they are mostly transported as passive particles until they reach the planula stage. During transportation, embryos can be exposed to variable environmental conditions which may affect their development (Melzner et al. 2009). This phenomenon can be even more pronounced in larvae of deep-sea species, which often display upward swimming, crossing water masses with very different physicochemical characteristics (Young et al. 1996, 2012, Arellano et al. 2014, Strömberg & Larsson 2017). In the case of deep-sea corals, the effect of natural fluctuations of environmental conditions, such as salinity and temperature, have only been addressed in the scleractinian *Lophelia pertusa* (Strömberg & Larsson 2017).

The aim of this study was to provide a detailed description of the early life history traits of the deep-sea broadcast spawning species *Dentomuricea* aff. *meteor,* a common habitat-forming, deep-sea octocoral in the Azores. More specifically, the objectives were (1) to describe the embryo and larval development, larval survival, swimming and settlement behaviour of the target species and (2) to determine the effect of natural temperature variability on its embryo and larval traits. To achieve these objectives, we employed an experimental approach with assisted fertilization and larvae rearing in aquaria under two temperature regimes (13 ± 0.5°C and 15 ± 0.5°C), representing the minimum and maximum temperatures experienced by the species in its natural habitat.

3.1.3. Materials and Methods

3.1.3.1. Target species and colony collection

The Azores Archipelago, located above the Mid-Atlantic Ridge, is a biodiversity hotspot for deep-sea octocorals (Sampaio et al. 2019). Coral gardens (OSPAR 2010) formed by deep-sea octocorals

are among the most prominent deep-sea communities on regional seamounts and island slopes (Braga-Henriques et al. 2013). *Dentomuricea* aff. *meteor* is an octocoral species of the family Plexauridae, so far only recorded on the seamounts of the North Mid-Atlantic Ridge. It is common in regional seamounts between 200-600 meters (Braga-Henriques et al. 2013), where it forms dense populations, often in combination with other octocoral species such as *Viminella flagellum* and *Callogorgia verticillata*. The species is gonochoristic and presents gametes all year round, with seasonal peaks of gamete maturation and spawning usually occurring in autumn (Rakka et al. 2021b).

A total of 11 colonies of the species *Dentomuricea* aff. *meteor* were collected as by-catch from experimental long-line fisheries on board RV Archipelago (ARQDAÇO monitoring programme). Collection was performed at the summit of Condor Seamount, between 200-280 meters, in September and October 2019. Colonies were divided in large fragments (20-30 cm height) and were kept at the DeepSeaLab aquaria facilities (Orejas et al. 2019), in six 33L aquaria positioned in a thermo-regulated room at 14°C. Aquaria were supplied continuously with seawater (SW) pumped from 5m depth, previously treated with UV light (P10 UVsystem & Vecton 600 TMC^{TM}) and passed through 50 µm and 1 µm mesh filters. Circulation within the aquaria was maintained by pumps. Seawater temperature was kept between 13-14°C with the aid of chillers and salinity was 35.8 ± 0.1, similar to the natural conditions at the collection site (Santos et al. 2013). Colonies were fed twice per day with a mixture of frozen zooplankton and microplankton which was frequently enriched with live microalgae (*Chaetoceros calcitrans* and *Nannochloropsis gaditana*) and live rotifers.

3.1.3.2. Larval rearing

Larvae were obtained by maintaining reproductively active female and male colonies in the same aquaria to achieve natural spawning and fertilization. Coral fragments were allowed to acclimatize in the above aquaria conditions for approximately one month. Subsequently, colonies with mature gametes were identified by dissecting two branchlets (3-5 cm height) from each colony and observing their tissue under a dissecting microscope. Reproductively immature

colonies and fragments in poor condition were excluded from further analysis. This procedure resulted in selection of six female and three male colonies. Coral fragments from the female colonies were distributed in two aquaria, referred to as spawning aquaria. Subsequently the fertile male colony with the higher number of available fragments was selected and four of its fragments were distributed in each of the two spawning aquaria. The remaining male colonies were not used to avoid polyspermy (Levitan et al. 2007).

To increase the potential of spawning, we enriched the aquaria water with free mature sperm, obtained from the selected male colony. This was achieved by dissecting mature spermatocysts from coral tissue, which were subsequently concentrated in 50 ml flasks with filtered (mesh size: 0.2 µm) SW, mixed by gently shaking and redistributed to the aquaria. Water inflow was paused and aquaria pumps were substituted with aeration to ensure water circulation without losing or harming potentially spawned gametes. Upon gamete release, which happened in batches separated by intervals of at least 2-3 hours, gametes/fertilized eggs from each batch were collected from the water column to a 750 ml-culture flask (20-100 fertilized eggs per flask), filled with filtered SW from the aquaria facilities (mesh size: 0.2 µm). Whenever more than 100 gametes/embryos were released in one batch, these were equally distributed to two flasks to avoid maintaining larvae in high densities. During the first four days of the study we collected a total of 688 gametes which were distributed to 7 batches. Three of these batches were large enough to be split to two flasks (total n= 10 flasks).

3.1.3.3. Temperature experiments

In order to choose appropriate temperature regimes for larval rearing, we utilized temperature data collected during annual CTD surveys, under the framework of the projects CONDOR (EEA Grants PT-0040) and SMaRT (SRECC- Azores Regional Government M.2.1.2/029/2011). Data were collected between 2010 and 2012, above the coral garden where specimen collection took place. Subsequently, we utilized the minimum and maximum recorded values during the spawning season of the target species (October-November) to define the target rearing temperatures (13 ± 0.5°C and 15 ± 0.5°C). Two water baths were set-up, each maintaining temperature within ± 0.5

°C of the corresponding target temperature, with the aid of an aquaria chiller and a heater, respectively. Each day, the collected batches were divided between the two temperature treatments: immediately after collection of the released fertilized eggs/embryos, culture flasks were randomly assigned to one of the two water baths (n= 5 in each water bath). This corresponded to a total of 346 and 342 embryos reared at 13°C and 15°C respectively. Culture flasks were equipped with glass pipettes connected to an aquaria air pump, achieving continuous light circulation, while the full volume of water in the flasks was exchanged daily.

3.1.3.4. Embryonic and larval development

Embryos were monitored every 3-4 hours during the first 48 hours and subsequently once a day until reaching the planula stage, to study their early development. In every monitoring event, all embryos were counted to estimate survival. Additionally, 10-15 embryos were randomly removed from each flask and photographed, with a digital camera (DIGICAM 5MEG LCMOS MAC) attached to a microscope (10x), to record their developmental stage and size. Embryos were subsequently returned to the flasks. Due to the frequently prolonged gamete release, gametes of the same batch were occasionally in slightly different developmental stages, therefore the timing of embryonic development is approximate. Moreover, since it was not possible to define the moment of fertilization, embryo development is presented in respect to the time of gamete release. To estimate size, we measured width and length (mm) of embryos and larvae (days 4 and 14) using the open software Fiji/Image J (Schindelin et al. 2012). The data were subsequently used to estimate volume (mm^3) assuming larvae had the shape of a prolate spheroid (Larsson et al. 2014). The ratio of length to width (LW ratio) was used as a proxy of sphericity.

3.1.3.5. Embryo and larval survival

After reaching the planula stage, larvae were counted every 2-3 days. The last count corresponded to day 34, 36 or 39, depending on the batch. The obtained data were joined to the dataset collected during embryo development to estimate larval survival during the whole experimental period. Survival analysis was performed using the Kaplan-Meier method (Kaplan &

Meier 1958), following the rationale of Graham et al. (2008). Since monitoring was done in time intervals and the exact time of death for each larva was not known (interval-censored data), we assumed that time of death was the moment at which each larva was observed for the last time. The remaining larvae at the last monitoring event were considered alive (censored data). As the Kaplan-Meier method does not allow for incorporation of replicate information into the analysis, we performed the analysis by pooling data from all batches together, for each rearing temperature. Subsequently the analysis was repeated separately for each batch, to provide information about the variability among batches (Graham et al. 2008). A log-rank test was performed to compare the survival curves between larvae reared under 13°C and 15°C. Survival analysis was performed by using the packages survival (Therneau & Grambsch 2000) and survminer (Kassambara et al. 2019) in R 3.5.0 (R Core Team, 2018).

3.1.3.6. Larval swimming behaviour

Data on swimming speed and behaviour were collected by video recording and analysis. Videos were recorded with a Canon EOS 600D digital camera, equipped with a regular 22-55mm lens, on day 4 and day 15 after spawning, which corresponded to the first day larvae reached the mature planula stage and the second day larvae started settling, respectively. To minimize larval handling, swimming behaviour was recorded in the same culture flasks used for larval rearing. Videos were captured in the dark, using lateral led lights for illumination (Strömberg & Larsson 2017). Flasks were positioned in front of a black slide with a calibrated grid that was used as background and a 2-minute waiting period was implemented to ensure no water movement was interfering with larval swimming. Subsequently, three videos (duration: 1 min) were recorded at three minute intervals.

Videos were converted to frames and were analyzed by an automatic particle tracking method, using the open software Fiji/Image J (Schindelin et al. 2012) and the plugin TrackMate (Tinevez et al. 2017) to record data on vertical swimming behaviour, namely swimming direction (up/down), displacement and swimming speed. Estimates of swimming speed only considered

tracks with displacement higher than 2 mm, to exclude data from larvae that did not move or moved minimally.

3.1.3.7. Pelagic phase and larval settlement

During the counts performed for survival, each larva was assigned to one of four stages: planula, settled, pelagically metamorphosed and deformed. Because counts were made simultaneously for all flasks and each flask contained a batch of different age, e.g. some batches were released with 1-3 day difference, when average counts were estimated these were sometimes heavily influenced by the available count for that day. To be able to estimate robust mean counts for each monitoring day, missing counts were regenerated for each batch separately by using linear interpolation between existing data points (Dong & Peng 2013), by using the R package VIM (Kowarik & Templ 2016). Extrapolation was performed only until the last datapoint that was available for each batch, i.e. there was no attempt to predict the trend past the last available count. Subsequently, counts of each stage were divided by the total number of living larvae in each batch. This resulted in estimates of the proportion of the surviving larvae in each stage and was used to analyze the behaviour of the remaining larvae. Lastly, on days 4 and 14 after spawning, five planulae were removed from each flask (total n= 25 for each temperature regime) and photographed with a digital microscope camera to estimate their size.

Since larvae did not display clear bottom probing behaviour, the onset of competency was defined by settlement or pelagic metamorphosis. After the first larval settlement (day 14), substrate was provided to the culture flasks in order to monitor settlement behaviour. Three flasks from each temperature regime were randomly selected and three pieces (approximate diameter: 5 mm) of basalt rock attached to a plastic slide (10 mm x 80 mm) were offered as potential substrate in each flask. Basalt was selected because it is an abundant hard substrate in the deep seafloor of the Azores and where the studied species is frequently observed. The substrate was not pretreated to develop biofilm. Settled larvae were observed and photographed every 2-3 days to assess and describe settlement and metamorphosis, during a period of approximately two weeks. After metamorphosis was observed, a mixture of live microalgae

(*Nannochloropsis gaditana* and *Chaetoceros calcitrans*) and rotifers was provided weekly as a potential food source.

3.1.3.8. Statistical analysis

For all the dependent variables in question, we firstly performed exploratory analysis (Zuur et al. 2010) to select the most appropriate modeling method. The effect of each independent variable was subsequently tested with linear models (LMs), by adding the independent variables progressively to the respective model and using maximum likelihood ratio (MLR) tests and the Akaike Information Criterion (AIC). Data collected from monitoring larvae stages (proportions) were modeled by means of Generalized Additive Models (GAMs) with a binomial distribution. Summarized results of the MLR test for each variable in question are provided in Table 3.1, while the results from each selected model are provided graphically as supplementary material (Figure S 1.1 - Figure S 1.5). Statistical analysis was performed in R (R Core Team, 2019).

3.1.4. Results

3.1.4.1. Spawning

Gamete release occurred for the first time on the 27th of November, one day after the new moon. Oocytes were encountered 15 minutes after enrichment with free live sperm, in both aquaria. Spawning was not synchronized among colonies, neither among polyps of the same colony. Despite careful observation, it was not possible to directly observe polyps releasing sperm or oocytes and determine whether one or more colonies participated in gamete release. Similarly, it was not possible to directly observe if fertilization was internal or external. All collected oocytes were fertilized, therefore fertilization was either internal, or external with very high fertilization rates. Oocytes were spherical, they had no visible germinal vesicle and were released in batches of 10-80 at a time. They were mostly negatively buoyant, however, they remained in suspension for several hours due to water movement within the aquaria. Average oocyte diameter was 365.4 ± 24.2 µm. Gamete release was slow and sometimes continued for 1-3 hours. It happened multiple times a day (every 2-3 hours) for a week and continued with lower frequency (every 1-

3 days) for approximately a month. Release occurred both during day and night hours and did not seem to follow any circadian pattern.

3.1.4.2. Embryonic and larval development

Cell division was always equal but cleavage varied highly among stages and embryos. It was not possible to determine the timing of the first division after spawning. Cytokinesis was never visible for the 2-cell stage, in which cleavage seemed to be always superficial (Figure 3.1B). During the following stages, cleavage varied from radial to pseudospiral and in some cases superficial, leading to embryos with substantial differences in shape. Development always led to a hollow blastula (Figure 3.1G) followed by gastrulation and the formation of planula larvae without visible oral pore (Figure 3.1I). Cleavage and cell division did not differ between the two rearing temperatures.

At 13°C, all embryos reached the blastula stage within 10h and the early gastrula stage within 48h (Figure 3.2). After 72h all embryos reached the late gastrula stage and could perform slow, mainly rotating movements by cilia, while fully competent, swimming planulae were formed after 96h (4 days). During their development, embryos were negatively buoyant and accumulated at

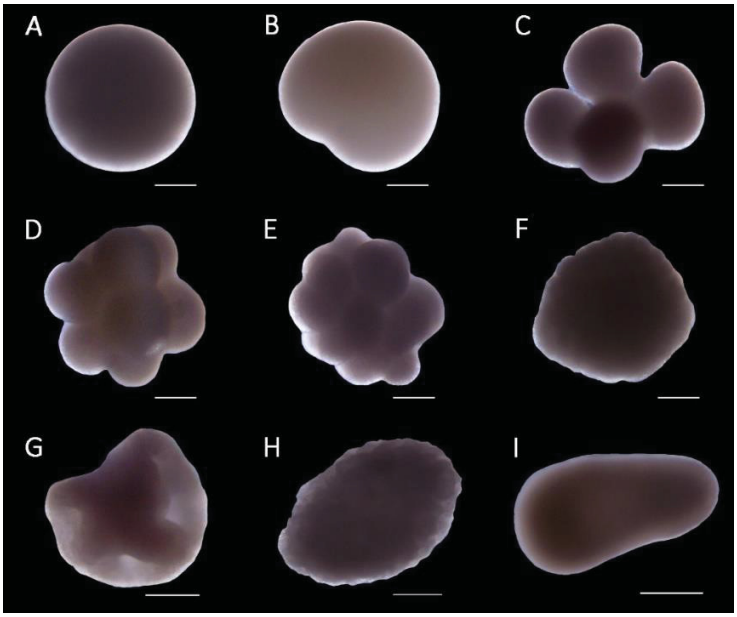

Figure 3.1: Stages of embryo development of the octocoral species *Dentomuricea* aff. *meteor* (A) fertilized oocyte; (B) 2-cell; (C) 4-cell; (D) 8-cell; (E) 16-cell; (F) 64-cell; (G) hollow blastula; (H) late gastrula; (I) planula.

the bottom of the flasks. In the first batch this resulted in the formation of embryo aggregations and abnormal embryo development. This issue was solved by adding slight aeration that ensured water and oxygen circulation within the flasks. At 15°C, during the first 6 hours cleavage seemed to be occurring at similar intervals until reaching the blastula stage (Figure 3.2), however, embryos reached the late gastrula and subsequently the planula stage approximately 24h (after 72h) earlier than embryos reared at 13°C (Figure 3.2).

Embryos between the 2-cell and 32-cell stage obtained variable shapes (Figure 3.1) and their volume was on average 0.03 ± 0.0073 mm^3. Subsequently, during the 64-cell stage and blastula they turned more spherical but had a similar volume range (0.03 ± 0.005 mm^3). After reaching the planula stage, embryos increased significantly in size (Table 3.1) and planulae reached 0.28 ± 0.1

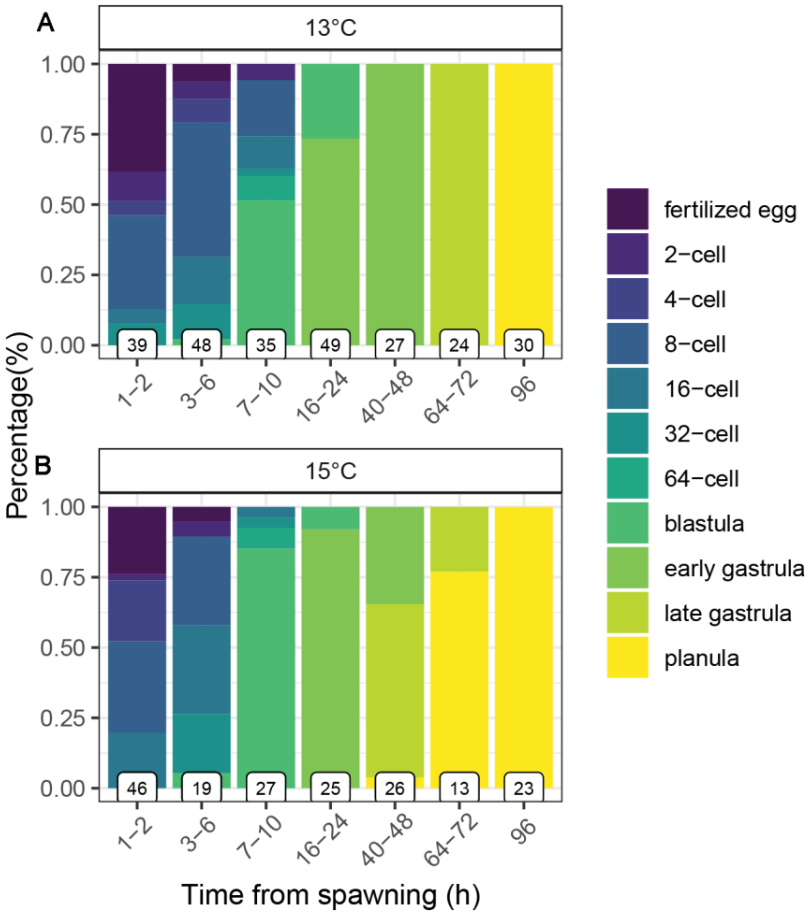

Figure 3.2: Early development of embryos of the octocoral species *Dentomuricea* aff. *meteor* reared at 13°C and 15 °C, displaying the proportion of embryos in each developmental stage over a course of 96 hours after spawning. Numbers at the base of each bar represent sample size (n).

mm^3 on day 4 and 0.67 ± 0.28 mm^3 on day 14, with measurements on day 14 displaying substantial variability. Mature planulae displayed the capacity to change their shape between spherical and elongated, and more elongated larvae were observed on day 14 compared to day 4 (Figure S 1.1). This was also confirmed from the LW ratio which presented a non-significant decrease from late gastrula embryos (1.49 ± 0.17 mm^3) to planulae on day 4 (1.22 ± 0.29 mm^3) but increased significantly (Table 3.1) on day 14 (2.02 ± 0.45 mm^3). Embryo sizes were not statistically different between the two temperatures (Table 3.1). Planulae on day 4 had significantly higher LW ratios at15°C (LW=1.59 ±0.39; Table 3.1), showing a tendency to maintain a more elongated shape than at 13°C (Figure S 1.1).

3.1.4.3. Embryo and larval survival

In both temperatures, survival differed substantially among batches (Figure S 1.2). In most batches reared at 13°C, a sharp decline in survival rates was observed during the first 48 hours, after which a more moderate mortality rate was established (Figure 3.3). In the same temperature treatment, median survival time, i.e. time when mortality reached 50%, was 11 days while survival after 36 days was 16.4%. At 15 °C, the average mortality rate seemed to be more constant (Figure 3.3). Median survival time was 5 days longer than at 13°C (16 days), however, final survival after 36 days was slightly lower (12.6%). Overall, these differences were not statistically significant according to the log-rank test (p= 0.05; Figure 3.3).

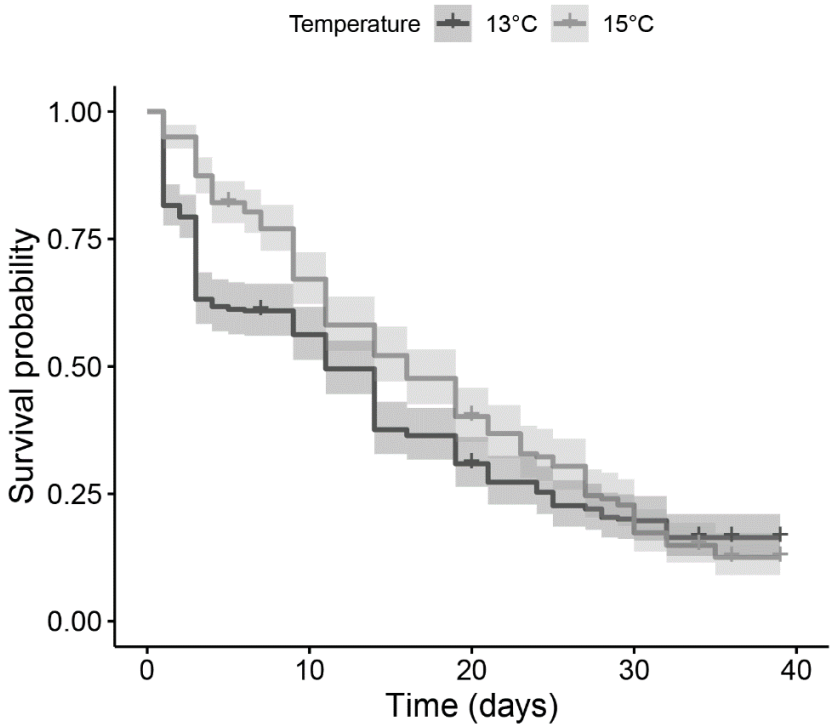

Figure 3.3: Comparison of Kaplan-Meier estimates of larvae survival of the species *Dentomuricea* aff. *meteor* under two temperature regimes. The initial pool of embryos corresponded to 346 and 342 embryos at 13°C and 15 °C, respectively.

3.1.4.4. Swimming behaviour

Planulae remained mostly at the bottom of the culture flasks, where they displayed slight rotational and unidirectional movements. They rarely became waterborne without the aid of water movement. Once in the water column, larvae did not show a specific swimming pattern but followed random trajectories. Overall, for larvae reared under 13°C, 51.2 ± 14.2 % of the recorded larval tracks were directed upwards while 50.7 ± 6.33 % were directed downwards. It was not clear if downward movement involved swimming or just sinking. The proportion of upward/downward swimming larvae did not change significantly with time (Table 3.1). Larvae displayed an average swimming speed of 0.24 ± 0.16 mm s^{-1} on day 4 and 0.36 ± 0.21 mm s^{-1} on day 15. Swimming speed did not differ significantly between upward and downward movements (Table 3.1) but it was significantly higher on day 15 compared to day 4 (Table 3.1).

Table 3.1: Results of Maximum Likelihood Ratio (MLR) tests, revealing significant effects of the independent variables in question. Selected models are highlighted with bold.

Dependent variable	Model type	Model	AIC	X^2	df	p
Size	LM	Null	-186.35			
		Stage	**-725.87**	**8.63**	**13**	**2.20×10^{-16}**
		Stage + Temperature	-724.69	0.004	12	0.37
		Stage x Temperature	-708.56	0.03	11	0.90
Length/width ratio	LM	Null	184.68			
		Stage	-132.57	21.66	13	2.20×10^{-16}
		Stage + Temperature	-130.58	0.0004	12	0.91
		Stage x Temperature	**-159.44**	**1.66**	**11**	**4.78×10^{-7}**
Swimming speed (13°C)	LM	Null	-141.32			
		Time	**-170.05**	**1.24**	**1**	**1.95×10^{-8}**
		Time + Direction	-168.18	0.005	1	0.71
		Time x Direction	-166.18	0.00001	1	0.99
Swimming speed (15°C)	LM	Null	77.12			
		Time	**63.7**	**1.02**	**1**	**7.91×10^{-5}**
		Time + Direction	64.11	0.10	1	0.20
		Time x Direction	65.75	0.23	1	0.54
Swimming speed	LM	Null	42.04			
		Time	-24.12	4.00	1	2.20×10^{-16}
		Time + Temperature	**-79.80**	**3.17**	**1**	**1.41×10^{-14}**
		Time x Temperature	-77.89	0.04	1	0.767
Swimming direction (13°C)	LM	**Null**	**80.31**			
		Time	82.31	0	1	1
		Time + Direction	84.15	5.35	1	0.70
		Time x Direction	82.90	95.15	1	0.11
Swimming direction (15°C)	LM	**Null**	**89.65**			
		Time	91.65	0.00	1	1
		Time + Direction	92.38	88.60	1	0.34
		Time x Direction	94.38	0.09	1	0.97
Proportion of planula	Binomial GAM	Null	4260.04			
		Time	811.09	3454.09	2.57	2.20×10^{-16}
		Time + Temperature	**749.43**	**63.61**	**0.97**	**1.5 x 10-15**
		Time x Temperature	749.68	2.06	1.16	0.15
Proportion of metamorphosed	Binomial GAM	Null	2658.9			
		s(Time, k=4)	467.12	2196.8	2.52	2.20×10^{-16}
		s(Time, k=4) + Temperature	468.32	0.80	1	0.36
		s(Time, k=4, by=Temperature)	**465.44**	**6.68**	**1.9**	**0.03**
Proportion of settled	Binomial GAM	Null	1359.07			
		s(Time, k=4)	670.27	694.69	2.95	2.20×10^{-16}
		s(Time, k=4) + Temperature	578.11	94.11	0.97	2.20×10^{-16}
		s(Time, k=4, by=Temperature)	**564.18**	**17.74**	**1.9**	**1.40×10^{-4}**
Proportion of deformed	Binomial GAM	Null	551.37			
		s(Time, k=4)	220.45	335	2.07	2.20×10^{-16}

Dependent variable	Model type	Model	AIC	X^2	df	p
		s(Time, k=4) + Temperature	220.05	2.44	1.02	0.11
		s(Time, k=4, by=Temperature)	**184.87**	**36.86**	**0.84**	**1.26×10^{-9}**

Swimming velocity for larvae reared under 15 °C was similar between upward and downward swimming (Table 3.1) and increased slightly but significantly with time (Table 3.1), from 0.4 ± 0.24 mm s^{-1} on day 4 to 0.44 ± 0.23 mm s^{-1} on day 15. Overall, 52.7% of the recorded tracks were directed downwards and the proportion of upward/downward swimming tracks did not differ significantly between dates (Table 3.1). Larvae swimming velocity was significantly higher under 15 °C compared to 13 °C, (Figure S 1.3) both on day 4 and day 15 (Table 3.1).

3.1.4.5. Pelagic phase and settlement

The proportion of planulae decreased substantially during the course of the experiment, mainly due to high mortality (Figure 3.4A). The surviving planulae followed slightly different trends between the two temperatures with planulae under 15°C remaining in the pelagic phase for a longer period (Figure 3.4B), a difference that was statistically significant (Table 3.1). In both temperatures, after day 36 only a minimal proportion of larvae remained (Figure 3.4A) and the last free swimming planulae were observed on day 39.

Larvae started settling on day 14 under 13°C and on day 17 under 15°C. Under both experimental temperatures, larvae settled on the flask walls and plastic slides whereas no larvae attached to the provided basalt rock. Since the addition of substrate did not have any effect on settlement behaviour, data from all flasks, i.e. with and without provided substrate, were pooled together for further analysis. All settled larvae underwent metamorphosis. Larvae firstly obtained a pear-like shape and subsequently became rounder, gradually forming a polyp base, mouth and mesenteries (Figure 3.5A). Fully developed primary polyps were formed within approximately 2-3 days, after the formation of tentacles, sclerites and tentacle pinnules (Figure 3.5B). In both

rearing temperatures, the number of settled larvae corresponded to a very low proportion of the initial pool of planulae,

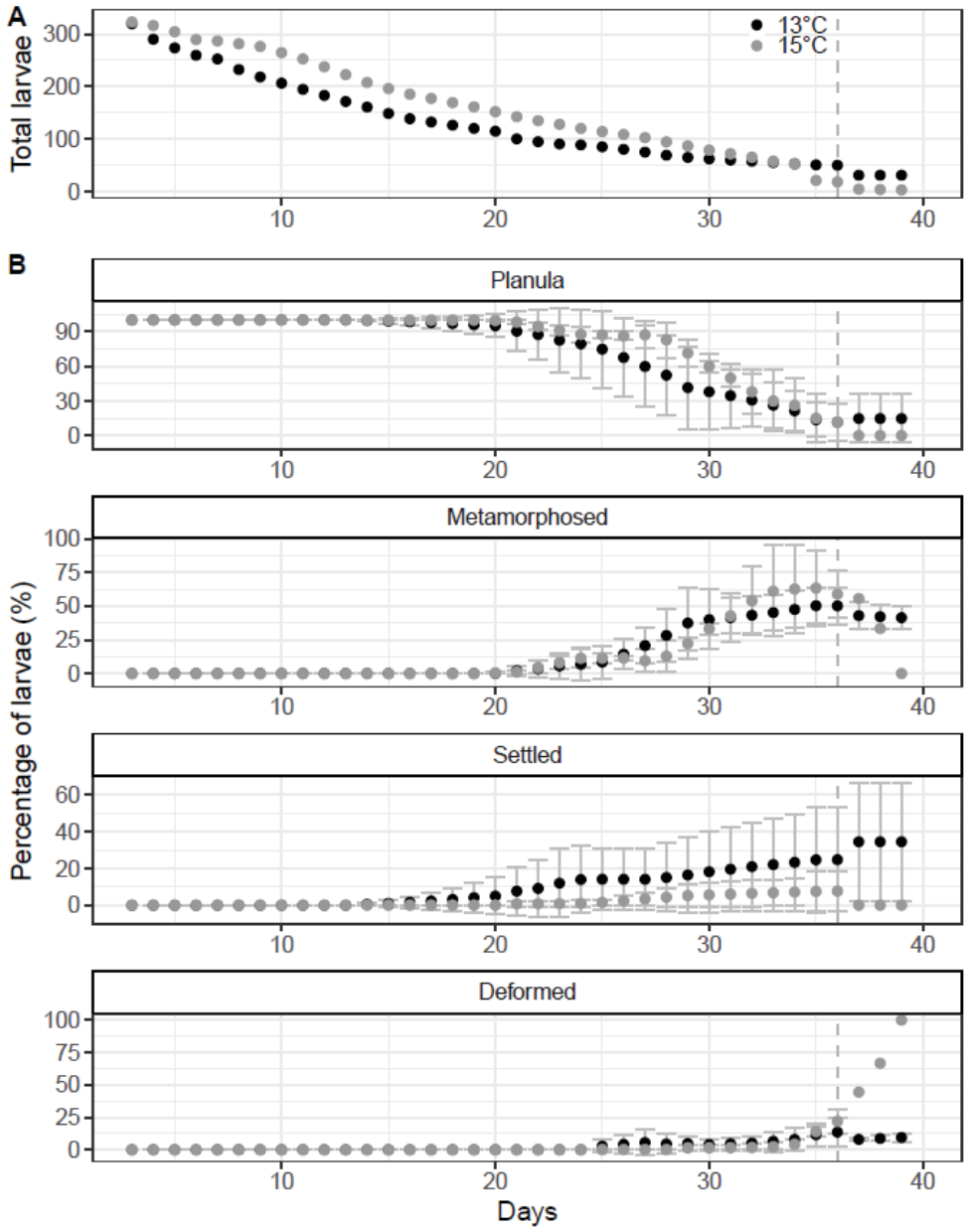

Figure 3.4. Larval behaviour of the octocoral species *Dentomuricea* aff. *meteor* during the pelagic phase under two experimental rearing temperatures. (A) Number of surviving larvae in each temperature; (B) proportion of larvae in different developmental stages (planula, metamorphosed, but not settled, settled, deformed)

corresponding to 3.21% (11 larvae) under 13°C and 1.46% (5 larvae) under 15°C. Nevertheless, surviving planulae displayed slightly but significantly different trends during the course of the study (Table 3.1), with a larger proportion of larvae settling earlier under 13°C than under 15°C (Figure 3.4). A high variance was observed on the estimates of the average proportion of settled larvae (Figure 3.4) among batches at 13°C, mainly due to a single batch in which very few larvae settled throughout the study period.

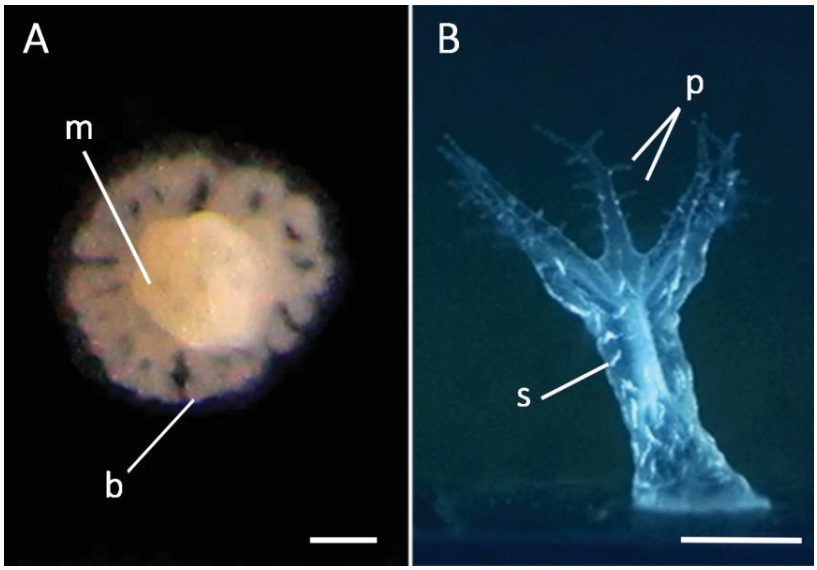

Figure 3.5: Formation of primary polyps from planula larvae of the octocoral *Dentomuricea* aff. *meteor*. A: Recently settled primary polyp with a polyp base (b) and formation of eight mesenteries (m); B: final primary polyp with sclerites (s), tentacles and tentacle pinnules (p). Scale bar 500 µm.

After day 20, an increasing proportion of the surviving larvae initiated metamorphosis without settling (Figure 3.4), in both temperature regimes. This form of pelagic metamorphosis started with planula larvae obtaining a pear shape (Figure 3.6A) and continued with formation of mouth, mesenteries, tentacles and finally sclerites (Figure 3.6B, Figure 3.6C). Metamorphosis from planula larva to primary polyp took approximately 2-3 days. None of the larvae that displayed pelagic metamorphosis settled during the course of the study. Metamorphosed larvae were still able to get transported by water movements but displayed limited swimming ability. The trend of pelagically metamorphosed larvae appeared to be significantly different between the two temperatures (Table 3.1), but the constructed model was heavily influenced by one batch under 15°C in which all remaining planulae on day 31 metamorphosed pelagically and subsequently

presented deformations and deceased (Figure 3.4B). Overall, during the experimental period 26 larvae metamorphosed pelagically under 13°C and 28 under 15°C, representing only 7.5% and 8.18% of the initial planulae pool. Deformed larvae were observed in both temperatures but represented a small proportion of the initial pool (2.02% under 13°C and 1.16% under 15°C). Under 13°C, they started appearing on day 24 (Figure 3.4B) but remained in low numbers throughout the experimental period. Under 15°C, they appeared 2 days later, but reached significantly higher proportions after day 35 (Table 3.1, Figure 3.4B). Most of these late deformations under 15°C were observed in pelagically metamorphosed larvae (Figure 3.6D).

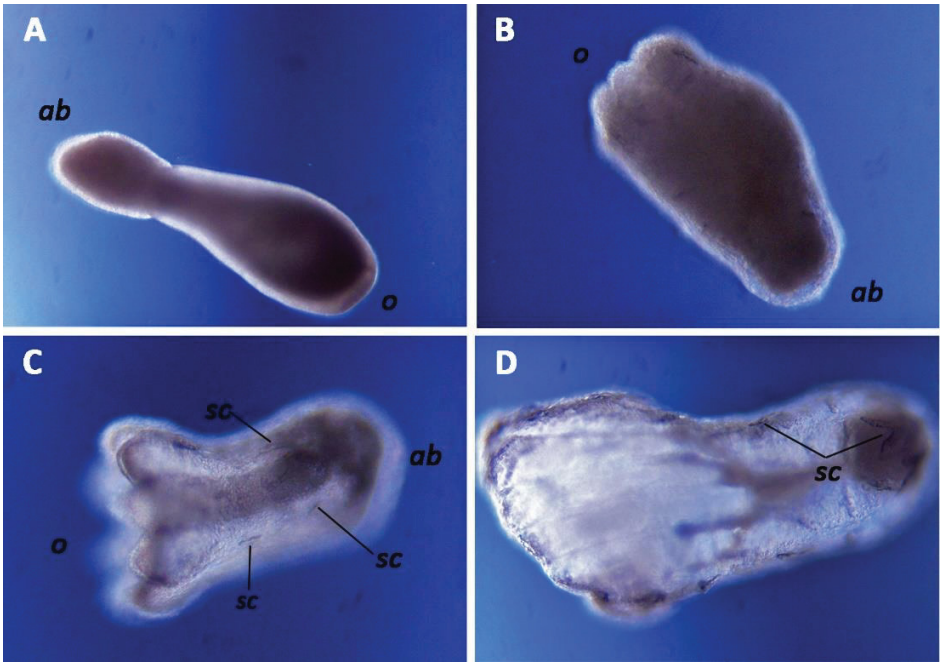

Figure 3.6: Pelagic metamorphosis of larvae of the octocoral species *Dentomuricea* aff. *meteor*. A: pear shaped larva with formed mouth in the oral side (o) and closed aboral side (ab); B: tentacle formation on the oral side; C: Fully formed tentacles, mesenteries and sclerites (sc); D: deformed larva with abnormal mesentery and tentacle formation.

3.1.5. Discussion

So far, studies on the biology and ecology of deep-sea octocorals have focused mainly on the adult stage (Watling et al. 2011), with very few studies tackling early life history stages (Cordes et al. 2001, Sun et al. 2010b, 2011). To our knowledge, the present study is the first to provide a

detailed insight to the larval biology of a deep-sea octocoral species including embryo and larval development, larval survival, swimming and settlement behaviour, which are essential variables to understand dispersal and connectivity in the deep-sea (Gary et al. 2020).

In our study, it was not clear if spawning was actually induced, assisted or just coincided with sperm enrichment, due to the limited time interval between sperm enrichment and the first gamete release. Repetitive release of gametes or planulae within a specific period is common among octocorals, including tropical broadcast spawning (Pakes & Woollacott 2008, Wells et al. 2020), temperate brooding (Weinberg & Weinberg 1979, Martínez-Quintana et al. 2015) and deep-sea brooding species (e.g. Sun et al. 2011b). This strategy may increase the probability that some embryos and larvae develop under optimal conditions (Kahng et al. 2011). In species with this behaviour, studying the effects of different environmental variables on embryo development is crucial, as embryos of different cohorts are likely to be released under different environmental conditions including temperature, salinity, pH and food availability.

Embryo and larval development of *Dentomuricea* aff. *meteor* had many similar characteristics with other octocoral species. Unequal cleavage that ranges from radial to pseudospiral is common among cnidarians (Fritzenwanker et al. 2007), including tropical brooding (Benayahu & Loya 1983, Dahan & Benayahu 1997) and broadcast spawning (Mandelberg-Aharon & Benayahu 2015) octocorals. Superficial cleavage, like the one observed in *D.* aff. *meteor* is frequently encountered in embryos with high amounts of yolk reserves (Scriba 2015), indicating a lecithotrophic larvae which was also confirmed by the absence of oral opening before metamorphosis. Larval size was also comparable to that of other octocoral species (Table 3.2). Overall, these findings suggest that some reproductive and larval characteristics might be conserved among taxonomically related groups, despite local adaptations due to depth and other habitat limitations.

Temperature is considered one of the main factors affecting larval biology, with higher temperatures usually resulting in faster developmental rates (Hoegh-Guldberg & Pearse 1995).

Our results were consistent with this premise, with larvae reaching the planula stage 24 hours earlier at 15°C when compared to 13 °C. This difference in developmental time is likely the drive between the differences in the LW ratios, since planulae tend to be more elongated with age. The different developmental rates did not affect survival which was similar and very low for both temperatures but varied substantially among batches. Since it was not possible to observe which female colonies participated in each gamete release, the possibility that first batches had lower survival cannot be excluded. Larval characteristics such as size and longevity have been shown to vary between cohorts in many marine larvae (Marshall et al. 2008, Cumbo et al. 2012). Such variability between offspring has been considered an adaptive strategy to increase offspring survival in species that inhabit unstable environments (Cooper & Kaplan 1982, Marshall et al. 2008).

Most deep-sea octocoral planulae studied so far, displayed low mobility, negative buoyancy and crawling (e.g. *Drifa glomerata*, Sun et al. 2010a; *Duva florida*, Sun et al. 2011b) or very limited swimming capacity (e.g. *Drifa sp*., Sun et al. 2010b). On the contrary, larvae of *D.* aff. *meteor* were active swimmers but initiated swimming only after stimulation, a behaviour also recorded in *Corallium rubrum* (Martínez-Quintana et al. 2015). Swimming in *D.* aff. *meteor* was random, as revealed by the similar proportion of upward and downward swimming. When compared to other deep-sea broadcast spawning corals, such as the scleractinian *Lophelia pertusa*, *D.* aff. *meteor* had lower swimming capabilities, especially since *L. pertusa* displayed intense, negative geotactic behaviour (Larsson et al. 2014). Swimming velocity of *D.* aff. *meteor* was comparable to that of *C. rubrum* (Table 3.2) and *L. pertusa* (Larsson et al. 2014), but larvae of these species were maintained under different temperature regimes (19-20 °C for *C. rubrum*, Martínez-Quintana et al. 2015; 8-12 °C for *L. pertusa*, Larsson et al. 2014). Temperature can affect both larval physiology and water characteristics since higher temperature often causes a decrease in viscosity and increase in larval metabolic rates (Herbing 2002). Both effects can result in higher swimming velocity and are likely to be associated with the higher larval swimming speed of *D.* aff. *meteor* under 15°C. Nonetheless, metabolism is not the only physiological process affected

by temperature and larvae display physiological limits, which need to be further studied for the target species.

Larval planktonic period can be divided in two phases, an obligatory phase that lasts until the onset of developmental competence (the ability to respond to settlement cues) and a facultative phase that depends on settlement behaviour in response to the existence of certain substrate characteristics (competency window, Elkin & Marshall 2007). In the present study, both phases were characterized by high mortality, leading to a loss of more than 50% of planulae before the defined onset of competency. Moreover, the onset of competency was inferred by the first larval settlement since larvae did not display any specific geotactic or bottom probing behaviour, but it is possible that larvae had entered competency before actually settling. Settlement rates were low and a higher proportion of the surviving larvae metamorphosed without settling. These are strong indications that adequate settlement surfaces and cues were not provided during the study. It is thus likely that larvae were forced to proceed to the next ontogenetic phases (settlement and metamorphosis) due to the lack of energy reserves. This phenomenon has been tentatively explained by the “desperate larvae hypothesis” (Gibson 1995, Marshall & Keough 2003), which states that the duration of the planktonic phase is likely determined by the availability of energetic reserves (Wendt 2000) and therefore non-feeding larvae can only delay settlement and metamorphosis until reaching a specific reserve level (Elkin & Marshall 2007).

Remarkably, settlement only took place on plastic surfaces while none of the larvae attached on the provided basalt rock. This was slightly unexpected since *D*. aff*. meteor* has been observed to colonize basalt rock in seamounts in the Azores. It is highly possible that this was due to the lack of bacterial biofilm on the rock, which has been shown as an important settlement clue for other invertebrates (Hadfield 2011). Moreover, the provided rock occupied a very small area compared to the flask walls. Settling on plastic is not uncommon among octocorals (Lasker & Kim 1996, Freire et al. 2019, Carugati et al. 2021) but further studies with more settlement surfaces are essential to clarify the settlement requirements of the target species. Pelagic metamorphosis of planulae into polyps has also been reported for many octocorals from shallow tropical (Lasker &

Kim 1996, Ben-David-Zaslow & Benayahu 1998), to temperate (Linares et al. 2008a) and deep-sea species (Sun et al. 2011). In some corals, pelagic polyps can display high survival and dispersal potential (Mizrahi et al. 2014) and have the ability to feed (Ben-David-Zaslow & Benayahu 1998, Linares et al. 2008a).

In our study, pelagic polyps displayed high mortality but this could be due to the absence of sufficient or adequate food sources. Nevertheless, pelagic metamorphosis might provide a way to acquire feeding structures and allows the acquisition of energy while waiting for the right settlement cue. In the case of *D.* aff. *meteor*, the high proportion of surviving larvae that displayed this behaviour supports the hypothesis that larvae had limited energy reserves and possibly reached their maximum longevity during the experiment.

Under higher temperature, larvae of *D.* aff. *meteor* remained longer in the pelagic phase and displayed lower settlement rates. This was contrary to the expected outcome, since the higher developmental rates observed under higher temperature are expected to be accompanied by earlier competency and higher settlement rates (O'Connor et al. 2007, Heyward & Negri 2010). Faster developmental rates, accompanied by decreased settlement under higher temperatures (+ 3°C) has been also reported for the tropical octocoral *Heliopora coerulea* (Conaco & Cabaitan 2020). It is possible that these results are related to temperature-induced changes in developmental and physiological mechanisms that were not evaluated in our study. For example, it is possible that faster development under higher temperature was accompanied by faster metabolic rates (O'Connor et al. 2007) and resulted in faster consumption of reserves, leading to high rates of pelagic metamorphosis and deformations under the absence of proper settlement cues. Ontogeny depends on certain developmental processes and their timing and while developmental rate can be plastic, changes in timing are likely to have consequences on structure and function, ultimately affecting individual performance (Kováč 2002).

Overall, the embryonic and larval characteristics of *D.* aff. *meteor* suggest a higher dispersal potential than most deep-sea octocorals studied so far (Table 3.2). However, when compared to

Table 3.2: Summary of embryo and larval characteristics of octocoral species in the order Alcyonacea. Depth: deep (>200m) and shallow (<200m); Repr. mode: Reproductive mode; T: Temperature. Competency refers to the period when larvae are competent to settle. Variables are provided either as range, average ± standard deviation, maximum (max) or median (median) values.

Family	Habitat	Depth	Repr. mode	Species	T (°C)	Larval size (mm)	Competency (days)	Longevity (days)	Swimming behaviour	Swimming speed (cm/s)	Reference
Alcyoniidae	Temperate	Deep	Internal brooding	*Anthomastus ritteri*		3.3 ± 1	2-3, 123^{max}				Cordes et al., 2001
Coralliidae	Temperate	Shallow	Internal brooding	*Corallium rubrum*	22	1.5					Ben-David-Zaslow & Benayahu 1998
					19-21	1		28.9 ± 3.3	Vertical swimming	0.045-0.056	Martínez-Quintana et al. 2015
Gorgoniidae	Tropical	Shallow	Broadcast spawning	*Antillogorgia americana*	24		36^{median}	>60	Vertical swimming	0.22 ± 0.01^{m}	Coelho & Lasker 2016
	Temperate	Shallow	Internal brooding	*Eunicella singularis*	22	2.5					Ben-Zaslow and Benayahu, 1998
					18-20			35 ± 11.6			Guizien et al. 2020
Neptheidae	Tropical	Shallow	Internal brooding	*Parerythropodium f. fulvum*	21-26		1-64	76 ^{max}			Ben-Zaslow and Benayahu, 1998
	Tropical	Shallow	Broadcast spawning	*Dendronephthya hemprichi*	21-26		2-74	81^{max}			Ben-Zaslow and Benayahu, 1998
	Tropical	Shallow	Internal brooding	*Litophyton arboreum*	21-26		1-57	92^{max}			Ben-Zaslow and Benayahu, 1998
	Tropical	Shallow	Internal brooding	*Nephthea sp.*	21-26		1-57				Ben-Zaslow and Benayahu, 1998
	Subpolar/ Temperate	Deep	Internal brooding	*Gersemia fruticosa*		1.5-2.5	40-70		Swimming		Sun et al., 2011
	Subpolar/ Temperate	Deep	Internal brooding	*Duva florida*	0-9	1-2.5	5		Crawling		Sun et al., 2011
	Subpolar/ Temperate	Deep	Internal brooding	*Drifa glomerata*	2	4-5					Sun et al., 2010

Family	Habitat	Depth	Repr. mode	Species	T (°C)	Larval size (mm)	Competency (days)	Longevity (days)	Swimming behaviour	Swimming speed (cm/s)	Reference
Plexauridae	Tropical	Shallow	Broadcast spawning	*Plexaura kuna*	28-30	2	4-21				Lasker & Kim 1996
	Temperate	Shallow	Surface brooding	*Paramuricea clavata*	18-20			32 ± 11	Crawling		Guizien et al., 2020
	Tropical	Shallow	Broadcast spawning	*Plexaura homomalla*	27-29	1	4		Swimming and crawling	0.5	Wells et al. 2020
	Temperate	Deep	Broadcast spawning	*Dentomuricea* aff. *meteor*	13	1.15 ± 0.28	25	11	Swimming and crawling	0.024-0.036	This study
					15	1.14 ± 0.28	29	16	Swimming and crawling	0.04-0.044	This study
Xenidae	Tropical	Shallow	Internal brooding	*Xenia umbellata*	21-26		2-76	155^{max}			Ben-Zaslow and Benayahu, 1998
	Tropical	Shallow	Internal brooding	*Heteroxenia tuscescens*	21-26		49 ^{max}	50^{max}			Ben-Zaslow and Benayahu, 1998

other deep-sea species, the dispersal capacity of *D.* aff. *meteor* appears to be limited. For example, the scleractinian *L. pertusa* delayed the onset of competency up to 3-5 weeks from spawning, displayed active upward swimming and survived without settlement for approximately a year (Larsson et al. 2014).Similarly, other deep-sea species such as the bivalve *Bathymodiolus childressi* and the gastropod *Bathynerita naticoidea* display longer longevities (approximately one year) and enhanced upward swimming which indicate much higher dispersal potential than *D.* aff. *meteor* (Arellano & Young 2009). The larvae of these deep-sea species are planktotrophic and therefore are not constrained by reserve availability. Our results highlight that the energy reserves of *D. aff. meteor* are a great limitation for many of its larval traits, especially its longevity and behaviour regarding settlement and metamorphosis. While its swimming behaviour is very likely to allow it to disperse among regional seamounts with the aid of local hydrodynamics, its short longevity is indicative of its narrow regional distribution in the North Mid-Atlantic Ridge, especially when compared with the wide distributions of *L. pertusa* and *B. childressi*.

Since the two temperature regimes used in this study are likely to be experienced by embryos of the target species in their natural environment, our results highlight how small changes in temperature can affect embryo development and larval characteristics, such as swimming velocity and settlement behaviour. Climate change is expected to cause changes in ocean circulation (Sweetman et al. 2017) which can modify the water mass dynamics and alter the physicochemical characteristics encountered by embryos and larvae (van Gennip et al. 2017, Claret et al. 2018). Under these circumstances, baseline information on the responses of early life history stages under variable conditions is essential to predict potential effects on dispersal and connectivity. For example, embryos and larvae of the Antarctic echinoderm *Sterechinus neumayeri* can withstand high pressures only under a narrow temperature interval which can be encountered in specific water masses that allowed the species to disperse to greater depths (Tyler et al. 2000). In the case of this species, potential changes in regional circulation, may affect or even disrupt connectivity between shallow and deeper populations. Moreover, larval dispersal and success are important features not only from an ecological but also from an evolutionary perspective, as their adaptive significance can define the selection of reproductive strategies such

as reproductive timing (Olive 1992, Crowder et al. 2014, Fan et al. 2017). In deep-sea corals, reproductive timing has been discussed in relation to the seasonal constraints of adult reproductive physiology (e.g. Orejas et al. 2002, Waller et al. 2014) but its relation to larval survival and success has not been addressed so far. Further studies on the effect of temperature on larval development, physiology and behaviour are therefore essential to obtain a holistic view of the potential impacts of climate change on deep-sea corals and communities.

3.1.6. Conclusions

In our study, we provided a detailed description of embryo and larval characteristics of the species *D.* aff. *meteor*. To our knowledge, this is the first systematic description of the early life history traits of a deep-sea octocoral. Our results suggest that *D.* aff. *meteor* larvae are lecithotrophic with development similar to other octocorals and low dispersal capacity compared to other deep-sea species. Rearing at different temperatures did not affect survival, but significant effects were detected on the rate of embryo development, swimming speed and settlement behaviour which in the field can potentially alter larval dispersal and ultimately success. Deep-sea octocorals are receiving increasing attention as a growing number of studies focus on the habitat requirements and environmental conditions shaping deep-sea communities (Radice et al. 2016, Barbosa et al. 2020, Morato et al. 2020c). However, understanding species distributions requires further knowledge on their early life history biology and dispersal, as these play a key role in the successful occupation of available suitable habitat (Schurr et al. 2007, Robinson et al. 2011). As attempts of biophysical dispersal modelling are increasing in the deep-sea (Hilário et al. 2015, Ross et al. 2016), further biological data to feed into these models are essential to obtain a better understanding of deep-sea ecosystems.

3.2. Larval biology of the deep-sea octocoral *Viminella flagellum*

3.2.1. Abstract

The whip coral *Viminella flagellum* is a common octocoral in deep-sea benthic communities throughout the Mediterranean, Macaronesia and eastern North Atlantic. Despite its importance as a habitat-forming species, there is currently very little information on its early life history and dispersal capacity. The aim of this study was to describe the embryo and larval characteristics of the species. Larvae were reared in aquaria conditions and closely observed to describe the embryo and larval development, longevity and swimming behaviour. Embryos were mostly neutrally buoyant and developed into planulae within 72h. Fully developed planulae lacked a mouth. On day five, larvae displayed active upward swimming behaviour which lasted until day 7-12, after which larvae returned to the bottom. Swimming speed was recorded on day 10 and was on average 2.06 ± 1.34 mm/s. After three months, only one larva survived and displayed a maximum longevity of one year. Swimming speed and longevity of *V. flagellum* are the highest reported for deep-sea octocorals so far and indicate a high dispersal potential.

3.2.2. Introduction

Embryo and larval biology are fundamental determinants of the ecology of marine species. They are key for species dispersal which affects their distribution, and a number of population characteristics such as recruitment, genetic structure, genetic diversity as well as connectivity among populations (Pineda et al. 2007, Cowen & Sponaugle 2009, Treml et al. 2015). Moreover, embryos and larvae constitute the early stages of the life cycle, and thus they have a central role in species persistence (Marshall et al. 2012, Przeslawski et al. 2015). These stages are frequently sensitive to disturbance and changes in environmental conditions (O'Connor et al. 2007, Przeslawski et al. 2015). Moreover, events during the early life history of an organism can have latent, or carry-over effects on subsequent stages, affecting species performance and survival (Pechenik 2006, Marshall & Morgan 2011). The importance of early life history stages is even more pronounced in sessile marine species, in which they constitute the only stage when dispersal is possible (Byrne 2012). Therefore, in deep-sea communities that are dominated by

benthic species, knowledge on early life histories is key to understand ecosystem dynamics (Hilário et al. 2015, Kenchington et al. 2019).

Deep-sea octocorals are major ecosystem engineers in the deep-sea (Buhl-Mortensen et al. 2017, Buhl-Mortensen & Buhl-Mortensen 2018). Their dense populations create habitat for a variety of species (Pham et al. 2015, De Clippele et al. 2015, Rueda et al. 2019). The species *V. flagellum* is a whip octocoral, commonly encountered in the Mediterranean, Macaronesia and eastern North Atlantic (Grasshoff 1972, Carpine & Grasshoff 1975). It creates dense aggregations, mostly on rocky substrates and detritic soft bottoms between 100- 350 m and has been reported up to 1000 m (Grasshoff 1972, Carpine & Grasshoff 1975, Brito & Ocaña 2004). In the Azores Archipelago, the species is very common on island slopes and seamounts, between 150-400 m of depth (Braga-Henriques et al. 2013).

Despite the wide distribution of *V. flagellum* and its importance as a common habitat-building species, there is currently no information on its embryo and larval biology. This is the case for most deep-sea octocorals, as information so far only exists for a few brooding (Cordes et al. 2001, Sun et al. 2009, 2010a) and one broadcast spawning species (Chapter 3.1). The objective of this study was to describe the embryo and larval biology of *V. flagellum*. This was achieved by performing observations on live embryos and larvae of *V. flagellum* that were reared in aquaria conditions, after spontaneous spawning and successful fertilization of gametes. The study provides information on the embryo and larval development, longevity and swimming behaviour of the target species.

3.2.3. Materials & Methods

Coral colonies of *V. flagellum* were collected as bycatch from experimental long-line fisheries in the framework of the ARQDAÇO monitoring program at Condor Seamount, in May-July 2017. A total of five colonies were collected, in depth ranging between 200-250 meters. Colonies were transferred to the DeepSeaLab aquaria facilities of IMAR-UAz (Orejas et al. 2019) in coolers. Upon reaching the aquaria, colonies were separated in fragments (length 15-30 cm) and placed in 33L aquaria that were kept in a thermoregulated room in darkness. The aquaria were continuously supplied with seawater pumped from 5 m of depth, that was previously treated with UV light

(P10 UV system and Vecton 600, TMC), and passed through filters with mesh size of 50 µm and 1 µm. Before entering the aquaria, water was refrigerated with the aid of coolers (14 ± 0.6 °C), matching the temperature recorded at the site of collection (Santos et al. 2013). Colonies were fed daily with a mixture of frozen microalgae, microzooplankton and frozen thawed macrozooplankton (Artemia nauplii, Mysis shrimps).

Spawning and successful fertilization of the target species occurred in a tank holding several coral fragments. A total of 29 fertilized oocytes were collected and transferred to a 250 ml beaker filled with filtered water (0.2 µm) and placed within a water bath keeping temperature stable at 14 ± 0.6 °C. Air bubbling was used to ensure water circulation within the beaker and water was renewed daily. In order to describe embryo and larval development, embryos were observed every 3-12h until reaching the planula stage and subsequently planulae were observed every 2-3 days. During observations, information on the developmental stage, number of larvae and position in the water column were noted. Data on swimming speed were collected on the 28th of August (day 10). To estimate swimming speed, larvae (n=8) were transferred in a 1 cm (diameter) labyrinth, one at a time. Larvae were left to acclimatize for 1 minute and subsequently three video segments with duration of 1 min were recorded with a calibrated dissecting microscope (DinoLite AM7013MT). During video recording, water was exchanged before introducing new larvae and temperature was closely monitored to match the rearing temperature (14 ± 0.6 °C). Videos were processed by using a manual tracking method within the software ImageJ.

After the first week from spawning, water was renewed every 3-4 days and small quantities of food were given weekly, consisted of a mixture of freshly harvested, mashed zooplankton and phytoplankton. Upon observing downward swimming behaviour, a piece of basalt was offered to the larvae as a potential substrate.

3.2.4. Results

Spawning occurred on the 18th of August 2017. Embryos had neutral buoyancy. The collected embryos were already on the 8-cell stage and had a diameter of 1216.1 ± 21.3 µm (average ± sd). Division was always holoblastic and cleavage was mostly spiral (Figure 3.7). Three hours after encountering the embryos most of them were already in the 32-cell stage. They reached the

blastula stage within 24 hours and the planula stage within 72 hours. During that period all embryos had neutral buoyancy.

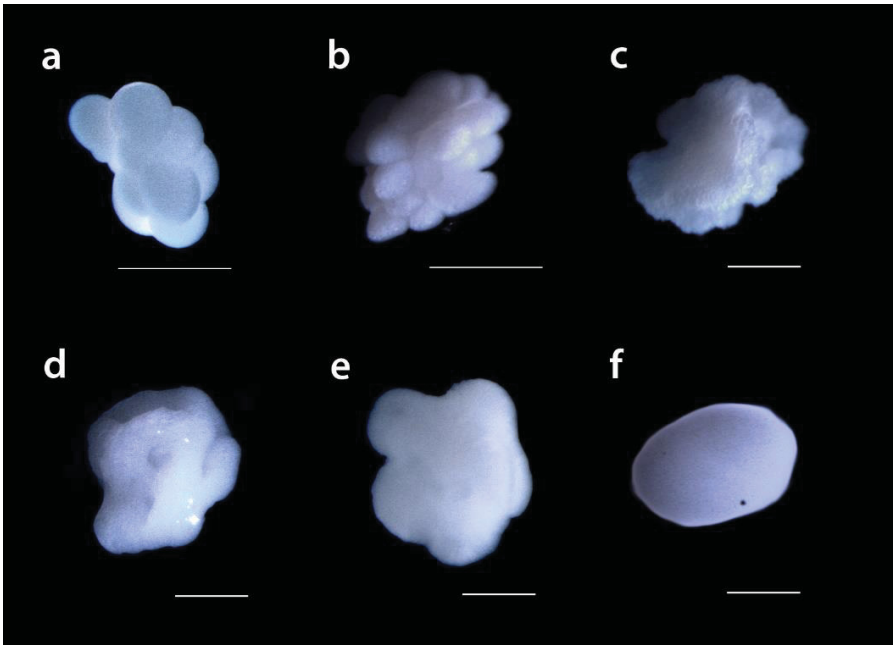

Figure 3.7: Embryo development of the species *Viminella flagellum*. (a) 8 cell embryo; (b) morula; (c) 1 day blastula; (d) 2 days gastrula; (e) 3 days gastrula; (f) early planula. Scale bar: 500 µm.

Early-stage planulae were mainly spherical with an average diameter of 1522.6 ± 19.3 µm. Mature planulae could alternate between a spherical and more elongated shape. Planulae after day 5 were mostly elongated and their length could reach 4-8 mm. On the fifth day all larvae were encountered on the surface of the beaker and displayed active upward swimming behaviour, even if moved lower in the water column. Swimming was frequently performed in rotational, counterclockwise movements, but upward swimming was mostly performed in a straight line. On the same day, survival was 41.3%, and only 12 larvae remained.

Bottom probing was first observed on day 7 and by day 12 all larvae had returned to the bottom, resulting to a medial pelagic larval duration (PLD) of 9.5 days. On day 10, swimming speed was 2.06 ± 1.34 mm/s, while minimum and maximum recorded speed corresponded to 0.12 mm/s and 6.5 mm/s, respectively. After reaching the bottom, most larvae performed horizontal movements, and attached temporarily in the provided substrate. The number of larvae declined

and after three months only one larva remained (survival 5%). This larva survived for a total 210 days. During this period, it performed very limited movements between crevices, attached temporarily and gradually became more transparent (Figure 3.8). On the sixth month, the larva metamorphosed partially and developed a mouth and a gastrovascular cavity (Figure 3.8). No permanent attachment was observed during the study period.

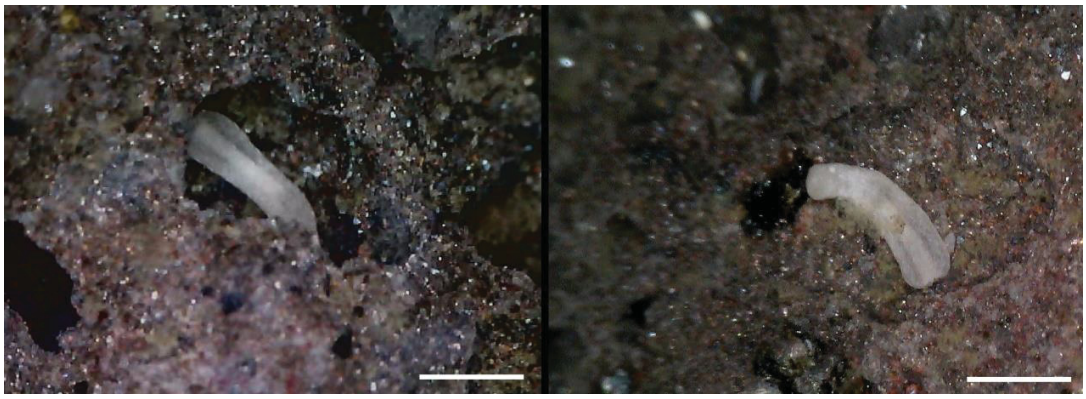

Figure 3.8: Bottom probing behaviour displayed by larvae of the gorgonian *Viminella flagellum*. Scale 7 mm.

3.2.5. Discussion

So far, our knowledge on the embryo and larval biology of deep-sea octocorals is scarce, and mainly limited to brooding species (Cordes et al. 2001, Sun et al. 2011). Embryo and larval development of *V. flagellum* had characteristics similar to the ones reported for other octocorals. Both in broadcast spawning and brooding species, the planula stage is typically reached within 24-96h (Coelho & Lasker 2016, Wells et al. 2020) and planulae are commonly large and lecithotrophic, both in deep-sea (Cordes et al. 2001, Sun et al. 2011) and shallow species (Martínez-Quintana et al. 2015, Wells et al. 2020).

To our knowledge, this is the first time that active upward swimming is recorded in a deep-sea octocoral. Up to date, studies on deep-sea octocorals report either crawling (Sun et al. 2010a, 2011) or swimming after stimulation (Chapter 3.1). Larvae of *V. flagellum* also displayed much higher swimming speed than broadcast spawning deep-sea (e.g. *Dentomuricea* aff. *meteor*, Chapter 3.1) and temperate octocorals, e.g. *Corallium rubrum*, in which swimming speed was

between 0.2-0.6 mm/s (Martínez-Quintana et al. 2014). In addition, *V. flagellum* displayed a late onset of competency compared to most deep-sea and shallow octocoral species studied so far, which appear to be competent to settle within 1-3 days from spawning (e.g. Ben-David-Zaslow & Benayahu 1998, Cordes et al. 2001). Larvae of *V. flagellum* were also able to delay settlement up to a year. Such a long larval longevity and competency period has not been recorded so far in deep-sea octocoral species (see Table 3.2).

Larval characteristics such as swimming ability, PLD and longevity are extremely important, as they determine the ability of a species for dispersal (Cowen & Sponaugle 2009). Overall, *V. flagellum* displayed characteristics that indicate a higher capacity for dispersal compared to all the known deep-sea octocorals. However, its dispersal potential appears to be lower compared to the that of *Lophelia pertusa* (syn. *Desmophyllum pertusum*, Addamo et al. 2016). *Lophelia pertusa* releases a large number of oocytes which after fertilization develop to relatively small planktotrophic larvae (127–178 µm) with active upward swimming (speed: 0.5 mm s^{-1}) and appearance of bottom probing behaviour only after 3-5 weeks (Larsson et al. 2014). These larvae are also capable of crossing salinity gradients and survive in surface water, suggesting that they possibly migrate to the photic zone during the pelagic phase (Strömberg & Larsson 2017). Although larvae of *V. flagellum* displayed upward swimming, it is unknown if they are able to perform vertical migrations to shallower depths and more further studies are essential to clarify this. *Viminella flagellum* releases a lower number of oocytes (Rakka et al. 2021b), and larvae are larger, lecithotrophic, with a higher swimming speed than *L. pertusa*. However, they remain in the pelagic phase only for 7-12 days. This might be a result of their feeding strategy, as they have limited reserves compared to larvae of *L. pertusa* which can feed in the water column. The lack of reserves was also corroborated by the fact that the single larva that survived for a year metamorphosed partially and obtained a mouth and gastrovascular cavity, very likely due to the lack of reserves.

Although larvae of *V. flagellum* displayed bottom probing behaviour and attachment, no settlement was observed during the study period. This is likely due to the absence of suitable substrate. Basalt rock is a common feature in the natural habitat of *V. flagellum* in the Azores (Tempera et al. 2013), however the substrate provided to the larvae was not previously treated

to develop biofilm which is an important component for larval settlement (Gleason & Hofmann 2011, Freire et al. 2019). Moreover, although in many shallow coral species care is taken to treat the substrates with suitable biofilm such as crustose coralline algae (e.g. Golbuu & Richmond 2007, Whitman et al. 2020) this process is much more challenging in the case of deep-sea species.

The present study provides essential information on the embryo and larval biology of the target species, however it is based on a very small sample size. Moreover, considering the low number of obtained embryos, it is very likely that these resulted from spawning of a single female fragment in the aquaria. *Viminella flagellum* displays low polyp fecundity (Rakka et al. 2021b) which poses an additional challenge in attempts to study embryo and larval biology. Early life history stages are extremely important not only for species dispersal, but for species persistence in a constantly changing ocean. Development is a complex procedure and species success requires the completion of all stages (Byrne 2012). Considering the importance of deep-sea octocorals as ecosystem engineers in the deep-sea, it is essential to improve our knowledge on early life history stages and further studies are essential to provide further insights on embryo development, larval behaviour and dispersal potential of deep-sea octocorals.

Chapter 4
Contrasting metabolic strategies of two co-occurring deep-sea octocorals[3]

4.1. Abstract

The feeding biology of deep-sea octocorals remains poorly understood, as attention is more often directed to reef building corals. The present study focused on two common deep-water octocoral species in the Azores Archipelago, *Dentomuricea* aff. *meteor* and *Viminella flagellum*, aiming at determining their ability to exploit different food sources. We adopted an experimental approach, with three different food sources, including live phytoplankton, live zooplankton and dissolved organic matter (DOM), that were artificially enriched with ^{13}C and ^{15}N (C and N tracers). The presence of tracers was subsequently followed in the coral tissue, C respiration and particulate organic C and N (POC and PON) release. In both species, feeding with zooplankton resulted in significantly higher incorporation of tracers in all measured variables, compared to the other food sources, highlighting the importance of zooplankton for major physiological processes. Our results revealed contrasting metabolic strategies between the two species, with *D.* aff. *meteor* acquiring higher amounts of prey and allocating higher percentage to respiration and release of POC and PON than *V. flagellum*. Such metabolic differences can shape species fitness and distributions and have further ecological implications on the ecosystem function of communities formed by different octocoral species.

[3] Rakka M, Maier SR, Van Oevelen D, Godinho A, Bilan M, Orejas C, Carreiro-Silva M (2021) Contrasting metabolic strategies of two co-occurring deep-sea octocorals. Sci Rep 11:10633.

4.2. Introduction

Octocorals are common benthic suspension feeders in tropical, subtropical, temperate and polar regions (Watling et al. 2011, Sánchez 2016, Rossi et al. 2017a). The majority of octocoral species are found in waters deeper than 50 m (Cairns 2007b) where they create dense single- or multi-species aggregations, structuring three-dimensional and highly heterogenous habitats known as coral gardens (Freiwald & Roberts 2005, Rossi et al. 2017a). These communities provide essential habitat for a variety of associated fauna (Rossi et al. 2017a, Buhl-Mortensen & Buhl-Mortensen 2018).

The Azores Archipelago, located in the central North Atlantic, harbors an extremely rich biodiversity of cold-water octocorals, reaching a total of 101 species which represent the highest octocoral species richness known so far in North Atlantic (Braga-Henriques et al. 2013, Sampaio et al. 2019). Coral gardens constitute the most prominent cold-water coral (CWC) habitat in the Azores, with monospecific or multispecific octocoral communities frequently colonizing seamounts and island slopes (Tempera et al. 2012, Braga-Henriques et al. 2013). Because of their life-history traits, including slow growth and high longevity, recovery of octocoral communities from fisheries and other disturbances can be very slow (Andrews et al. 2009, Neves et al. 2015) and thus coral gardens have been classified as vulnerable marine ecosystems (VMEs) in need of protection (FAO 2009, OSPAR 2010). However, effective conservation of VMEs requires knowledge on the species biology and ecology which in the case of deep-sea octocorals is scarce (Watling et al. 2011).

Resource acquisition is a key factor in the biology of suspension feeders (Kim & Lasker 1998, Gori et al. 2013) ultimately determining population dynamics and species distributions (Coma & Ribes 2003, Nisbet et al. 2008, Sebens et al. 2017). Thus, knowledge on feeding biology of key habitat formers such as octocorals is pivotal to understand local ecosystems. Octocorals can feed on a variety of prey including microplankton, nanoeukaryotes, as well as detritus (Ribes et al. 1999, 2003, Orejas et al. 2003, Cocito et al. 2013). In some cases, their diet varies seasonally following the cycles of local phytoplankton and zooplankton communities (Gori et al. 2013, Leal et al.

2014a). Although most octocoral species seem to be able to ingest and utilize phytoplankton (Fabricius et al. 1995a, Orejas et al. 2003, Leal et al. 2014a) small zooplankton with low mobility is the main component of the natural diet of many temperate species (Rossi et al. 2004, Cocito et al. 2013, Coma et al. 2015). While considerable knowledge exists on the feeding biology and ecophysiology of shallow octocorals, such information is scarcer for deep-sea octocorals with a few studies so far focusing mainly on Antarctic ecosystems (Orejas et al. 2001, 2003).

In this study, we examined the feeding biology of two common habitat-forming deep octocoral species in the Azores Archipelago: *Dentomuricea* aff*. meteor* and *Viminella flagellum*. The two species form dense coral gardens (Figure 4.1) on seamounts between 200-600 m and very frequently occur in mixed populations (Tempera et al. 2012, Braga-Henriques et al. 2013). The objective of the study was to determine (1) the ability of the two species to exploit different food sources and (2) whether assimilation of different food sources affects their metabolic activity. We employed an experimental approach, with the use of aquaria flumes with steady flow velocity and four different food treatments including provision of live phytoplankton, dissolved organic matter (DOM) and live zooplankton, as well as fasting (deprivation of particulate food). Food sources were artificially enriched with ^{13}C and ^{15}N and food utilization was quantified (a) as the appearance of $^{13}C^{15}N$ in the coral tissue, indicating tracer carbon (C) and nitrogen (N) incorporation from the provided food, (b) as the production of ^{13}C-enriched dissolved inorganic carbon (DIC) by the coral, indicating tracer C respiration, and (c) as the production of $^{13}C^{15}N$-enriched particulate organic carbon and nitrogen (POC, PON) by the coral, indicating tracer POC and PON release.

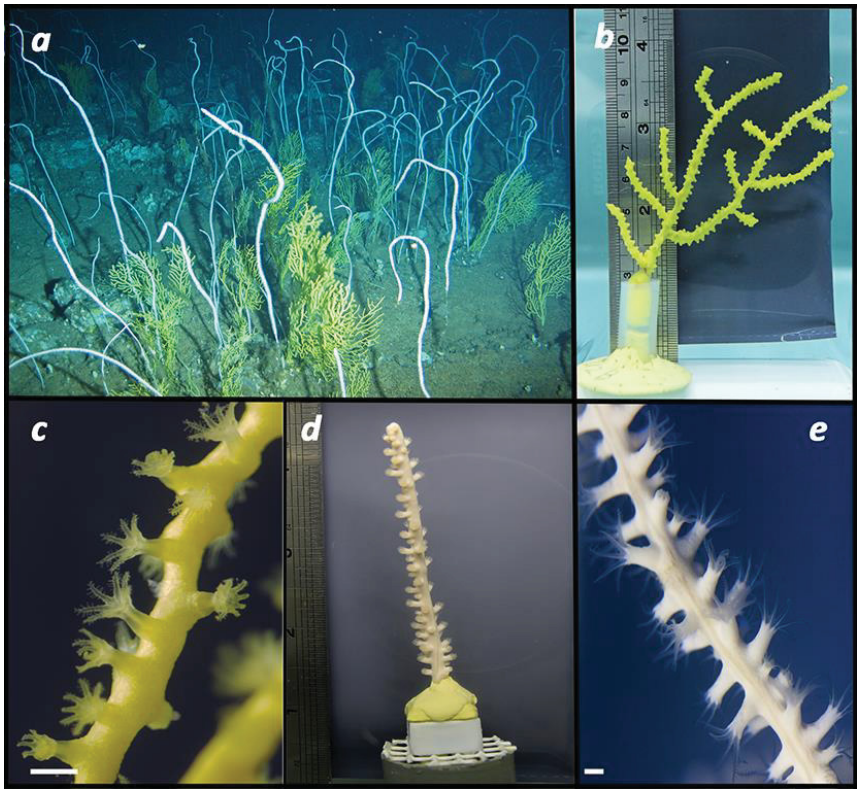

Figure 4.1: (a) Mixed coral garden of the octocorals *Viminella flagellum* and *Dentomuricea* aff. *meteor* (Gavin Newman, Greenpeace); coral fragment of *D.* aff. *meteor* (b) and its polyps (c); coral fragment of *V. flagellum* (d) and its polyps (e). Scale bar 1 mm.

4.3. Methods

4.3.1. Target species

The species *Dentomuricea* aff*. meteor* is a fan-shaped alcyonacean of the family Plexauridae. Its known distribution is limited to seamounts close to the Mid-Atlantic ridge where it is typically encountered between 200-400 m depth (Braga-Henriques et al. 2013). It can reach heights of up to 1.5 m and can create dense monospecific or mixed populations with other species (Figure 4.1a).

Viminella flagellum (Johnson 1863) is a whip coral of the family Ellisellidae. It creates monopodial colonies without branches which can grow up to 3 m height (Weinberg & Grasshoff 2003). Its distribution includes the eastern North Atlantic coast, islands of the Macaronesia and the

Mediterranean Sea (Carpine & Grasshoff 1975, Brito & Ocaña 2004, Cau et al. 2015). It is usually encountered between 120-500 m depth (Carpine & Grasshoff 1975, Brito & Ocaña 2004) and can form monospecific or mixed coral aggregations, often with branching octocorals such as *D.* aff*. meteor*, *Callogorgia verticillata and Acanthogorgia armata* (Tempera et al. 2012).

4.3.2. Colony collection and maintenance

Live colonies of both *V. flagellum* and *D.* aff*. meteor* were collected as by-catch from long-line fisheries on R/V *Archipelago* (ARQDAÇO monitoring program, University of the Azores) and on commercial fishing vessels through a fisheries observer program, during September-November 2017. Collection was performed in various seamounts within the Azorean EEZ (Table S 2.1). Colonies were transferred to the aquaria facilities of IMAR (DeepSeaLab) in coolers and distributed in three 170 L aquaria in a thermo-regulated room, in darkness. Corals were inspected for potential tissue injuries from the collection process and colonies with unhealthy tissue were discarded. Aquaria were supplied with seawater pumped from 5 m depth in continuous flow-through open systems. Before entering the aquaria, water was treated with UV-light (P10 UVsystem and Vecton 600, TMC) and was repeatedly filtered (mesh size: 50 µm and 1 µm). Temperature was maintained at 14 ± 0.7 $^{\circ}$C, which is similar to the temperature recorded at coral gardens of the two species (Santos et al., 2014), by cooling systems connected to temperature controllers. Corals were fed daily with a frozen mixture of microalgae, microzooplankton and frozen thawed macrozooplankton (*Artemia* nauplii, *Mysis* shrimps), which was enriched with live microalgae and rotifers 2-3 times per week. The collected colonies were left to acclimatize for approximately three months in the aforementioned conditions. Subsequently, colonies were divided in 8-10 cm fragments and mounted to bases made of epoxy one month before the experiments (Figure 4.1b, Figure 4.1d). During this period, fragments were closely monitored to ensure that they had vibrant colour, intact tissue, and displayed polyp activity.

4.3.3. Feeding experiment

Four different food treatments were created, based on: a phytoplankton derived source (PHYTO), a zooplankton derived source (ZOO), a dissolved organic carbon (DOM) derived source and a fourth treatment where no particulate food source (size >1µm) was provided (fasting, FAST). Food treatments were created based on current knowledge of the species biology and on available food sources in their natural environment. The diatom species *Chaetoceros calcitrans* was selected as a phytoplankton-derived food source. Species of the genus *Chaetoceros* are common components of spring blooms in some of the sampling sites, e.g. Condor Seamount (Santos et al. 2013). The rotifer *Branchionus plicatilis* was selected as zooplankton-derived food source due to its small size (140-330 µm) and slow swimming capacity, which correspond to the characteristics of zooplankton prey usually captured by octocoral species (Cocito et al. 2013, Coma et al. 2015). Due to the known capacity of cnidarians to utilize DOM (Sorokin 1973, Gori et al. 2014) this food source was also used as a food treatment. Lastly, the FAST treatment aimed at measuring the basal metabolic activity of the corals, in the absence of particulate food.

The feeding experiment was run in four 33 L flumes designed to keep live prey in continuous circulation (Figure S 2.1), which allowed the use of a multilevel experimental design (Figure 4.2). The different number of available specimens for the two species led to a slightly different experimental design for each (Figure 4.2), however the same rationale was followed for both species. One month before the experiment, each mother colony (n=5 for *D.* aff*. meteor* and n=12 for *V. flagellum*) was divided in smaller fragments (n=5 for each colony of *D.* aff *meteor* and n=4 for each colony of *V. flagellum*) and these were randomly distributed to the four food treatments. The characteristics of fragments in the different treatments are presented in Supplementary Table S 2.2. Because of the limited number of experimental flumes, we repeated the experimental work several times, in order to have more than one aquaria replicates for each food treatment (n=3 for *D.* aff*. meteor* and n=4 for *V. flagellum*). Each repetition is referred to as experimental cycle (Figure 4.2). At the beginning of each experimental cycle, coral fragments were randomly positioned in the available aquaria for each treatment. This led to a total of 15 coral fragments for each food treatment for the case of *D.* aff*. meteor* and 12 coral fragments for

each food treatment for *V. flagellum*. Dependence among fragments on the colony and aquaria level was treated statistically, by the use of mixed effects models (see statistical analysis).

Before each experimental cycle, zooplankton and phytoplankton food sources were prepared by adding enhanced levels of the stable isotope tracers ^{13}C and ^{15}N to the respective culture media. Two microalgae species, *C. calcitrans* and *Nannochloropsis gaditana* were cultured using artificial

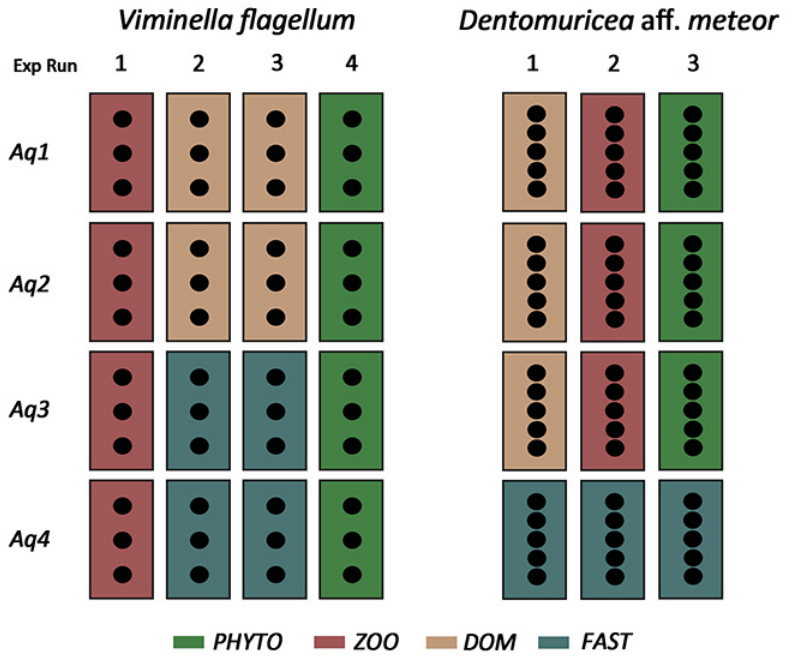

Figure 4.2: Experimental design of the two feeding experiments with the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum*. Exp cycle: Experimental cycles; Aq: Aquaria; PHYTO: phytoplankton *Chaetoceros calcitrans*; ZOO: zooplankton *Branchionus plicatilis*: DOM: dissolved organic matter; FAST: no food provision. Rectangles represent experimental aquaria and black dots represent coral fragments.

seawater and an F/2 culture medium containing 50 % ^{15}N-sodium nitrate ($NaNO_3$, Cambridge Isotopes) and 100 % ^{13}C-bicarbonate ($NaHCO_4$, Cambridge Isotopes) for three weeks. Subsequently, cultures were harvested by filtering with membrane filters (0.2 µm), rinsed with filtered SW (0.2 µm) and re-suspended in artificial SW. Rotifer starter cultures (concentration: 45 rotifers ml^{-1}) were inoculated in filtered seawater (1 µm), and continuously fed with $^{13}C^{15}N$-enriched *N. gaditana*, cultured as described above, for 6 days. Rotifer cultures were harvested

by filtering (nylon filters, 40 µm), rinsed and re-suspended in artificial seawater. Preliminary analysis was performed to ensure that harvesting procedures did not affect cell concentration significantly. An algal-derived product of dissolved amino-acids (Cambridge Isotopes, U 13C 97–99 %, U ^{15}N 97–99 %, CNLM-452-0.5) was used as DOM food source.

Prey was provided to a target carbon (C) concentration of 10 µmol L^{-1}, similar to POM concentrations previously recorded in mixed gardens of the two species (A. Colaço, pers. comm.). Cultures were scheduled to reach the desired prey concentration, corresponding to the desired target C concentration, on the day of delivery and were harvested a few hours before provision. To monitor the experimental food concentrations, aliquots of the provided food were taken before provision and analyzed *a posteriori* for DW and carbon content.

For both species, each experimental cycle consisted of five days. At the start of each cycle, fragments were positioned in the aquaria one next to the other, perpendicular to the current, avoiding branch overlapping. Once per day a predefined quantity of food was provided to reach a concentration of 10 µmol C L^{-1}. Flow of 4 cm s^{-1} was established for one hour and water renewal was paused just before supplying aquaria with food. This flow speed was selected as it allowed both species to capture live prey (Rakka et al., unpublished data) and kept prey in suspension for 12 hours without affecting its concentration. After 12 hours, water renewal was reestablished and all remaining food was cleaned by siphoning.

In the last day of each experimental cycle and immediately after the end of feeding, closed cell incubations were performed to measure oxygen consumption, DIC respiration and POC/PON release. Seven coral fragments from each food treatment were transferred to 450 ml glass chambers with pre-filtered seawater (0.7 µm) and glass-coated magnetic stirrers. All chambers were placed in a water bath keeping temperature at 14 ± 0.5 °C. Another two chambers were left without coral fragments and served as controls. Respiration rates were derived from depletion of dissolved O_2 during the incubation, measured by a single channel oxygen meter (Fibox4) with a PSt3 sensor (PreSens, Germany). The chamber with the larger fragment was continuously

connected to the sensor to monitor oxygen saturation levels during the incubations. Each incubation lasted for approximately 14 hours in which oxygen saturation never dropped below 80%. Oxygen consumption was standardized to the tissue organic carbon content (OC), i.e. without taking into account the main skeletal axis. These values were adjusted for rates recorded in chambers without coral fragments to account for microbial respiration. Water samples were taken before and after each incubation to determine the concentration of DIC and ^{13}C-DIC. Samples were kept in 10 mL headspace vials with 10 µL of a saturated mercury chloride solution and stored at 4°C until analysis. Lastly, the remaining water (300 ml) from each chamber after the end of the incubation was filtered through precombusted, preweighted GF/F (0.7 µm, WHATMAN) filters to estimate POC, ^{13}C-POC, PON and ^{15}N-PON release. Filters were freeze-dried and kept at room temperature until analysis.

Upon completion of each experimental cycle, fragments were freeze-dried and stored at -80 ºC. Fragments were dissected to separate the tissue from the skeleton. The tissue was ground by mortar and pestle, and a subsample was analyzed for total C and N content and isotopic ratios using an elemental analyzer (Thermo Electron Flash 1112) coupled to an isotope ratio mass spectrometer (EA-IRMS, DELTA-V, THERMO Electron Corporation). A second subsample was acidified stepwise with drops of HCl to remove the inorganic C fraction, and all remaining material was analyzed on the elemental analyzer for organic C content and isotopic ratio.

Calculation of tracer C and N incorporation, i.e. calculation of the amount of C and N which came from the provided food source and was incorporated in coral tissue, was performed as described in Maier et al. (2019b). Tissue C and N content of each fragment (i.e. without coral skeleton) was standardized to DW, and expressed as mmol C or N (g DW)$^{-1}$. The heavy/light isotope ratio (e.g. ^{13}C: ^{12}C) of each coral fragment (Rsample) was calculated as Rsample = ([δtracerCsample/1000] + 1) × Rref, where Rref = 0.0111802 for organic C (OC) and Rref = RN_2 = 0.0036782 for organic N (ON). Fractional abundance of ^{13}C and ^{15}N (e.g. $F^{13} = {}^{13}C/[{}^{12}C + {}^{13}C]$) was expressed as Ftracer = Rsample/(Rsample + 1). Experimental ^{13}C and ^{15}N enrichment of each coral fragment tissue was expressed in relation to the fractional abundance

of the respective fasting (non-enriched) fragment or average of fasting fragments of the same colony. Finally, tracer ^{13}C incorporation was calculated by multiplying ^{13}C enrichment with tissue OC content (µmol ^{13}C fragment^{-1}) and tracer ^{15}N incorporation was obtained by multiplying ^{15}N enrichment with tissue ON content (µmol ^{15}N fragment^{-1}). The total amount of C or N incorporated into coral tissue from the provided labelled food source (tracer C and N incorporation) was calculated by dividing the tracer C or N incorporation of each fragment with the fractional abundance (F^{13} or F^{15}) of the respective food source. Final tracer C and N incorporation rates were normalized to the OC (mmol) of each coral fragment.

Concentration of DIC was determined on an Apollo SciTech AS-C3 analyzer, after transforming DIC to gaseous carbon dioxide by addition of concentrated phosphoric acid (H_3PO_4, volume: 10 µL mL^{-1}) in each headspace vial. Subsequently, a 10 µL subsample of headspace gas was obtained from each vial and analyzed in the isotope ratio mass spectrometer, as described above, to obtain measurements of $\delta^{13}C$. Filters collected for POC and PON measurements were weighted (accuracy 0.1 mg) and analyzed with the isotope ratio mass spectrometer to obtain concentrations of POC and PON, as well as $\delta^{13}C$ of and $\delta^{15}N$ respectively. Determination of fluxes, i.e. DIC respiration, POC and PON release was estimated in two steps. Firstly, the bulk fluxes, i.e. the amount of total C and N released during the incubation period were calculated as the respective concentration difference between start and end water sample. Subsequently, tracer fluxes, i.e. the amount of C and N derived from the provided food were estimated, by multiplying bulk fluxes by their relative enrichment in ^{13}C and ^{15}N during the incubation, and dividing by the food enrichment following Maier et al. (2019b). A final tracer C budget was compiled by estimating tracer C incorporation, tracer C respiration and tracer C release for the duration of the whole experiment for each treatment and is reported as percentage of the provided C.

4.3.4. Statistical analysis

Data exploration was done following Zuur et al. (2010) to select the most appropriate statistical modelling method. To test if independent factors had a significant effect on the dependent variables in question, the former were added progressively to the models and the Akaike

Information Criterion (AIC) along with maximum likelihood ratio (MLR) tests were used to select the most appropriate model. Model diagnostics were inspected to detect potential violation of model assumptions. Statistical analysis was performed in R 3.5.0 (R Core Team, 2018). We provide detailed results of the MLR tests in the supplementary information (Table S 3.1) and coefficients of the best models in Table 4.2.

Linear Mixed Effects Models (LMEs) and GLSs were used to analyze all response variables. Colony and experimental aquaria were incorporated as crossed random factors to deal with dependence related with: (1) the existence of multiple coral fragments that originated from the same colony and (2) the fact that multiple coral fragments were positioned in the same aquaria. Whenever the assumption of homogeneity of variance was not fulfilled, variance structure components were added to the models to allow the variance to differ among tested treatments (Zuur et al. 2009b). LME models were build using the packages LME4 (Bates et al. 2015) and nlme (Pinheiro et al. 2019).

4.4. Results

All utilized food sources were significantly enriched above background levels, i.e. above non-labelled food (Table 4.1). Target C concentrations within the aquaria were successfully achieved in the case of the DOM and ZOO treatment, however they were below the target for the PHYTO treatment, by 34 % for *D.* aff*. meteor* and 44 % for *V. flagellum* respectively (Table 4.1).

Table 4.1: Characteristics of enriched food sources used in feeding experiments of *Dentomuricea* aff. *meteor* and *Viminella flagellum*, including average ± SD of the created C concentrations in the aquaria, total provided C per coral fragment and per coral organic carbon, fractional abundance (F13, F15) and carbon to nitrogen (C/N) ratio. CC: *Chaetoceros calcitrans*, NG: *Nannochloropsis gaditana*, BP: *Branchionus plicatilis*.

	D.* aff. *meteor				***V.flagellum***			
Food source	***CC* (PHYTO)**	***NG***	**DOM**	***BP* (ZOO)**	***CC* (PHYTO)**	***NG***	**DOM**	***BP* (ZOO)**
C Concentration (µmol L^{-1})	7.28 ± 0.2	-	9.19	11.24 ± 0.5	5.59 ± 0.2	-	9.19	10.06 ± 0.5
Total provided C (µmol coral $fragment^{-1}$)	287.93 ± 8.2	-	364.20	445.41 ± 22	369.09 ± 18.9	-	607.0	664 ± 33
Total provided C (µmol mmol coral C^{-1})	150.75 ± 5	-	190.68	233.94 ± 13	54.51 ± 2.7	-	89.66	98.14 ± 4.8
F^{13}	0.58	0.49	0.96	0.21	0.59	0.58	0.96	0.37
F^{15}	0.42	0.31	0.88	0.12	0.41	0.30	0.88	0.15
C/N	11.86	9.94	4.41	5.44	9.95	10.83	4.41	5.62

Coral fragments incorporated tracer C and N from all food sources in their tissue (Figure 4.3). All data on tracers are presented as average ± standard deviation. Both species incorporated significantly higher tracer C and N under the ZOO treatment followed by the DOC treatment (Figure 4.3, Table 4.2). Coral fragments of *D.* aff. *meteor* and *V. flagellum* under the ZOO treatment incorporated 422% and 453% more tracer C than under the PHYTO treatment, respectively. *Viminella flagellum* displayed lower tracer incorporation compared to *D.* aff. *meteor* in all treatments, reaching on

Table 4.2: Coefficients of constructed models to explore the effect of four food treatments (FAST: fasting; PHYTO: diatom *Chaetoceros calcitrans*; DOM: dissolved organic matter; ZOO: rotifer *Branchionus plicatilis*) on each dependent variable after analysis of collected data on tissue, respiration and excretion of two octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum*. If food source was excluded from the respective model during model construction, it was assumed it had no significant effect on the response variable in question and therefore no coefficients are provided. SE: Standard error

Species		*Dentomuricea aff. meteor*						*Viminella flagellum*					
Variable group	Dependent variable	Treatment	Fixed effects			Random effects		Variance Structure	Fixed effects			Random effects		Variance Structure
			Value	SE	p-value	Colony	Residual		Value	SE	p-value	Colony	Residual	
Tissue	Tracer C Incorporation	PHYTO	0.18	0.091	0.040			0.27	0.05	0.006	0.000			1.00
		DOM	1.61	0.166	0.000	0.172	0.602	1.00	0.54	0.140	0.002			23.70
		ZOO	80.26	6.359	0.000			39.49	21.80	4.030	0.000			655.10
	Tracer N Incorporation	PHYTO	0.01	0.009	0.130			0.12	0.01	0.004	0.001			1.00
		DOM	0.40	0.041	0.000	0.018	0.152	1.00	0.12	0.126	0.001			16.30
		ZOO	16.66	1.159	0.000			28.45	3.36	0.447	0.000			355.90
Respiration	Oxygen consumption	FAST	1.08	0.199	0.001				0.11	0.047	0.040			
		PHYTO	0.98	0.290	0.716	0.070	0.430		0.23	0.050	0.278	0.080	0.090	
		DOM	1.61	0.290	0.089				0.14	0.050	0.514			
		ZOO	1.93	0.290	0.011				0.25	0.050	0.012			
	DIC Bulk respiration	FAST							0.24	3.090	0.017			
		PHYTO							0.18	0.540	0.605			
		DOM							0.26	0.140	0.887			
		ZOO												
	Tracer C respiration	PHYTO												
		DOM	0.00	0.001	0.002				0.00	3.770	0.013			0.06
		ZOO	0.23	0.055	0.003				0.06	3.110	0.264			1.00
POC/PON release	POC bulk release	FAST	0.03	0.013	0.062			1.00						
		PHYTO	0.07	0.026	0.207			1.06						
		DOM	0.09	0.015	0.002			0.45						
		ZOO	0.21	0.060	0.002			3.78						
	Tracer C release	PHYTO	0.00	0.002	0.104			1.00	0.00	0.000	0.060			2.49
		DOM	0.00	0.001	0.440			0.69	0.00	0.000	0.419			1.00
		ZOO	0.10	0.029	0.003			20.92	0.01	0.001	0.000			9.49
	PON bulk release	FAST	0.01	0.011	0.240									
		PHYTO	0.07	0.014	0.728									
		DOM	0.04	0.014	0.054									

Species		*Dentomuricea aff. meteor*						*Viminella flagellum*					
Variable group	Dependent variable	Treatment	Fixed effects			Random effects		Variance Structure	Fixed effects			Random effects		Variance Structure
			Value	SE	p-value	Colony	Residual		Value	SE	p-value	Colony	Residual	
		ZOO	0.03	0.014	0.192									
	Tracer N release	**PHYTO**	0.00	0.000	0.000			1.00	0.00	0.000	0.000			0.18
		DOM	0.00	0.001	0.050			13.50	0.00	0.000	0.532			1.00
		ZOO	0.02	0.004	0.000			102.70	0.00	0.001	0.003			3.06

average 64 % lower C incorporation and 70 % lower N incorporation than *D.* aff*. meteor* (Figure 4.3). Total tracer C incorporation varied between 0.19 ± 0.24 µmol tracer C mmol tissue C^{-1} under the PHYTO treatment to 80.2 ± 24.5 µmol tracer C mmol tissue C^{-1} under the ZOO treatment for *D.* aff*. meteor* and from 0.05 ± 0.02 µmol tracer C mmol tissue C^{-1} under the PHYTO treatment to 21.8 ± 13.9 µmol tracer C mmol tissue C^{-1} under the ZOO treatment for *V. flagellum*.

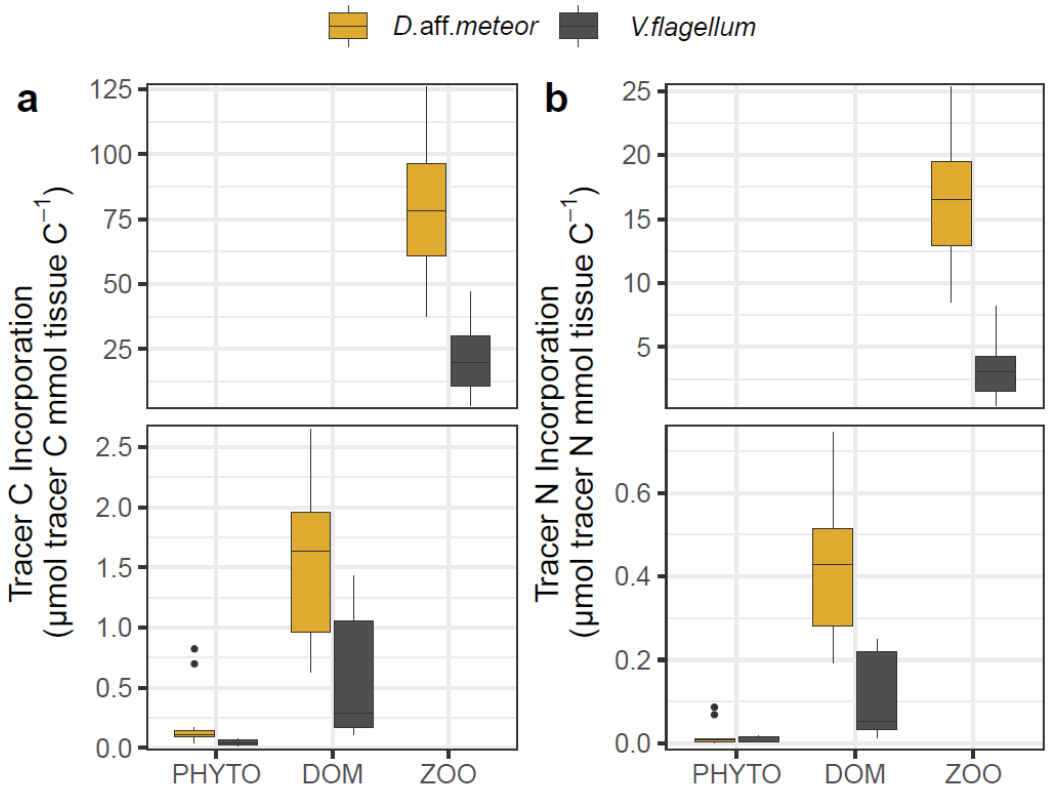

Figure 4.3: Tracer C (a) and N (b) incorporation of the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum* upon provision of different food sources enriched with 13C and 15N. Axis breaks are used to highlight the large differences of tracer among treatments. PHYTO: phytoplankton *Chaetoceros calcitrans*; DOM: dissolved organic matter; ZOO: zooplankton *Branchionus plicatilis*.

In both species, oxygen consumption was significantly higher in the ZOO treatment compared to the other food treatments (Table 4.2), reaching on average 0.308 ± 0.042 µmol O_2 mmol tissue C^{-1} h^{-1} for *D.* aff*. meteor* and 0.151 ± 0.036 µmol O_2 mmol tissue C^{-1} h^{-1} for *V. flagellum* (Figure 4.4). Overall, oxygen consumption was almost two times higher in fragments of *D.* aff*. meteor* compared to *V. flagellum*.

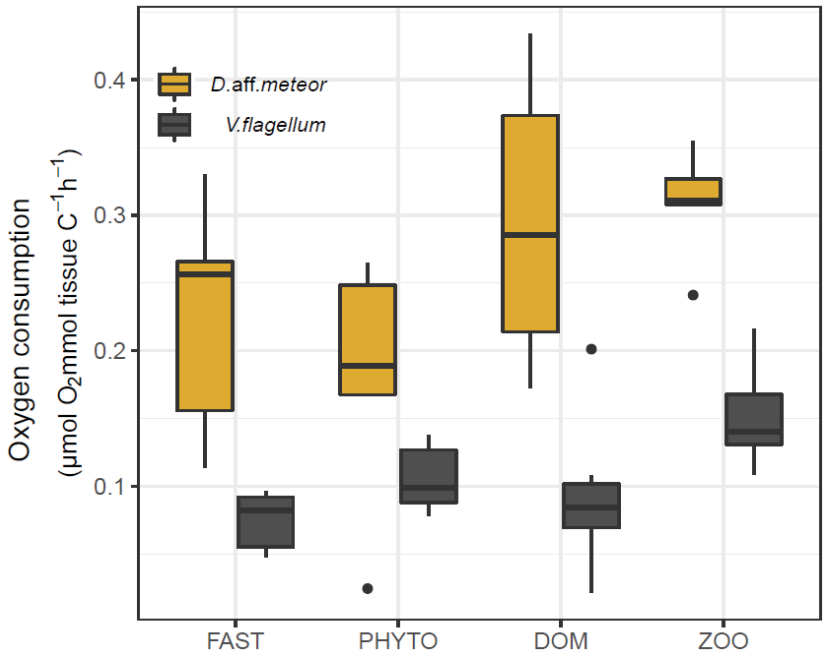

Figure 4.4: Oxygen consumption of the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum* upon provision of different food sources. Coral fragments were fed with the respective food source for four days and oxygen was measured in closed-cell incubations that took place immediately after feeding on day four and lasted for approximately 12-14h. FAST: no food provision; PHYTO: phytoplankton *Chaetoceros calcitrans*: ZOO: zooplankton *Branchionus plicatilis*; DOM: dissolved organic matter.

Both species utilized tracer C and N derived from the provided food for C respiration, POC and PON release (Figure 4.5). Tracer C respiration was significantly higher under the ZOO treatment for both species (Figure 4.5Figure 4.4, Table 4.2). Similarly, for both species tracer POC and PON release were higher under the ZOO treatment while fragments under the DOM and PHYTO treatments displayed very low average values of POC and PON release, which did not differ significantly from zero (Table 4.2).

To take into account differences in the provided C quantity, the amount of utilized C tracer is provided as a percentage of the total provided C in Figure 4.6. For *D.* aff. *meteor* 45 % of the provided zooplankton-derived tracer C could be traced back in tissue incorporation, DIC respiration and POC release, with phytoplankton-derived and DOM-derived tracer C reaching 1.08 % and 0.26 %, respectively (Figure 4.6). Under the PHYTO treatment, corals utilized most of the C tracer for POC release, while in the two other treatments they utilized most of the C tracer for tissue incorporation, followed by tracer C respiration (Figure 4.6). In *V. flagellum,* a smaller percentage of the provided C was traced back, reaching 23 % under the ZOO treatment, 0.48 % under the DOM treatment and 0.17 % under the PHYTO treatment.

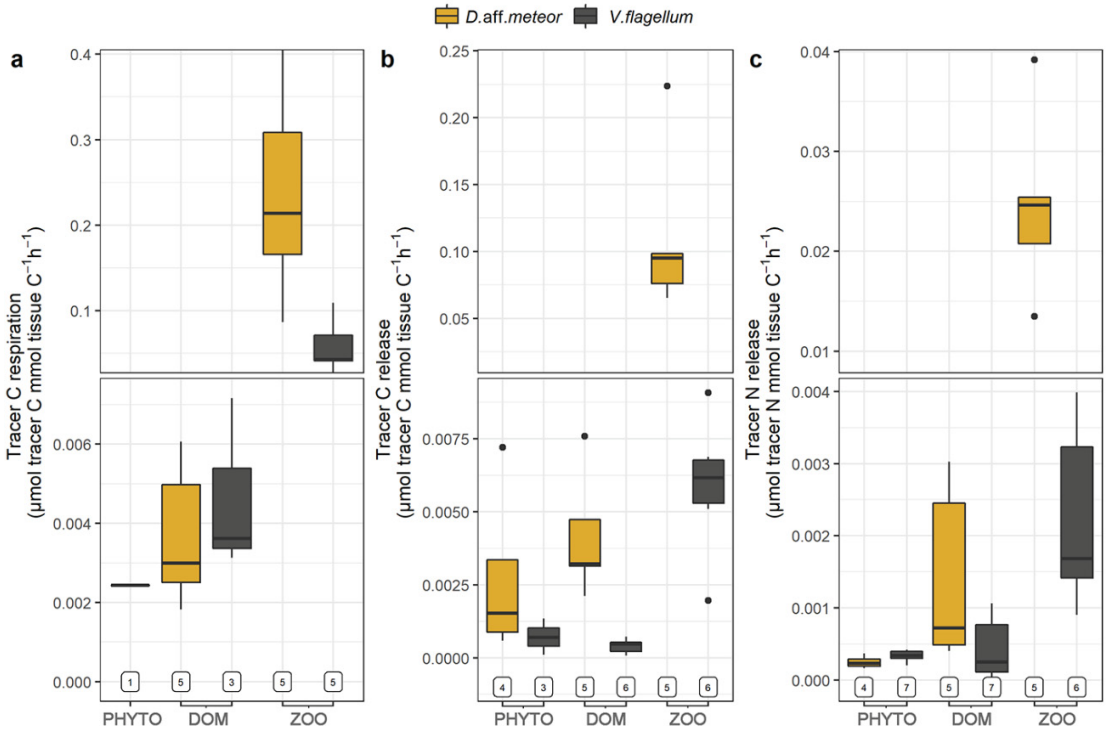

Figure 4.5: Tracer fluxes, including tracer C respiration (a), tracer C release (b) and tracer N release (c) of the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum* upon provision of different food sources. Numbers below bars represent the number of coral fragments for which positive estimates were obtained (max 7). Axis breaks are used to highlight large differences in scale among some treatments. PHYTO: phytoplankton *Chaetoceros calcitrans*; DOM: dissolved organic matter; ZOO: zooplankton *Branchionus plicatilis*.

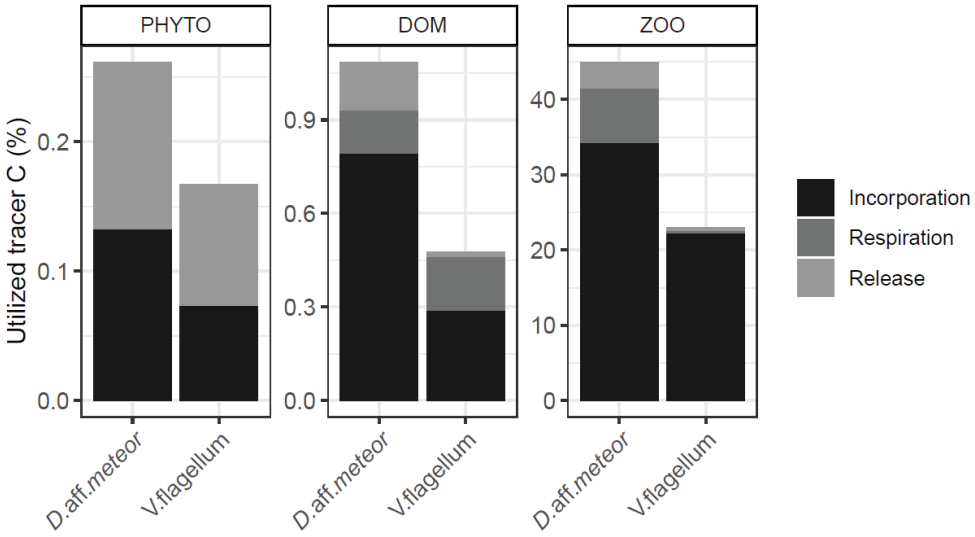

Figure 4.6: Tracer utilized by fragments of two octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum*, expressed as a percentage of the provided carbon of different food sources: PHYTO: phytoplankton *Chaetoceros calcitrans*; DOM: dissolved organic matter; ZOO: zooplankton *Branchionus plicatilis*.

4.5. Discussion

To our knowledge this is the first study addressing the feeding biology of deep octocoral species by employing an experimental approach with the use of stable isotopes. The strong differences in tracer C and N incorporation among the food treatments suggest a higher efficiency in capture and ingestion of zooplankton prey when this resource is available, compared to the other food treatments. Previous studies have highlighted zooplankton as an important dietary component of deep-sea scleractinians (Kiriakoulakis et al. 2005, Sherwood et al. 2008, Naumann et al. 2011, 2015). However, only a few deep-sea octocorals, mainly of the family Primnoidae, have been shown to base their diet on microzooplankton (Sherwood et al. 2005, Imbs et al. 2016) while most studied species rely mostly on phytodetritus and particulate organic matter (POM) (Sherwood et al. 2008, Salvo et al. 2018). Our results provide a direct demonstration of the importance of zooplankton for some deep-sea octocorals and highlight that they might be more selective than previously thought.

In areas with strong hydrodynamics, such as seamounts, fresh phytoplankton can be directly transported to great depths by rapid downwelling and tidal waves (Davies et al. 2009, Agusti et al. 2015), and therefore it might be often available for the two target octocorals. The closely positioned pinnules of octocoral tentacles and their relatively weak nematocysts generally suggest herbivory (Fabricius & Klumpp 1995, Fabricius et al. 1995a, Widdig & Schlichter 2001). Moreover, in previous studies, both target octocorals have been shown to occupy low trophic levels, placed between primary and secondary consumers (Colaço et al. 2013). Thus, the lower incorporation of phytoplankton was unexpected. It is possible that during the experiment, coral fragments fed additionally on small particles (<1 μm) that passed the filtration system. This could explain the lower DOM and phytoplankton utilization, but it further supports the hypothesis that both species can display selective feeding.

The feeding behavior of the two target octocorals is in strong contrast to the results of similar feeding experiments with the deep-sea scleractinian *Lophelia pertusa* (recently synonymized to *Desmophyllum pertusum* (Addamo et al. 2016)) which displayed a rather unselective feeding behavior under feeding with similar food sources and flow velocities (Mueller et al. 2014). These differences might be due to the use of processed (freeze-dried) versus live prey. The use of isotopically enriched live prey has been used before to study the feeding preferences of octocorals (Roushdy & Hansen 1961, Sorokin 1991) and scleractinians (Seemann 2013, Orejas et al. 2016). It allows more realistic comparisons compared to dry food, as it takes into consideration both the capture and ingestion ability of the study species. On the other hand, it includes a considerable error in determining and standardizing provided C quantities since C content can vary among culture batches. In the present study, the available C in the aquaria of the DOM and ZOO treatments was 30-40 % higher than in the PHYTO treatment, thus a proportionately higher utilization of the DOM and ZOO food sources was expected. While this can explain the small differences in tracer C utilization between the PHYTO and DOM treatments, it cannot explain the disproportionally larger tracer utilization under the ZOO treatment, strongly indicating more efficient feeding on zooplankton.

Since zooplankton dynamics in the study area follow seasonal phytoplankton productivity cycles (Carmo et al. 2013), it is likely that this important food source displays strong seasonal fluctuations. Both target species displayed the ability to utilize food sources variable in size and composition, therefore during the rest of the year it is very likely that the two species sustain their metabolism through feeding on other sources such as phytoplankton and DOM. Dissolved organic matter has proven to be an important food source for cold-water scleractinians when particulate food sources are scarce (Gori et al. 2014). The ability to utilize DOM can be very important in oligotrophic deep-sea environments where food availability is seasonal and is likely to be further affected by climate change (Sweetman et al. 2017). A number of previous studies have highlighted the variable and seasonally-dependent diet of octocoral species in temperate ecosystems (Sebens & Koehl 1984, Ribes et al. 1999, Migné & Davoult 2002) while similar seasonality has been also reported in benthic Antarctic ecosystems (Gili et al. 2001, Orejas et al. 2001).

The two target octocorals displayed higher oxygen consumption and tracer C respiration upon feeding with zooplankton, highlighting the importance of zooplankton to meet their respiratory and metabolic demands. Similar results have been reported for *Desmophyllum dianthus* which displayed lower oxygen respiration, calcification and TOC release after exclusion of zooplankton from available food sources (Naumann et al. 2011). Because of the seasonal availability of zooplankton and its importance for tissue incorporation and metabolism, it appears likely that physiological processes that require the development of C and N rich tissues, such as growth and reproduction, may also undergo strong seasonality. This is a common phenomenon for octocorals in temperate areas, which display seasonal cycles in their biochemical levels e.g. Rossi et al. 2006) and often pass through periods of metabolic dormancy in summer months when available seston is scarce and temperature rises (Coma et al. 2000, Coma & Ribes 2003). D*entomuricea* aff*. meteor* and *V. flagellum* display gamete presence all year round with frequent seasonal peaks in spring and autumn (Rakka et al. 2021b), which could be related to higher zooplankton availability (Carmo et al. 2013), but more studies on abiotic conditions and physiological cycles are essential to unravel their ecophysiology.

Fragments fed with zooplankton also displayed higher tracer POC and PON release in both species, which in corals is associated with mucus production, essential in processes such as feeding, cleansing and protection from epibionts and pathogens (Bythell & Wild 2011). Mucus production can be extremely important to protect the corals against mechanical and chemical disturbance due to bottom trawling, oil extraction and mining (e.g. mine tailing and drill cutting resuspension) (Brooke et al. 2009, Larsson et al. 2013, Ragnarsson et al. 2017). Moreover, coral mucus has been identified as an ecologically important element for CWC communities, since it enhances microbial activity and therefore mineralization, recycling and overall ecosystem productivity (Bythell & Wild 2011, Rix et al. 2016). The increased POC release under the ZOO treatment showcases how feeding on zooplankton can enhance the contribution of octocoral species to C recycling and highlights their importance for bentho-pelagic coupling.

Overall, *D.* aff*. meteor* appeared to acquire higher percentage of the provided C compared to *V. flagellum*. Moreover, when fed with the most effectively utilized food source (zooplankton), *D.* aff*. meteor* allocated a higher proportion of captured C to respiration and POC release compared to *V. flagellum*. This highlights the different strategies adopted by the two species. While *V. flagellum* appeared more conservative in resource allocation, storing most of the captured C in tissue, and minimizing losses, the pattern displayed by *D.* aff*. meteor* is indicative of "sloppy feeding", in which high amounts of the captured C are lost during the feeding process (Lampert 1978, Moller 2004) Feeding behaviour and metabolic rates can be influenced by an array of factors, such as environmental variables, physiological status and life stage. The two target species were haphazardly collected, maintained in similar conditions and were expected to be in similar stages in their reproductive cycle. Thus, variations in colony health, age and maturity are more likely to explain the observed variance within each species, while the marked difference between the two octocorals may be attributed to species-specific characteristics, such as morphology and growth pattern (Burton et al. 2011). The species *D.* aff*. meteor* has a branching pattern with high surface to volume ratio and possesses a large number of small polyps which can increase both capture rates and metabolic costs (Kim & Lasker 1998, Burgess et al. 2017) On

the other hand, *V. flagellum* displays an erect growing pattern with bigger polyps and a lower surface to volume ratio that may have lower maintenance costs.

Both target species incorporated a lower amount of tracer from phytoplankton compared to *L. pertusa* (Maier et al. 2019b). In contrast, fragments fed with zooplankton displayed higher tracer incorporation than *L. pertusa*, i.e. 10 times higher for *D.* aff*. meteor* and two times higher for *V. flagellum*. Although these differences might be attributed to the use of dry versus live prey, they highlight that resource acquisition strategies are species-specific, as also demonstrated for several tropical and cold-water coral (CWC) species (Sherwood et al. 2008, Imbs et al. 2016, Sebens et al. 2017). Metabolism is tightly connected to the ecological niche of a species, and different responses to food supply can explain distributions of species and species assemblages (Okie et al. 2015). For example, *D.* aff*. meteor*, due to its metabolic strategy presented herein, is expected to have an advantage under high food concentration, however, it is unlikely to outperform *V. flagellum* under low food conditions.

Similarly to reefs formed by cold-water scleractinian species (Oevelen et al. 2009, Cathalot et al. 2015) the rich organic excretion of octocorals promotes organic cycling and plays an important role supporting a diverse community of associated fauna in coral gardens and adjacent deep-sea communities (Coppari et al. 2019). Taking into account that the two species displayed different strategies in respect to the respired and released C, we hypothesize that the communities dominated by *D.* aff*. meteor* are likely to be characterized by higher C and N recycling whereas communities of *V. flagellum* will likely have higher residence time of C and N in the coral tissue, with further consequences for the local C cycle. Sloppy feeding is known as an important behavior for the support of C cycles, fueling the microbial loop and supporting local food webs (Moller & Nielsen 2001, Titelman et al. 2008) Thus, the role of these species to local and global marine biogeochemical cycles should be further investigated.

In conclusion, the present study provides important knowledge on the resource utilization and metabolic strategies of two important habitat forming octocorals, that can help understand

patterns at the species, population and community level (Violle & Jiang 2009). Species distribution modelling has shed light to the distribution of different deep-sea coral groups, including octocorals (Yesson et al. 2012), but comparatively little effort has been made to delve further into the biological and physiological characteristics which shape these distributions (Violle et al. 2007, Kearney et al. 2010). Taking into account these traits will not only improve predictions on species occurrences and help to identify priority areas for conservation and management (Evans et al. 2015), but will also provide a more robust understanding of the ecology of deep-sea corals. Coral resource use and metabolism is likely to change under future conditions of increased seawater temperature, stratification of water masses and consequent reduction in the quantity and quality of POM flux to the seafloor (Sweetman et al. 2017). Further studies on the ecophysiology of octocoral species under present and future scenarios of climate change are therefore essential to improve our understanding of the distribution and ecological function of deep-sea communities.

Chapter 5
Resource acquisition and metabolism of the deep-sea octocoral *Viminella flagellum* under acidification and variable food availability

5.1. Abstract

Ocean acidification (OA) and a decrease in food supply are among the pressures that climate change is expected to pose to deep-sea species and ecosystems. Although increased concern has been raised on the potential of deep-sea corals to withstand such changes, attention has focused largely on scleractinian, reef building species. The objective of this study was to determine the impacts of ocean acidification and variable food availability on the whip coral *Viminella flagellum*, a common octocoral species in deep-sea benthic communities in the Mediterranean, Macaronesia and eastern North Atlantic. A multifactorial experiment was performed with live colonies of the species which were subjected to two pCO_2 (ambient and elevated levels projected for the end of the century) and three food availability treatments (fasting, low and high food availability) for six weeks. Isotopically labelled food was provided to evaluate the ability of the species to acquire and incorporate C and N tracers from the provided food in the coral tissue. Prey capture, polyp activity and oxygen consumption were also determined throughout the experiment. The species displayed lower metabolic activity under conditions of high pCO_2 which were reflected in lower polyp activity and oxygen consumption, a response that was accentuated under fasting. However, no significant effects of pCO_2 were observed on tracer incorporation in the coral tissue. The presence of food helped the species to maintain higher metabolic rates, however higher food availability did not counteract the negative effects of ocean acidification on coral metabolism. To our knowledge, this is the first study to determine the effects of multiple climate change stressors to a deep-sea octocoral.

5.2. Introduction

Ocean acidification (OA), the decrease in water pH due to the absorption of high CO_2 levels that are rapidly released in the atmosphere, has been identified as a major threat for marine ecosystems (Dupont & Pörtner 2013). Marine calcifiers such as corals are considered among the most vulnerable species, as a potential pH decrease can affect the precipitation of $CaCO_3$ which is essential for the formation of their skeletons (Orr et al. 2005, Hofmann et al. 2010). However, calcification is not the only physiological mechanism affected by OA (Fabry et al. 2008). The regulation of pH levels within animal tissues is essential for a variety of physiological processes (Pörtner 2008), and constitutes an energetically demanding mechanism that can be extremely costly for species that are sessile, have low metabolism, and thrive in environments with low food availability such as the deep-sea (Pörtner 2008, Sokolova et al. 2012).

According to climate projections, deep-sea benthic ecosystems will be subjected to additional stressors, including warming and a concomitant enhancement of stratification, which may cause a substantial decrease in the input of organic carbon to deep-sea organisms (Yool et al. 2013, Sweetman et al. 2017, Bindoff et al. 2019). These potential changes have brought increased attention to the cumulative impacts of acidification and food availability on deep-sea corals (Maier et al. 2016, Georgian et al. 2016, Büscher et al. 2017, Gómez et al. 2018). To date, most of these studies support that increased food concentration does not alter the negative effects of acidification on deep-sea corals (Maier et al. 2016, Büscher et al. 2017, Gómez et al. 2018). However, Georgian et al., (2016) reported contrasting results among populations of the scleractinian *Lophelia pertusa* (syn. *Desmophyllum pertusum*, Addamo et al. 2016) highlighting that our understanding of the underlying mechanisms is still limited. Important to note is that work to date has focused on reef-building scleractinians, and no information exists on other deep-sea coral species.

During the geological history of corals, octocorals have been shown to be more resilient to low pH conditions than scleractinians (Quattrini et al. 2020, Conci et al. 2021). This capacity has also been highlighted in current-time field observations (Lasker et al. 2020, Inoue et al. 2013) and laboratory experiments of certain species (Gabay et al. 2013, Gómez et al. 2015,

Vargas et al. 2020). However, most of these studies consider tropical, shallow-water octocorals. Species that live in cold-water environments are expected to be more vulnerable to changes in water pH, partly due to the decrease in carbonate saturation levels in lower temperatures (which can lead to skeletal dissolution (Hofmann et al. 2010)), and also due to their relatively low metabolism (Sokolova 2013). To our knowledge, studies with non-tropical octocorals have only been performed with the temperate species *Corallium rubrum*, reporting high vulnerability to lower pH (Bramanti et al. 2013, Cerrano et al. 2013).

Deep-sea octocorals are the most common cold-water corals (CWCs) in the Azores Archipelago (Braga-Henriques et al. 2013, Sampaio et al. 2019), forming diverse and structurally complex communities in seamounts and island slopes (Tempera et al. 2012). Because of the paucity of studies on the impacts of climate change on deep-sea octocorals, there is currently limited ability to predict the potential response of these communities to projected environmental changes.

The aim of this study was to determine the impacts of OA and food availability on the physiology of *Viminella flagellum* (Johnson 1863), a common octocoral not only in the Azores, but also in the eastern North Atlantic and Mediterranean (Carpine & Grasshoff 1975, Brito & Ocaña 2004, Braga-Henriques et al. 2013). We employed an experimental approach to simulate OA and food availability scenarios with the use of isotopically enriched live zooplankton prey, with the following objectives: (1) determine the impacts of OA on the resource acquisition and metabolism of *V. flagellum*; (2) describe the metabolic response of the species under variable food availability and (3) determine whether food availability can alter the metabolic responses of the species to OA.

5.3. Materials and methods

5.3.1. Sampling site and colony collection

In the Azores, *V. flagellum* is frequently encountered in seamounts where it creates dense coral gardens between 200-500 m (Tempera et al. 2012, Braga-Henriques et al. 2013). Live colonies of the octocoral *V. flagellum* were obtained as bycatch from

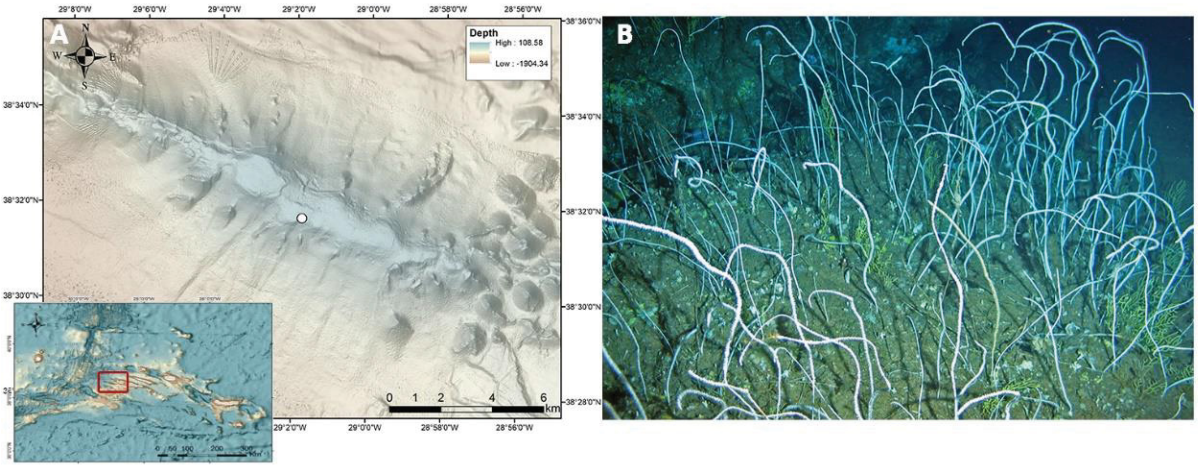

Figure 5.1: Study area. (A) Condor Seamount with the location of coral sampling and (B) coral garden of the target species *Viminella flagellum* (Gavin Newman, Greenpeace).

experimental longline fisheries (CONDOR monitoring program, University of the Azores) onboard of the RV "Arquipélago", in October-November 2018 at Condor Seamount (Figure 5.1A). A total of 19 colonies were collected and transported to the DeepSeaLab aquaria facilities (Orejas et al. 2019) in coolers. Colonies were examined and parts with injuries or non-healthy tissue were discarded. Healthy colonies were kept in 170 L tanks continuously supplied with seawater through a flow-through open system in a temperature controlled room (14 ± 0.7 ºC) under darkness. Incoming water was repeatedly filtered (1 µm mesh size) and treated with UV-light (P10 UVsystem and Vecton 600, TMC^{TM}) prior to entry into experimental tanks. Corals were fed daily with a mixture of frozen zooplankton (*Artemia salina* adults and nauplii, mysids, copepods) and microplankton (OceanNutrition), which was enriched with live microalgae and rotifers three times a week. Colonies were fragmented into 8-10 cm fragments, mounted to epoxy bases and left to acclimatize in the aquaria conditions for approximately one month.

Additionally, environmental conditions, such as temperature, salinity and Particulate Organic Matter (POM) were studied during a short survey performed in July 2017 on the site of collection (Rovelli et al., in press). Water samples were also collected to measure alkalinity (TA), as described below (see 5.3.3.Carbonate chemistry).

5.3.2. Experimental design and system

A fully crossed factorial experimental design was used to test for the interactive effects of OA and food availability on the physiology of *V. flagellum* over a period of six weeks. Two levels of pCO_2 conditions were considered: present day (~385 atm) and IPCC RCP8.5 scenario (1000 atm; IPCC, 2013), corresponding to pH_T values of 8.09 and 7.73 respectively. Subsequently, three food availability scenarios were recreated: (1) a condition of high food availability (HIGH), corresponding to carbon (C) concentrations recorded during the spring bloom and considered as sufficient to cover the physiological and metabolic demands of the species; (2) a low food availability (LOW) corresponding to C conditions recorded during the summer which is considered as a period of low productivity in oligotrophic systems such as the Azores (Carmo et al. 2013, Santos et al. 2013); and (3) an extreme scenario where no particulate organic food was provided (FAST). The LOW treatment corresponded to C concentration of 1.6 µmol C/L, based on the water sampling described above (see 5.3.1. Sampling site and colony collection). The HIGH treatment corresponded to C concentration of 10.12 µmol C/L and was based on collected data obtained by a sediment trap deployed between November 2017-June 2018 and showed that C input was on average six times higher during the spring months than during the summer months (Rovelli et al., in press).

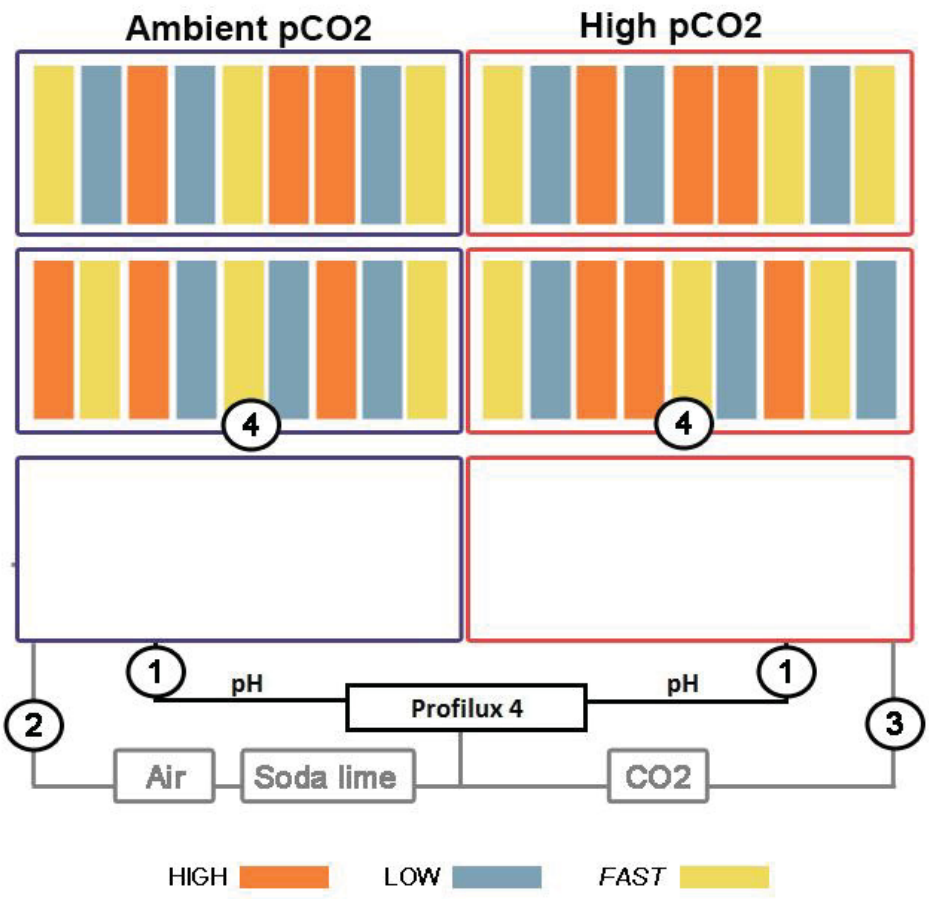

Figure 5.2: Experimental design to study the effects of OA and variable food availability on the physiology of the deep-sea octocoral *Viminella flagellum*. The system included two aquaria tanks, equipped with pH electrodes (1) connected to an aquaria controller (Profilux 4). The device controlled the provision of CO_2-free air using a soda lime filter to manipulate seawater for the ambient pCO2 scenario (2) and pure CO_2 used to manipulate seawater for the increased pCO2 scenario (3). Seawater from each tank supplied a total of 18 aquaria, placed in four water baths (4) and distributed to three food availability scenarios (HIGH: increased food availability; LOW: decreased food availability; FAST: no particulate food provided). Each aquarium contained a single coral fragment.

The experimental system included two storage 150L tanks (Figure 5.2) continuously supplied with seawater as described above. The two tanks were used to simulate the two pCO_2 scenarios referred above using pure CO_2 or CO_2-free air depending on the treatment: the control pCO_2 treatment tank (400 atm) was bubbled with CO_2-free air, supplied by an air pump connected to soda-lime filters, while the high pCO_2 treatment tank (1000 atm) was bubbled with pure CO_2 (Figure 5.2). Bubbling in both tanks was controlled by a central aquaria controller (Profilux 4, GHL) connected to glass electrodes which continuously monitored the pH values in the two storage tanks. Each tank provided water continuously to 18 experimental aquaria (capacity:13L). All aquaria were equipped with a heavy-duty motor (313 rpm; Servocity) connected to an 8 cm propeller to create continuous laminar flow between 3-6

cm/s (Rakka et al. 2021a). Aquaria were distributed in four water baths (Figure 5.2), keeping temperature stable at 14.5 ± 0.5 ºC. Care was taken to distribute the aquaria of the experimental treatments evenly among water baths.

A total of 36 fragments of *V. flagellum* were randomly distributed among the 36 experimental aquaria (Figure 5.2). Seawater pH was gradually adjusted to the required pCO_2 levels within two weeks, time referred to as acclimatization period (W_{ac}), and kept stable for another six weeks (W_1-W_6). During this period, conditions of the three food levels referred above were recreated in the corresponding aquaria, by using rotifers of the species *Branchionus plicatilis* as live prey: aquaria of the FAST treatment did not receive any prey, aquaria of the LOW food treatment received approximately 250 prey/L (corresponding to 1.6 µmol/L) and aquaria of the HIGH food treatment received 1500 prey/L (corresponding to 10.12 µmol/L). Food conditions were recreated six times a week, each time following the same feeding protocol, referred to as food cycle. During each food cycle, prey was provided in the morning after closing the water inflow and establishing continuous laminar flow with flow velocity of 6 cm/s. This flow regime guaranteed high prey capture (Rakka M, unpublished data). Coral fragments were left to feed for approximately 6 hours, before subsequent removal of excess food with a 2/3 water change, and reconnection to flow through conditions. Subsequently, coral fragments were left to rest overnight in flow velocities < 4 cm/s created by mini pumps (Hailea, HL-BT200).

The provided prey was enriched with ^{13}C and ^{15}N in 9 of the 30 food cycles that took place during the experiment. The enriched food cycles were randomly assigned to the 6 experimental weeks: twice per week for the weeks W_1, W_2 and W_6, and once per week in W_3, W_4 and W_5. Labeled cultures of the rotifer species were obtained by inoculating starter cultures (concentration: 45 rotifers/ml) in filtered seawater (1 µm) with continuous presence of enriched microalgae *Nannochloropsis gaditana*, for six days. The enriched microalgae were prepared by using artificial seawater and an F/2 culture medium containing 50% ^{15}N-sodium nitrate (NaNO3, Cambridge Isotopes) and 100% ^{13}C-bicarbonate (NaHCO4, Cambridge Isotopes) for three weeks.

5.3.3. Carbonate chemistry

Temperature, pH and salinity were measured manually in each aquaria every day using a Mettler-Toledo S8 glass electrode and an S30 SevenEasyTM conductivity meter calibrated every other day. The pH measurements were conducted in the NBS scale but were transformed to the total scale by measuring standard TRIS buffer (Batch 13.187) after each calibration and adjusting corresponding values (Carreiro-Silva et al. 2014). Water samples for total alkalinity (TA) were collected once per week and analyzed immediately after collection using a spectrophotometric method (Dionísio et al. 2017, adapted from Sarazin et al. 1999) with an average estimated error of 4.15 ± 3.39%. Seawater carbonate chemistry parameters (Table 5.2) were calculated from the measured TA, pH, temperature and salinity using the R software (R Core Team, 2018) and the package SeaCarb (Gattuso et al. 2020) with the thermodynamic constants of Mehrbach et al. (1973) as refitted by Dickson & Millero (1987).

5.3.4. Measured variables

5.3.4.1. Polyp activity

Polyp activity was determined to assess if coral fragments were able to actively feed on the provided prey. It was evaluated twice per day, one hour after the start of the feeding cycle and one hour before its end. Fragments were considered open if at least one polyp was extended (value= 1) and closed if all polyps were retracted (value= 0).

5.3.4.2. Resource acquisition

Prey capture was used as an indicator for the ability of the coral fragments to capture available prey. Prey capture was estimated by counting prey in water samples that were taken at the beginning and the end of the feeding cycle in three aquaria from each crossed treatment, twice a week. Only aquaria with open fragments at the beginning of the feeding cycle were used for prey capture estimates. To determine prey concentration, four replicate water samples were taken from each aquaria, and prey was counted under a dissecting microscope. Estimates of prey at the beginning of the feeding cycle were also used to monitor the recreated C concentrations (HIGH vs LOW) in different aquaria.

The fate of the provided isotopically enriched food was assessed by determining the incorporation of C and N tracer in the inorganic and organic fraction of the coral coenenchyme and coral skeleton. To determine tracer incorporation, coral fragments at the end of the experiment were collected, stored at -20°C for a week and subsequently lyophilized. Coenenchyme was separated from the main skeletal axis by dissection and both parts were weighed and ground with pestle and mortar to fine powder.

Isotopic ratio and C/N content using an elemental analyzer (Thermo Electron Flash 1112) coupled to an isotope ratio mass spectrometer (EA-IRMS, DELTA-V, THERMO Electron Corporation), as described in Chapter 4. Calculation of tracer incorporation was performed as described in Maier et al., (2019). Tissue carbon and nitrogen content of each fragment was standardized to dry weight of the corresponding part (tissue, axis) and expressed as mmol C or N $(g\ DW)^{-1}$. Final tracer C and N incorporation rates were also normalized to the DW (g) of each coral fragment. Incorporation of C in the inorganic part was determined by subtracting the organic carbon fraction from the total carbon pool.

5.3.4.3. Oxygen consumption

Oxygen consumption was monitored as a proxy of the metabolic rate of the coral fragments. Measurements were performed on four out of the six fragments of each crossed treatment, in closed-cell incubations at the end of W_{AC}, W1, W2, W4, W5 and W6. Incubations took place right after the end of the feeding cycle with isotopically enriched prey. Four coral fragments from each treatment were transferred to 170 ml glass chambers with pre-filtered seawater (0.2 µm) that was previously treated with pure CO_2 or CO_2-free air to match the corresponding pCO_2 treatment. Two chambers did not contain coral fragments and served as controls, one for each pCO_2 treatment. PTFE-coated magnetic stirrers were used to ensure circulation within the chambers and all chambers were placed in a water bath keeping temperature at 14 ± 0.5 °C. Chambers were equipped with sensor spots and oxygen concentration was monitored manually every 20 minutes by using a single channel oxygen meter (Fibox4) with a PSt3 sensor (PreSens, Germany), calibrated before each incubation. Incubations lasted for approximately 4-5 hours during which saturation never dropped below 80%. Oxygen consumption was standardized to the dry weight of each coral fragment. These values were

adjusted for rates recorded in chambers without coral fragments to account for microbial respiration. Measurements of pH were performed before and after each incubation to ensure that pCO_2 treatments were maintained during the incubations. In addition, water samples were collected from all chambers before and after each incubation for the determination of TA. Water was filtered through syringe filters (WHATMAN 0.2 µm) into 40 mL borosilicate vials and were kept at 4°C pending analysis.

5.3.4.4. Statistical Analysis

Collected data were evaluated visually following Zuur et al. (2010) to select the most appropriate statistical modelling method. Generalized Linear Models (GLMs) were used to analyze all variables. Standard linear regression was used for variables that were normally distributed. Binomial regression was used to analyze polyp activity which had binary values (0 for closed fragments and 1 for open fragments) and Gamma regression was used to analyze capture rate and tracer incorporation which did not include negative values. To test if the experimental factors (pCO_2 and food availability) had a significant effect on the dependent variables in question, the former were added progressively to the models and the Akaike Information Criterion (AIC) along with maximum likelihood ratio (MLR) tests were used to choose the most appropriate model. Model diagnostics were inspected to detect potential violation of model assumptions and model coefficients were used to evaluate the effect of each experimental factor. Statistical analysis was performed in R 3.5.0 (R Core Team, 2018). Detailed results of the MLR tests are provided in the supplementary information (Table S 3.1) and coefficients of the best models are presented in Table 5.1.

Table 5.1: Coefficients of the constructed models to analyze the effect of ocean acidification (pCO2) and food availability scenarios on physiological parameters of the deep-sea coral *Viminella flagellum*. SE: Standard error; p: p value

Variable	Model	Family	Coefficients	Estimate	SE	z value	p
Feeding activity	DW x pCO_2 x Food availability	GLM, binomial distribution	Intercept	-2.25	0.29	-7.77	0.00
			DW	0.97	0.36	2.67	0.07
			$pCO_{2\text{-}400ppm}$	-0.04	0.40	-0.12	0.90
			$Food\ availability_{FAST}$	1.71	0.35	4.88	0.00
			$Food\ availability_{LOW}$	-1.00	0.48	-2.08	0.04
			DW : $pCO_{2\text{-}400ppm}$	-0.58	0.44	-1.30	0.19
			DW : $Food\ availability_{FAST}$	-1.36	0.42	-3.22	0.00
			DW : TreatmentFoodlow	0.19	0.54	0.34	0.73
			$pCO_{2\text{-}400ppm}$ x $Food\ availability_{FAST}$	-0.25	0.51	-0.49	0.62
			$pCO_{2\text{-}400ppm}$ x $Food\ availability_{LOW}$	2.62	0.58	4.46	0.00
			DW: $pCO2_{\text{-}400ppm}$ x $Food\ availability_{FAST}$	0.37	0.53	0.69	0.48
			DW: $pCO2_{\text{-}400ppm}$ x $Food\ availability_{LOW}$	-1.28	0.64	-1.99	0.04
Prey capture	DW + Food availability	GLM, gamma distribution	Intercept	6.70	0.08	75.18	0.00
			DW	-3.36	0.36	-9.31	0.00
			$Food\ availability_{LOW}$	-1.30	0.17	-7.37	0.00
Tracer C organic	log(DW)	GLM, gaussian distribution	Intercept	327.70	43.40	7.55	0.00
			log(DW)	-84.07	38.57	-2.18	0.04
Tracer C inorganic	log(DW)	GLM, gaussian distribution	Intercept	76.48	10.69	7.15	0.00
			log(DW)	-24.08	9.50	-2.53	0.02
Organic C content, tissue	DW + Food availability	GLM, gaussian distribution	Intercept	7.42	0.77	9.68	0.00
			DW	-0.61	0.48	-1.27	0.21

Variable	Model	Family	Coefficients	Estimate	SE	z value	p
			Food availability$_{LOW}$	1.67	0.77	2.18	0.04
			Food availability$_{HIGH}$	1.97	0.77	2.57	0.02
N content, tissue	DW + Food availability	GLM, gaussian distribution	Intercept	2.06	0.17	11.67	0.00
			DW	-0.19	0.11	-1.75	0.09
			Food availability$_{LOW}$	0.39	0.17	2.24	0.03
			Food availability$_{HIGH}$	0.49	0.17	2.78	0.01
C content, tissue	DW + Food availability	GLM, gaussian distribution	Intercept	16.73	0.47	35.06	0.00
			DW	-0.42	0.29	-1.43	0.16
			Food availability$_{LOW}$	1.20	0.47	2.50	0.01
			Food availability$_{HIGH}$	1.30	0.47	2.76	0.01
C/N ratio, tissue	DW + Food availability	GLM, gaussian distribution	Intercept	9.82	0.55	17.60	0.00
			DW	0.59	0.34	1.71	0.09
			Food availability$_{LOW}$	-1.21	0.56	-2.15	0.04
			Food availability$_{HIGH}$	-1.50	0.55	-2.69	0.01
Oxygen consumption	DW x pCO_2 x Food availability	GLM, Gamma distribution with log link	Intercept	-0.21	0.28	-0.75	0.45
			DW	-1.16	0.34	-3.43	0.00
			$pCO_{2\text{-}400ppm}$	-0.61	0.41	-1.49	0.14
			Food availability$_{LOW}$	0.51	0.36	1.42	0.16
			Food availability$_{FAST}$	-1.30	0.38	-3.39	0.00
			DW: $pCO_{2\text{-}400ppm}$	0.94	0.48	1.94	0.05
			DW: Food availability$_{LOW}$	-1.94	0.63	-3.03	0.03
			DW: Food availability$_{FAST}$	-0.84	0.44	-1.89	0.06
			$pCO_{2\text{-}400ppm}$: Food availability$_{LOW}$	-0.01	0.50	-0.03	0.98

Variable	Model	Family	Coefficients	Estimate	SE	z value	p
			pCO2-400ppm : Food availability$_{FAST}$	-0.87	0.61	1.41	0.16
			DW: $pCO_{2\text{-}400ppm}$: Food availability$_{LOW}$	1.38	0.73	1.88	0.06
			DW: $pCO_{2\text{-}400ppm}$: Food availability$_{FAST}$	-0.02	0.60	-0.04	0.96

5.4. Results

5.4.1. Carbonate chemistry

Estimated mean values of TA *in situ* at the coral garden were 2555 ± 52 µmol/kg respectively. Recreated experimental conditions in the aquaria were similar to the ones recorded at the field (Table 5.2). Seawater manipulation throughout the experiment caused a decrease of pH_T from 8.07 ± 0.02 (control pCO_2 treatment) to 7.73 ± 0.02 (low pCO_2 treatment) and a respective decrease in Ω_{CAL} from 4.07 ± 0.2 to 2.15 ± 0.08. All seawater CO_2 system parameters remained relatively stable throughout the experiment (Table 5.2).

5.4.2. Colony part and measured variables

Data analysis revealed that coral fragments that originated from different parts of the colony displayed different morphological characteristics in respect to DW, polyp number and size which affected the outcome of all measured variables. Fragments from the lower part of the colony possessed a thick skeletal axis which accounted for 79 ± 15% of their DW and led to high DW values (>1g). In these fragments, polyps were usually smaller and not densely packed (Figure 5.3). In contrast, fragments from the tips of the colonies included thin skeleton, which only accounted for 30 ± 16% of their DW and had lower DW (<0.5 g). These fragments possessed densely packed polyps which were very often larger than the ones encountered in the colony bases (Figure 5.3). The different characteristics of the coral fragments created a very strong effect of DW in all measured variables (Figure S 3.1-Figure S 3.5) which had to be taken into account statistically and is described in detail for each variable.

Table 5.2: Carbonate system parameters (average ± SD) for all experimental treatments during the six weeks of the experiment.

pCO_2	Food	Salinity	Temperature (°C)	pH_T	pCO_2 (µatm)	HCO_3 (µmol/kg)	DIC (µmol/kg)	T_A (µmol/kg)	Ω_{CAL}
400ppm	**HIGH**	35.77 ± 0.12	14.31 ± 0.12	8.07 ± 0.02	396.22 ± 22.33	1991.32 ± 59.9	2177.55 ± 65.23	2413.46 ± 70.89	4.06 ± 0.21
400ppm	**LOW**	35.79 ± 0.11	14.32 ± 0.12	8.07 ± 0.02	395.22 ± 20.21	1992.47 ± 52.42	2179.42 ± 58.02	2416.38 ± 64.77	4.08 ± 0.21
400ppm	**FAST**	35.79 ± 0.1	14.31 ± 0.12	8.07 ± 0.02	395.94 ± 20.68	1991.83 ± 57.85	2178.31 ± 63.87	2414.59 ± 70.84	4.07 ± 0.22
1000ppm	**HIGH**	35.79 ± 0.1	14.32 ± 0.13	7.73 ± 0.01	973.42 ± 39.93	2273.32 ± 60.37	2401.04 ± 63.71	2495.8 ± 65.19	2.15 ± 0.09
1000ppm	**LOW**	35.8 ± 0.1	14.32 ± 0.13	7.73 ± 0.02	977.33 ± 43.28	2287.52 ± 57.07	2416.21 ± 60.13	2511.76 ± 61.46	2.17 ± 0.09
1000ppm	**FAST**	35.81 ± 0.1	14.32 ± 0.14	7.73 ± 0.01	968.59 ± 35.94	2263.41 ± 45.79	2390.63 ± 48.17	2485.33 ± 48.94	2.14 ± 0.07

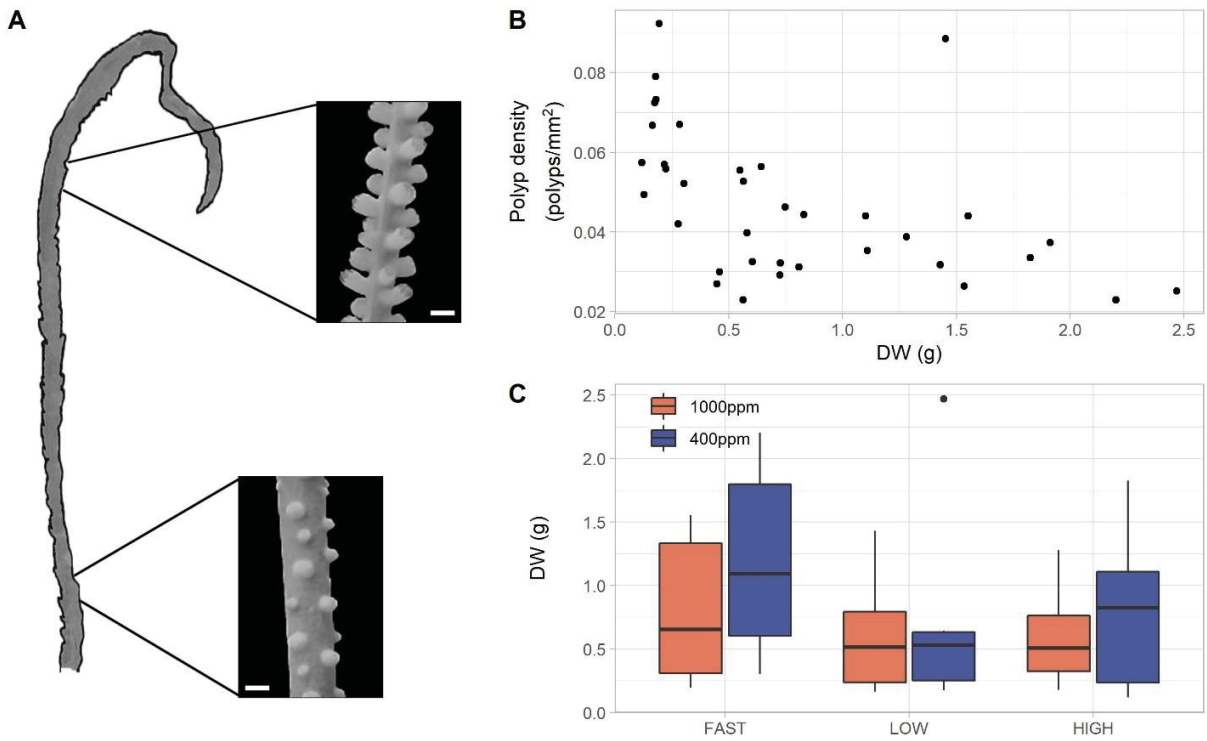

Figure 5.3: Characteristics of coral fragments used to determine the effects of ocean acidification on the deep-sea octocoral *Viminella flagellum*. (A) Coral fragments originating from the basal and apical part of the colony with the latter possessing a thin skeletal axis and densely arranged polyps and the former possessing thick axis and less dense polyps; (B) Relation between polyp density and dry weight (DW) of coral fragments. Fragments originating from the base of the colonies displayed high DW values; (C) Dry weight (DW) of coral fragments distributed to the different experimental treatments. HIGH: High food availability; LOW: low food availability; FAST: no particular food provided.

5.4.3. Polyp Activity

Overall, a significantly higher percentage of fragments remained open under 400 ppm compared to 1000 ppm across food treatments (Figure 5.4; Table 5.1). More specifically, under ambient pCO_2 conditions (400 ppm) 99.4 ± 3.04% and 98.9 ± 4.23% of the fragments were recorded open in the HIGH and LOW food treatments respectively, compared to 88.9 ± 13.4% and 91.7 ± 10.5% under acidified conditions (1000ppm), for the HIGH and LOW food availability respectively. Under the two FAST treatments, polyp activity decreased substantially within the first week of the experiment (Figure 5.4) and was significantly lower compared to the other food treatments over

time (Table 5.1; Figure 5.4). During the experimental period, 77.8 ± 22% and 88.9 ± 12.6% of the coral fragments remained open under the 400ppm/FAST and 1000ppm/FAST treatments

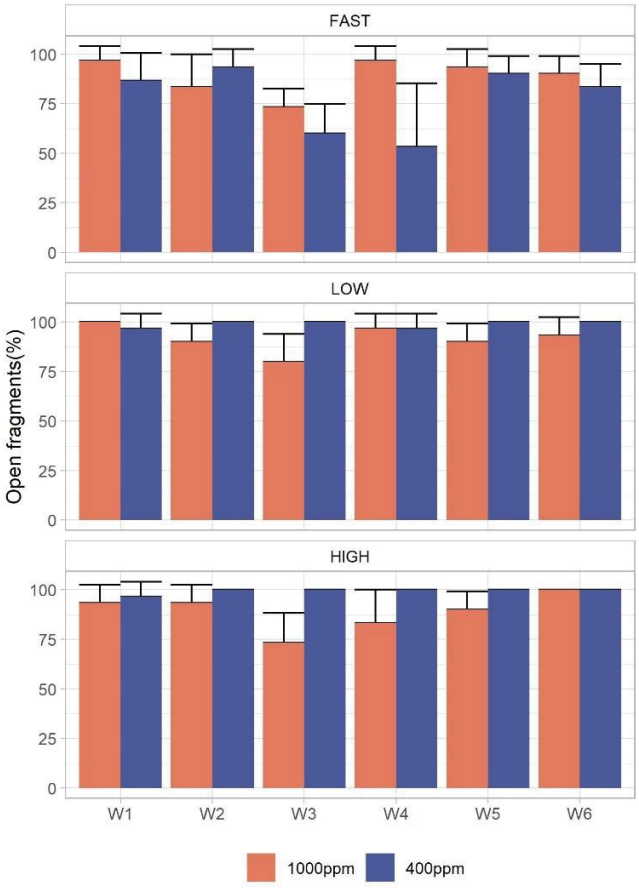

Figure 5.4: Polyp activity (average ± SD) of the deep-sea octocoral *Viminella flagellum* under two OA and food availability treatments. The OA treatments correspond to ambient (400ppm) and increased (1000ppm) pCO_2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and conditions with no provision of particulate food (FAST).

respectively, although differences between the two pCO_2 treatments were not statistically significant (Table 5.1, Figure 5.4). Under acidified conditions (LOW and HIGH food treatments) there was a significant relationship between coral DW and polyp activity (Table 5.1, Figure S 3.1), where the frequency of encountering closed fragments was higher (53% higher under HIGH food and 32% higher under LOW food treatment) for bigger fragments (DW>1) than for smaller fragments.

5.4.1. Resource acquisition

Fragments captured on average 160 ± 201 µmol C/coral DW/d under the HIGH food treatment and 54.4 ± 51.1 µmol C/coral DW/d under the LOW food treatment, which corresponded to 36.4 ± 18.6% and 75 ± 17.7% of the offered C quantity respectively.

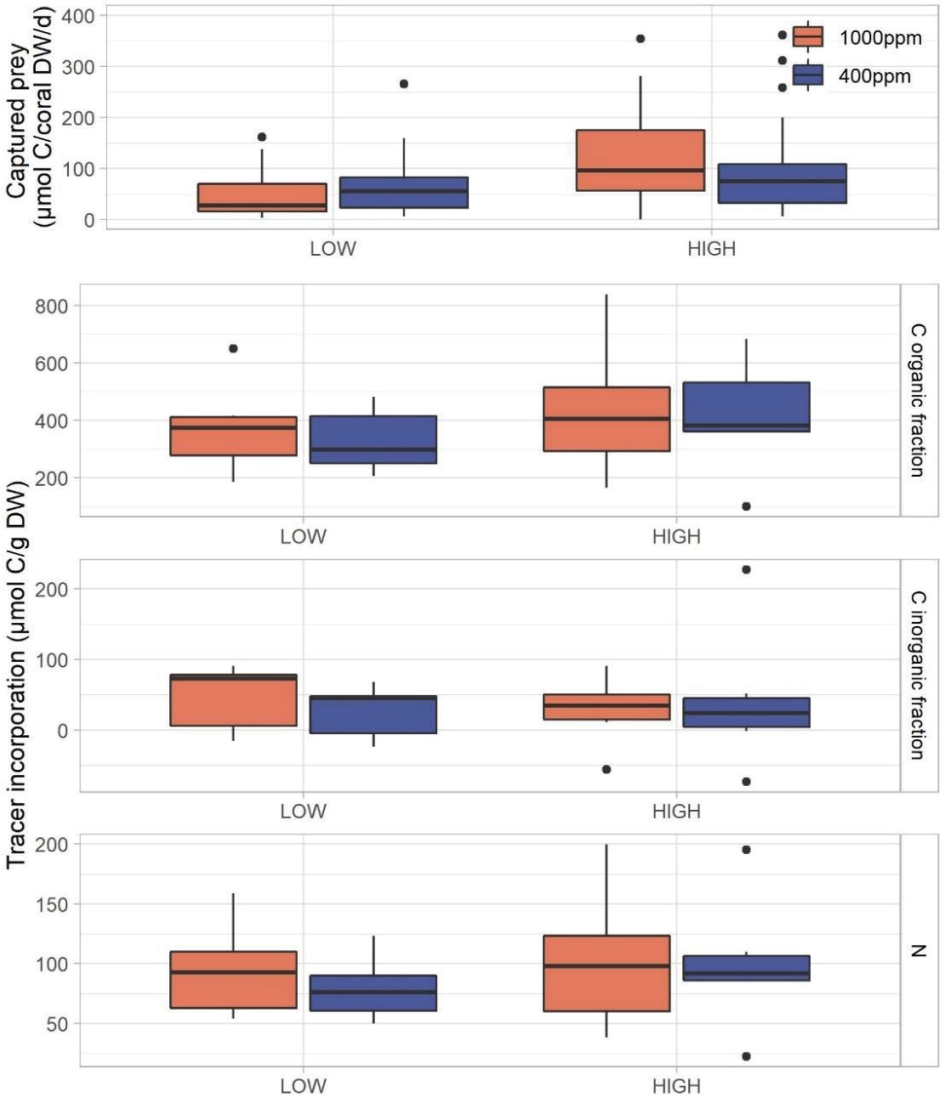

Figure 5.5: Resource acquisition of the deep-sea octocoral *Viminella flagellum*, including capture of live zooplankton prey (A) and incorporation of C and N tracers in the organic and inorganic fraction of the coral coenenchyme (B), under two acidification and food availability treatments. Acidification treatments correspond to ambient (400ppm) and increased (1000ppm) pCO_2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and conditions with no provision of particulate food (FAST).

While this difference was statistically significant for the different food treatments (Figure 5.5A, Table 5.1), we did not detect any significant effect of pCO_2 (Table 5.1). Prey capture was strongly negatively correlated to coral DW (Table 5.1, Figure S 3.2) in all treatments.

No statistically significant effect of pCO_2 was detected on tracer incorporation across food treatments (Table 5.1). In fragments under HIGH food availability, tracer C incorporation in the organic fraction of the coenenchyme as well as tracer N incorporation were higher than in fragments under LOW food availability (by 20.7% for C and 16.3% for N incorporation), however these differences were not statistically significant (Table 5.1). Similarly, tracer C incorporation in the inorganic fraction of the coenenchyme did not display any significant differences among food treatments (Table 5.1). Tracer C incorporation was negatively correlated to dry weight (Table 5.1, Figure S 3.3), i.e. basal fragments had lower tracer incorporation than apical fragments. The interaction of pCO_2 and DW (Table 5.1) appeared to be significant, however this was due to a single, very influential datapoint (Figure S 3.3). It was not possible to determine accurately tracer incorporation in the coral axis due to the high number of negative values.

5.4.2. Tissue condition

No significant pCO_2 treatment effects were detected in the total C content, Organic C content, N content and C/N ratio of coral coenenchyme. Fragments under FAST conditions displayed significantly lower values in all these variables, except for the C/N ratio where they displayed significantly higher values compared to other food treatments (Table 5.1, Figure 5.6). None of the tissue condition proxies were correlated to coral DW (Table 5.1, Figure S 3.4). Concerning the coral axis, no statistical differences were recorded on total C content, organic C, N content and C/N ratio among treatments (Table 5.1). There was also no significant relationship with coral DW (Table 5.1, Figure S 3.4).

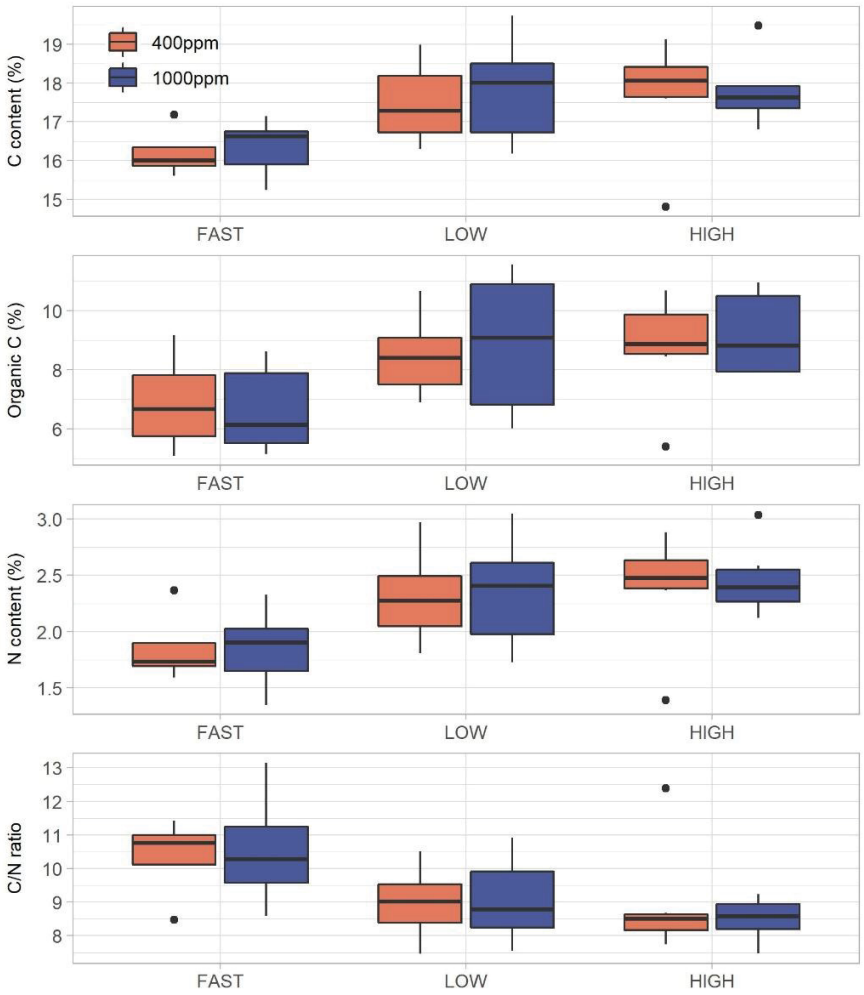

Figure 5.6: Proxies of the condition of the coral coenenchyme of the deep-sea octocoral *Viminella flagellum*, including total carbon (C) content, organic C content, nitrogen (N) content and C/N ratio, under two ocean acidification and food availability scenarios. Ocean acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO_2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and conditions with no provision of particulate food (FAST).

Oxygen consumption

Oxygen consumption depended on pCO_2, food treatments and DW of coral fragments (Table S 3.1, significant interaction). Under 1000ppm, in both the HIGH and LOW food treatments, oxygen consumption did not change significantly throughout the experiment (Table 5.1). On the contrary, in fragments of the HIGH food/400ppm treatment, oxygen consumption increased

significantly (Table 5.1) by 175% after the acclimatization period and remained higher than fragments in the HIGH food/1000 ppm treatment throughout the experiment (Figure 5.7). Under the LOW food/ 400ppm, oxygen consumption also increased by 169% during the first week and remained at high levels throughout the experiment (Figure 5.7) but due to high standard deviation among fragments of the LOW food/1000ppm, especially during W2 and W6, no significant difference was detected among pCO_2 treatments (Table 5.1). Under the FAST treatment oxygen consumption decreased significantly (Table 5.1) throughout the course of the experiment (Figure 5.7). During the first week (W1) oxygen consumption decreased by 26.7% and 42.5% under the FAST/400ppm and the FAST/1000ppm treatment, respectively. It continued decreasing, and on W4 reached 34.1% (FAST/400ppm) and 2% (FAST/1000ppm) of the initial oxygen consumption rates (estimated on W_{AC}). Throughout the experiment, oxygen consumption rates under the FAST/400ppm treatment remained significantly higher (Table 5.1) than under the FAST/1000ppm treatment (Figure 5.7).

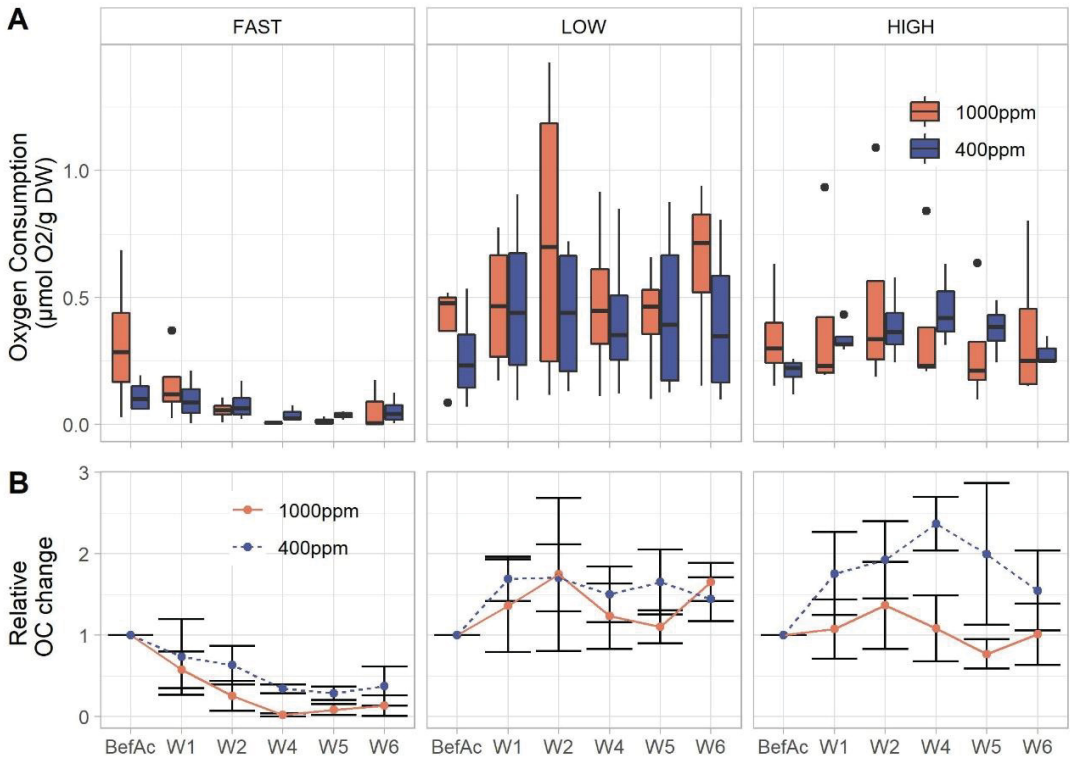

Figure 5.7: Oxygen consumption of the deep-sea octocoral *Viminella flagellum* under different ocean acidification and food availability scenarios. (A) Oxygen consumption of all measured coral fragments before acclimatization to experimental conditions (BefAc) and during the course of the experiment (Week

1-Week 6); (B) Relative oxygen consumption (OC) change, in respect to the OC measured before acclimatization. Acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and conditions (FAST) with no provision of particulate food.

Oxygen consumption was negatively and significantly related to coral DW except under the HIGH/400ppm treatment. However, in this treatment the subsample of fragments used to determine oxygen consumption had a very narrow range of dry weight (Figure S 3.5).

5.5. Discussion

In this study, we focused on the metabolic response of a deep-sea octocoral to OA (pCO_2) scenarios predicted for 2100, under conditions of different food availability. Our results revealed that the target species has the capacity to lower its metabolism in response to stressful environmental factors, including high pCO_2 and the absence of particulate food.

Under projected future high pCO_2 conditions, *V. flagellum* displayed reduced polyp activity and lower oxygen consumption, compared to current pCO_2 levels. The response of *V. flagellum* was much more pronounced under absence of particulate food, including significantly lower polyp activity, abrupt decrease in oxygen consumption and changes in tissue condition. The lower C/N ratios under fasting suggest some extent of protein catabolism which, along with the very low oxygen consumption rates, are indicative of metabolic arrest (Wilmer et al. 2004). A decrease in oxygen consumption after 3 weeks of zooplankton deprivation has been also recorded for the deep scleractinian *Desmophyllum dianthus* (Naumann et al. 2011). However, *V. flagellum* adjusted its polyp activity and oxygen consumption within a week, a very quick response to fasting that to our knowledge has not been reported before in deep-sea corals (Baussant et al. 2017, Maier et al. 2019b).

Several marine invertebrates, including filter and suspension feeders, have responded to high pCO_2 with a decrease in metabolic rates (Langenbuch & Pörtner 2004, Michaelidis et al. 2005, Hu

et al. 2014, Lee et al. 2020). In many cases, these responses were accompanied by a decrease in protein synthesis and general metabolic depression and were linked to the low ability of the studied species for acid/base regulation (Fabry et al. 2008). In the present study, tissue condition proxies did not provide any indication of protein catabolism due to exposure to high pCO_2. This might be due to relatively short duration of the experiments which may not have been sufficient to unveil impacts of metabolic processes on the condition of coral tissue (Pörtner 2008). Alternatively, tissue condition might have been maintained through acquisition of new resources, since despite the indications of a lower metabolic rate, no effects of pCO_2 were detected on prey capture and tracer incorporation.

Decreased polyp activity under exposure to high pCO_2 conditions has been reported before in the octocoral *C. rubrum* and was further accompanied by a decrease in calcification (Cerrano et al. 2013). Moreover, a decrease in oxygen consumption has been recorded for the deep-sea scleractinian *L. pertusa* (Hennige et al. 2014), however in this case growth and calcification were not affected. The difference between the two species may be attributed to their differing capacities to upregulate pH at the site of calcification. Scleractinian species appear to be able to upregulate the pH of calcifying media, allowing the maintenance of calcification rates under high pCO_2 (McCulloch et al. 2012, Anagnostou et al. 2012). The opposite has been reported for *C. rubrum*, which does not seem to possess such mechanism (Le Goff et al. 2017). In our study, tracer C incorporation in the inorganic fraction of the coenenchyme, which includes octocoral's calcitic skeletal elements (sclerites), was not affected by elevated pCO_2. However, this variable does not represent the process of calcification and therefore further studies are needed to unravel the responses of calcification and growth of octocoral species under high pCO_2.

The presence of particulate food helped the species to maintain higher metabolic rates during the experimental period, however, high food concentration did not seem to alter the response of *V. flagellum* to acidification, compared to the low food concentration. This is consistent with previous studies on the deep-sea scleractinian *Lophelia pertusa* (Büscher et al. 2017, Gómez et al. 2018). According to our results on resource acquisition, not all coral fragments under the HIGH

food availability were able to utilize the higher amount of provided prey. This was especially true for fragments that originated from the base of coral specimens, as they possess low number of smaller polyps and captured less prey, compared to fragments originating from tips. These fragments sustain the thickest part of the coral axis and despite their high DW, prey capture, tracer assimilation and respiration were not proportional to their DW (Figure S 3.1-Figure S 3.5). The described particularities of different parts of the colony constitute the main limitation of this study, as the presence of specific fragments with very high or very low DW in some experimental treatments masked treatment effects, especially when these were not strong. The relationship between DW and metabolic variables originates from metabolic scaling in modular animals (Burgess et al. 2017) which are beyond the scope of the current study. Nevertheless, these results showcase how different parts of the colony may respond differently to environmental factors.

The C amount provided under the LOW food treatment, corresponded to C concentrations recorded during the summer, but it consisted solely of zooplankton-derived C, which might not be present in such high concentrations during this period (Carmo et al. 2013). The amount of zooplankton prey provided under the LOW food treatment, was enough to sustain the respiration rates of the species to levels similar to the ones recorded before acclimatization, even under high pCO_2. These results showcase the importance of zooplankton prey for the metabolic demand of this species (Rakka et al. 2021a) and highlight the capacity of *V. flagellum* to adapt to the variable C conditions in its natural environment. This species is typically encountered in areas with oligotrophic waters, where octocorals often go through periods of dormancy, especially in summer months when food availability is scarce (Coma et al. 2000, Rossi & Tsounis 2007).

Metabolic responses of benthic invertebrates to stress can be classified in two broad strategies: compensation and conformity (Wilmer et al. 2004). The former includes energetically demanding processes that result in a shift of energy allocation from maintenance to repairing stress-induced damage, while the latter includes a decrease of metabolic rates to conserve energy for future, more favorable conditions (Sokolova et al. 2012). Our findings indicate that *V. flagellum* employed the conformity strategy under the most stressful treatments, including fasting and high

pCO_2. This strategy has been reported before in octocoral species, e.g. decreased polyp activity and respiration were recorded in four common Mediterranean octocoral species upon exposure to suboptimal temperatures (Previati et al. 2010), as well as in cold-water octocorals (Gugliotti et al. 2019). Similarly, molecular markers suggested decreased metabolism as a seasonal response to tolerate high temperatures in the temperate octocoral *Veretillum cynomorium* (Madeira et al. 2015). However, this is the first time that this physiological response to OA is reported for a deep-sea octocoral and studies with more species are essential to determine if it is a generalized or a species-specific strategy.

Although metabolic depression is a common response of invertebrates to short-term reversible stress (Guppy & Withers 1999), very low metabolic rates cannot be sustained for long periods, due to the need to fuel basic metabolic demands and export toxic metabolic byproducts (Hand & Hardewig 1996, Sokolova et al. 2012). Moreover, metabolic depression can have serious impacts on major physiological processes such as growth and reproduction and reduce survival of the species if it is maintained for long periods (Langenbuch et al. 2006, Fabry et al. 2008). Climate change is expected to affect water stratification and productivity with negative impacts on plankton communities (Bopp et al. 2005) and export of carbon to the deep-sea (Yool et al. 2013, Sweetman et al. 2017). A potential prolongation of periods with low food availability or low zooplankton abundance might not directly affect the survivorship of *V. flagellum*, but may drive the species to extended periods of low metabolic rates with adverse consequences on its overall fitness (Hofmann et al. 2010).

Collectively, *V. flagellum* was able to withstand a short (6-week) exposure to high pCO_2 levels projected to the end of the century and appeared to be resilient to the stress of fasting and the combination of fasting and high pCO_2. This was achieved by a decrease in metabolic rate which was relative to stress intensity, with the most extreme response appearing under fasting and high pCO_2 conditions. The presence of low food quantities alleviated the metabolic response in respect to pCO_2. Higher food concentration led to higher oxygen consumption under ambient pCO_2, however it did not seem to alter the species reaction to acidification.

Octocoral species are major habitat-formers in the deep-sea, supporting rich biodiversity (Guizien & Ghisalberti 2017, Buhl-Mortensen et al. 2017) and fueling the local carbon cycle (Rossi et al. 2017b, Coppari et al. 2019). Therefore, a possible decrease in their growth, metabolism, reproduction and fitness may have deleterious effects to the communities they form. Future climate change scenarios predict that deep-sea species will have to face multiple stressors, such as acidification, rise in temperature, lower oxygen concentration and decreased carbon input (Sweetman et al. 2017, Levin et al. 2019a). Species respond differently to environmental variables (Kroeker et al. 2010, Dupont & Pörtner 2013) and this is expected to cause shifts in higher ecological levels, including species distributions (Morato et al. 2020c) and community composition (Birchenough et al. 2015). Under these circumstances, a more complete understanding of the physiological responses of deep-sea corals to upcoming changes, including multiple stressors and processes beyond calcification, are essential to comprehend and manage potential shifts in deep-sea communities and ecosystems.

Chapter 6
General Discussion

During the past 20 years, studies on deep-sea corals have increased exponentially, enhancing our knowledge on deep-sea ecosystems (Freiwald et al. 2004, Roberts et al. 2009, Rossi et al. 2017a). However, attention is very frequently drawn on reef-building corals. This is especially pronounced in the case of experimental studies on ecophysiology and the impacts of climate change, which have primarily focused on deep-sea scleractinian species (e.g. Movilla et al. 2014, Carreiro-Silva et al. 2014, Maier et al. 2016), with very few studies existing so far on deep-sea octocorals (Gómez 2018, Rossin et al. 2019). This severely undermines our understanding of deep-sea ecosystems, especially considering the importance of deep-sea octocorals as ecosystem engineers in the deep-sea, and their high levels of diversity (Chapter 1). The present study aimed at (1) contributing to our knowledge on the biology of deep-sea octocorals across different life history stages and (2) assessing the potential impacts of climate change on deep-sea octocorals. It focused on two of the most common habitat-forming octocoral species in seamounts of the Azores Archipelago, the sea fan *Dentomuricea* aff. *meteor* and the whip coral *Viminella flagellum*. The region hosts a remarkable diversity of deep-sea octocorals (Braga-Henriques et al. 2013, Sampaio et al. 2019), constituting an ideal case study for the development of the current thesis.

A two-step comparative approach was adopted to fulfil the thesis aims. Firstly, in chapters 2-4, the thesis focused on gathering baseline knowledge on the species traits across three major processes: reproduction, early life development and energy metabolism. These processes and their associated traits are of great importance for species performance and persistence. Reproduction is a fundamental process for the continuity of species over time (Holt et al. 2003). Early life history characteristics determine a species ability to disperse (Metaxas & Saunders 2009, Cowen & Sponaugle 2009). Lastly, energy has been characterized as the “currency of fitness” (Burger et al. 2019) and therefore metabolism, referring to the way an organism acquires

and utilizes energy, is crucial for its ecology and evolution (Brown et al. 2004). The produced information constitutes an important contribution to our knowledge on deep-sea octocorals and can be used to assess their vulnerability upon various stressors on a conceptual level. Subsequently, the thesis utilized an experimental study (Chapter 5), to add on existing knowledge about the actual capacity of one of the target species to deal with specific climate stressors.

Climate change is expected to introduce multiple changes to the deep-sea, including temperature rise, altered carbonate chemistry, reduced oxygen concentration and food availability. These changes are expected to pose complex challenges to deep-sea organisms (Sweetman et al. 2017, Levin et al. 2019a). Deep-sea corals are generally considered vulnerable to climate stressors because they are sessile, calcifying, with relatively low metabolic rates and long lifespan (Guinotte et al. 2006, Roberts et al. 2009). Nevertheless, experimental studies have highlighted several deep-sea coral species that are resistant to some of the projected ocean changes such as increased pCO_2 and temperature (reviewed in Maier et al. 2019). These results highlight that even within groups that are considered vulnerable, there is great variability in the response of different species to climate change (Kroeker et al. 2010, Wittmann & Pörtner 2013). In this final chapter (General discussion), the generated information on life history traits is combined with experimental data produced herein and from the literature, to (1) discuss the potential fate of deep-sea octocorals in a changing world and (2) utilize the two species studied herein as a case study to assess the potential outcomes of climate change in deep-sea octocorals and the communities they form.

6.1. Will deep-sea corals be able to cope with climate change?

Most deep-sea organisms have evolved in relatively stable conditions, compared to their shallow-water counterparts, resulting in a relatively lower tolerance to disturbance (Levin & Le Bris 2015). Moreover, the deep-sea exhibits characteristics that make climate change even more challenging for deep-sea organisms, such as low temperature and low food availability. Due to these characteristics, the metabolic rates of deep-sea organisms are generally low, decreasing their ability for physiological regulation to withstand adverse conditions (Pörtner et al. 2004, Pane &

Barry 2007). The low temperature and high pressure that prevail in deep-sea environments cause a decrease in the saturation state of calcium carbonate, especially under acidified conditions (Guinotte et al. 2006, Murray Roberts et al. 2016). This will pose a challenge, especially for calcifying species that use calcium carbonate polymorphs with higher solubility, such as high magnesium calcite and aragonite (Ries et al. 2009, Bostock et al. 2015). Deep-sea octocoral species display a remarkable diversity of skeleton formation strategies and can use a variety of polymorphs to calcify (Chapter 1). Thus, some species might be more resilient to the difficulties that climate change will pose to calcification, as also showed by their evolutionary history (Conci et al. 2021). Although recent studies suggest octocoral species do not seem to upregulate the pH at the site of calcification like scleractinians (Le Goff et al. 2017), octocoral tissue protects their skeletal elements from acidification (Gabay et al. 2013), providing an additional advantage to cope with the adverse conditions caused by climate change.

In temperate regions, deep-sea ecosystems are often characterized by seasonal or episodic input of organic carbon from shallower depths (Davies et al. 2009, Duineveld et al. 2012). Some deep-sea octocorals have developed an opportunistic feeding strategy with the ability to exploit rapidly food sources of high quality and high energetic content, such as zooplankton (Chapter 4). However, a potential decrease in the organic carbon (Yool et al. 2013, Sweetman et al. 2017) or a change in zooplankton dynamics (Brun et al. 2019) due to climate change may prove detrimental for their physiology. For example, due to the seasonal or episodic carbon input, many octocoral species have developed an opportunistic reproductive strategy with the continuous existence of a gamete pool that can be further developed under optimum conditions (Chapter 2). Species that adopt this reproductive strategy may be able to cope with a decrease in food availability, however this may result in lower reproductive output, longer reproductive cycles and a decrease in oocyte size. These may have concomitant implications for recruitment and larval biology (Przeslawski et al. 2015), affecting negatively the adaptive capacity of the species. The two target species have developed lecithotrophic larvae which do not depend on the water column to feed and may not be directly affected by lower food availability (Gibson et al. 2011). However, maternal condition is extremely important to provision larvae with energetic reserves

(Marshall & Morgan 2011), causing indirect effects of climate stressors on larval characteristics such as survival, pelagic duration and size, and limiting their dispersal. Lastly, the direct impacts of climate change stressors to early life history stages of deep-sea octocorals are, so far, unknown. Ocean acidification, warming and deoxygenation might have direct impacts on fertilization and embryo development, especially for broadcast spawning species, as well as on larval physiology (Gibson et al. 2011). Therefore, more experimental studies are essential to understand the potential impacts of climate change on early life processes of deep-sea octocorals.

A growing number of studies suggest that organism responses to climate change are species-specific and depend on species life history traits (Kroeker et al. 2010, Dawson et al. 2011). Species of the subclass Octocorallia display a remarkable diversity of traits throughout their life cycle (Chapter 1) and thus will likely respond differently to upcoming changes.

6.2. Comparative analysis of species sensitivity to climate change

In recent years, more and more studies have highlighted that species responses to climate change stressors depend on their biological traits (Dawson et al. 2011, Doney et al. 2012). More specifically, the biological characteristics of an organism determine its sensitivity and adaptive capacity to various stressors. Sensitivity refers to the extent to which a stressor can affect the performance and survival of a species, while adaptive capacity refers to the species potential to avoid the stressor, either by shifting its distribution or by micro-evolutionary adaptation (Foden et al. 2013, Moritz & Agudo 2013).

As a result, a solid background on the biology and ecology of a group of organisms, along with their potential exposure, i.e. the degree to which the environmental conditions will change, allows us to evaluate their potential vulnerability to climate change (Figure 6.1). For example, high sensitivity to disturbance is frequently linked to habitat specialization and narrow environmental tolerance (Foden et al. 2013, Jones & Cheung 2018). In this case, species that have a very narrow distribution, trophic niche, or are specialized in specific habitats are adapted to

specific conditions and are expected to have higher sensitivity to stressors. Adaptive capacity, on the other side, is frequently associated with the dispersal ability of a species, and its potential to evolve (Dawson et al. 2011, Foden et al. 2013). Species with larvae that display low dispersal capacity, are less likely to reach more adequate habitats compared to species with larvae that can disperse longer. Moreover, species with low fecundity or low genetic diversity have low potential to evolve and therefore have intrinsically low adaptive capacity. Recent implementation of these concepts in deep-sea ecosystems, including coral gardens, has identified wide gaps in our knowledge of species biological and ecological traits, and the ways these are influenced by biotic and abiotic factors (Xavier et al. 2019). This hinders a proper assessment of their vulnerability to climate change and highlights the importance of the information gathered in this thesis for the assessment of climate change impacts on deep-sea species and ecosystems.

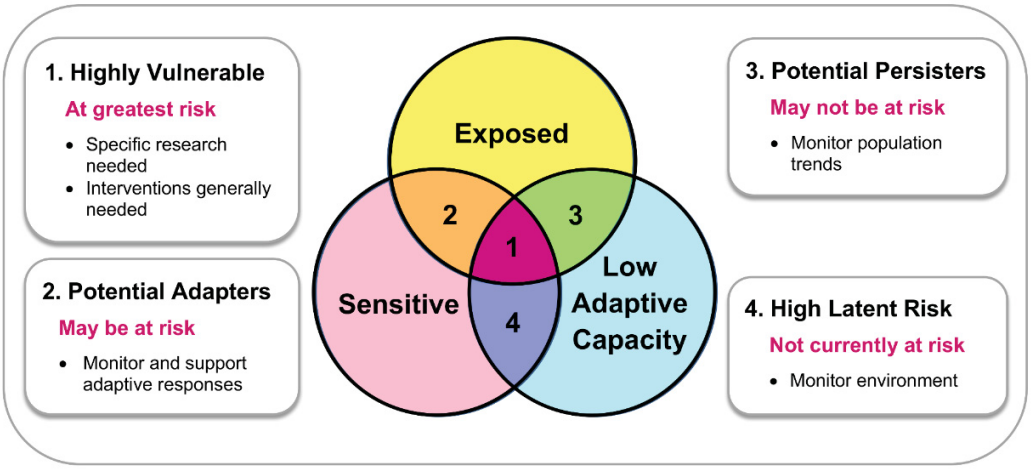

Figure 6.1: Assessing the vulnerability of species to climate change, according to three dimensions: Exposure, sensitivity, adaptive capacity. Foden et al., 2013.

Although the two species that were studied herein are encountered at similar depths and environments in the Azores, (Chapter 1), they display several differences that may determine their sensitivity and adaptive capacity to climate change stressors (Figure 6.1). *Dentomuricea* aff. *meteor* is a species with narrow distribution, so far only known to occur in the Azores and adjacent seamounts to the Mid-Atlantic Ridge. On the other hand, *V. flagellum* has much wider

distribution and can be encountered in the Mediterranean, Macaronesia and eastern North Atlantic, and therefore has a wider niche compared to *D.* aff. *meteor*. Species with wider geographic and bathymetric distributions, such as the case of *V. flagellum* are generally considered more flexible as they are expected to have wider environmental tolerance (Dawson et al. 2011, Jones & Cheung 2018). Moreover, when considering specific stressors such as OA, *V. flagellum* seemed to be able to withstand short-term changes (Chapter 5). Previous experimental studies with *D.* aff. *meteor* under similar OA scenarios (RCP8.5) for comparative experimental periods, showed that the species displayed lower metabolic rates, but also tissue mortality, indicating higher sensitivity to low pH conditions (Carreiro-Silva, upublished data, MERCES project). In addition, a scenario that combines ocean acidification and low food availability, which is very likely to occur in the Azores (Sweetman et al. 2017), will pose additional challenges to *D.* aff. *meteor*, due to its high metabolic requirements (Chapter 4). This is indicative that *D.* aff. *meteor* is likely more sensitive to the projected scenarios in the Azores for 2100.

Despite the capacity of *V. flagellum* to persist through experimental conditions of low pH/high pCO_2 for a month, its response included a decrease in metabolism which may prove costly in the long term, especially for fundamental processes such as growth and reproduction. As shown in Chapter 2 and Chapter 3.2, this species displays high maternal investment to its lecithotrophic larvae. Previous studies on the effect of OA on the reproduction of deep-sea corals revealed a decrease in oocyte size and fecundity under high pCO_2 levels, predicted for the end of the century (Rossin et al. 2019). It is therefore possible that long-term exposure to ocean acidification may have adverse effects on the sexual reproduction of the species. *Viminella flagellum* is capable of reproducing through fragmentation (Giusti et al. 2012), which may allow it to persist even when sexual reproduction is impaired (Foster et al. 2007, Wangensteen et al. 2016). However, sexual reproduction is important to maintain genetic diversity (Wangensteen et al. 2016) and maintain a high adaptive potential to changes in the natural environment. Further studies on the genetic structure of populations of the species are therefore essential to determine the contribution of asexual reproduction to recruitment at present and its potential as an alternative mode of reproduction under future climate change scenarios.

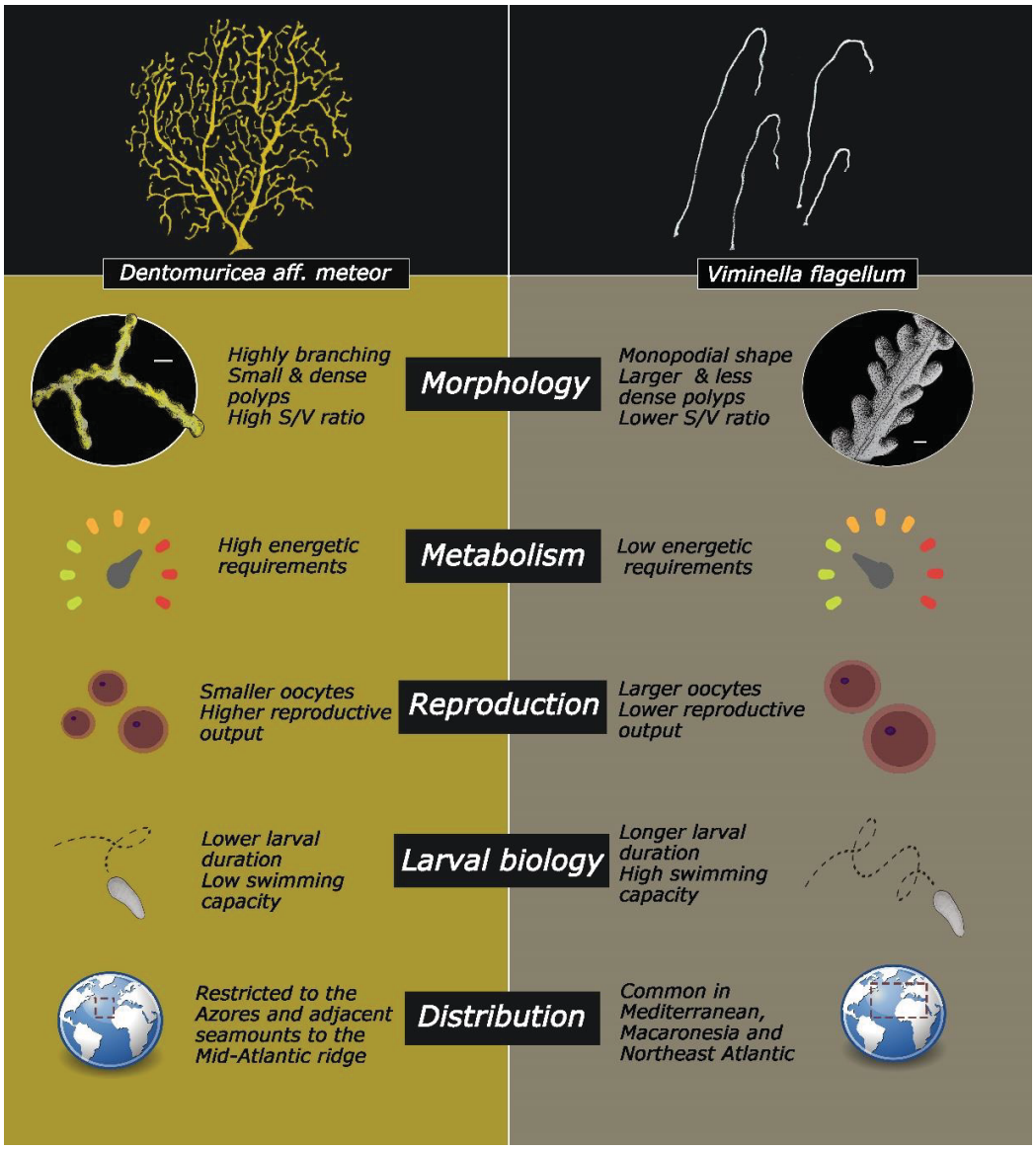

Figure 6.2: Differences in biological and ecological traits between the octocoral species *Dentomuricea* aff. meteor and *Viminella flagellum*.

The adaptive capacity of a species depends additionally on its dispersal potential, as species with longer dispersal may be able to shift their distributions to areas with more favourable conditions (Dawson et al. 2011, Foden et al. 2013). *Dentomuricea* aff. *meteor* displayed lower dispersal capacity than *V. flagellum*, due to its lower duration in the pelagic phase and weak swimming abilities compared to *V. flagellum* which displayed active, upward swimming and longer duration

in the pelagic phase (Chapter 3). Considering that larvae of *D.* aff. *meteor* presented indications of limited energy reserves (Chapter 3.1), it is possible that under conditions of low food availability the available reserves will be even more limited, decreasing even more the dispersal potential of the species. Nevertheless, this may present a problem for both species, as they both display high maternal investment which may be impaired under conditions of low food availability or low metabolic rates caused by ocean acidification.

Another key characteristic of a species adaptive capacity is its microevolutionary potential, i.e. the capacity of a population to go through changes in its genetic composition to adapt to specific conditions (Gienapp et al. 2008). This capacity is higher in species with high reproductive output and short generation lengths, as these traits increase the number of offspring that will go through the process of natural selection. Both species displayed very low polyp fecundity compared to other deep-sea octocorals (Chapter 2) and especially compared to deep-sea scleractinians which release hundreds of oocytes of smaller size (Waller & Tyler 2005, Harrison 2011). As a result, in each spawning event the number of produced juveniles that will be subjected to environmental conditions is low, decreasing the likelihood of adaptation for both target species (Aitken et al. 2008).

Studies on the recovery capacity of the two species after transplantation of colony fragments from aquaria to natural areas in the deep-sea point out towards a greater capacity for tissue regeneration and growth for *V. flagellum* than for *D.* aff. *meteor* (Carreiro-Silva, upublished data), demonstrating the high physiological resilience of *V. flagellum.* Overall, the comparison between the two species highlights that *V. flagellum*, presents characteristics of a generalist and appears to be more resilient to projected changes for the end of the century. On the other side, *D.* aff. *meteor* has a more specialized niche, and a relatively higher vulnerability to prospective changes due to climate change. The prevalence of such changes for long-term periods are likely to pose substantial challenges to both species and more studies are essential to create more certainty regarding the considerations presented herein. However, the present study is in line with existing literature on climate change effects on communities, which suggest that climate change favours

generalist taxa and is more likely to cause declines of rare and specialized species (Doney et al. 2012, Colossi Brustolin et al. 2019). The dominance of generalist species very often causes a decrease in genetic and functional diversity, with negative effects in ecosystem function (McKinney & Lockwood 1999, Alvarez-Filip et al. 2013). This is also exemplified in the current study: as mentioned in Chapter 4, *D.* aff. *meteor* likely has a larger contribution to C recycling than *V. flagellum* and provides more habitat through its highly branched colonies, thus a potential removal of this species, or a decrease of its population is likely to disrupt the local C cycling and ability of the community to sustain other species.

6.3. Conclusions and further considerations

Climate change is not the sole disturbance that deep-sea organisms have to cope with. Deep-sea ecosystems are already suffering impacts by numerous anthropogenic stressors, such as fisheries, oil and gas extraction, while additional pressure is expected to be posed by deep-sea mining (Clark et al. 2016, Ragnarsson et al. 2017, Levin et al. 2019b, 2020, Smith et al. 2020). Studies on both terrestrial and marine ecosystems recognize that human disturbance and climate change have complex impacts on ecosystems that start with species responses and have concomitant impacts on community structure, trophic interactions, energy flow and ultimately ecosystem function (Doney et al. 2012, Blois et al. 2013, Nagelkerken & Connell 2015). One of the recurrent themes, both in current cases of disturbance and in past geological periods that were marked by environmental changes, is biotic homogenization, i.e. the dominance of generalist species with broad niche within ecological communities (Blois et al. 2013, Magurran et al. 2015). Such shifts may disrupt trophic relationships and ecosystem function, with concomitant impacts on ecosystem services (Colossi Brustolin et al. 2019). While the establishment of these communities are often transitory when viewed in a geological scale, they are relatively permanent in the shorter time scale perceived by humans (Blois et al. 2013). As a result, the potential decrease in ecosystem services due to the combined effects of multiple human disturbances is likely to have long-term economic and social consequences.

Biotic homogenization has received increased attention in shallow marine ecosystems (Magurran et al. 2015, Garciá Molinos et al. 2016, Pecl et al. 2017), however, it is rarely discussed as a potential outcome of anthropogenic disturbance in the deep-sea. So far, deep-sea corals have been perceived as a homogenous group of species that are vulnerable to disturbance. However, their geological and evolutionary history, along with recent physiological and experimental studies (Maier et al. 2019a) suggest that some species might be capable to withstand certain stressors. Consequently, further attempts need to be made to attain a more holistic knowledge of deep-sea coral communities and a better understanding of species biology, including widespread generalists and more specialized or rare species. Without this knowledge, it is unlikely that we will be able to understand the impacts of climate change, determine whether these are already affecting deep-sea coral communities and predict if deep-sea corals will be able to survive in future conditions.

The current thesis provided essential fundamental knowledge on several life history traits of deep-sea octocorals, as well as their physiological responses to climate change stressors. It further demonstrated how this knowledge can be used as a baseline to assess the sensitivity and vulnerability of these species to climate change, highlighting potential implications on the ecosystem level. Trait based approaches are increasingly utilized in terrestrial and marine ecosystems to assess the impacts of multiple anthropogenic disturbances on ecological communities (Costello et al. 2015, Butt & Gallagher 2018) and inform conservation actions (Miatta et al. 2021). In the deep-sea, similar approaches have only recently been implemented (Xavier et al. 2019, Chapman et al. 2019, Murillo et al. 2020, Boschen-Rose et al. 2021). Especially in the case of long-lived species, such as deep-sea corals and other deep-sea habitat formers which have long generations and therefore lower capacity for microevolutionary adaptation, intrinsic traits determine their capacity to withstand certain conditions and can be very informative to assess their vulnerability upon climate change. As a result, trait-based approaches can be very useful for this kind of species. Lastly, trait-based methods use mechanistic approaches and provide a more holistic and strong knowledge baseline of the studied ecosystems. This is essential in the deep-sea, where the urge for management and governance

surpasses our current understanding, putting at risk not only these fragile environments but also future generations.

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APPENDIX A

Supplementary data-chapter 2

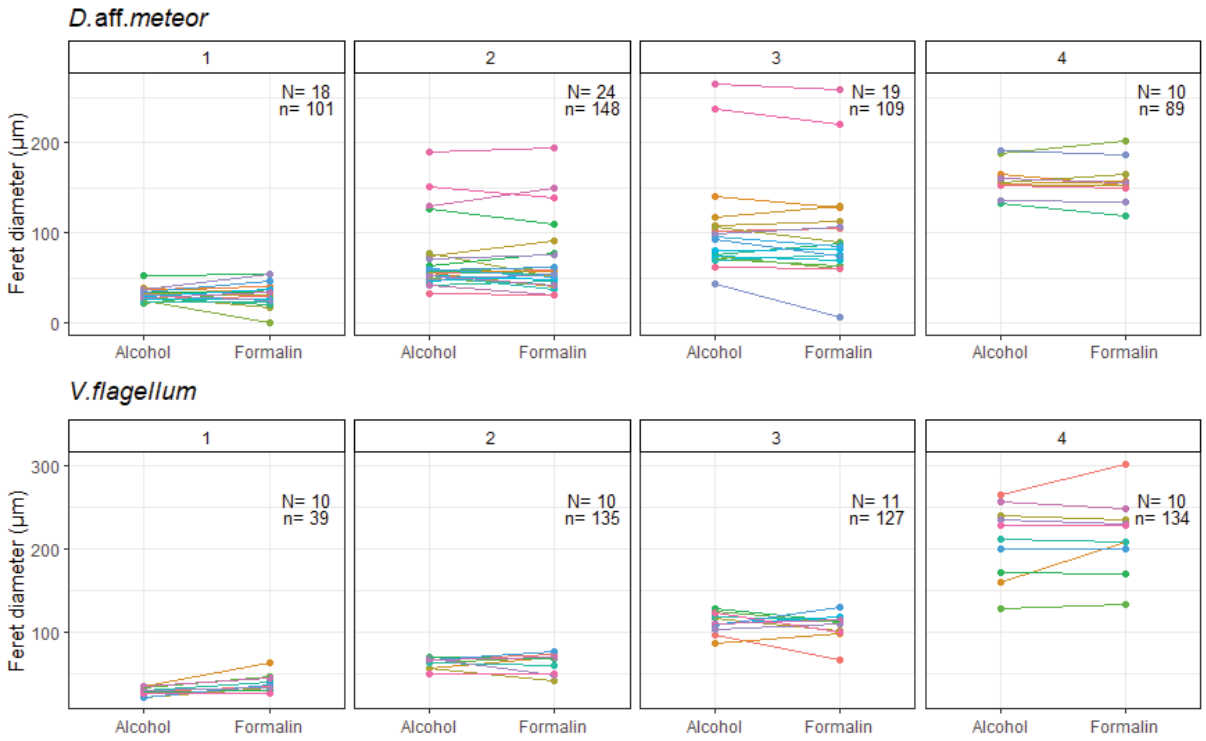

Figure S 0.1: Comparison of oocyte size (feret diameter) of the species *Dentomuricea* aff. *meteor* and *Viminella flagellum*, estimated from histologically processed tissue subsamples that were fixed in alcohol and formalin. Oocytes were in various developmental stages (1-4). Colored lines connect subsamples of the same specimen. Number of specimens (N) and oocytes (n) are provided for each stage.

Table S 0.1: Results of paired t-test analysis to compare oocyte size estimated from histologically processed subsamples fixed in alcohol and formalin, showing that rehydration and a posteriori fixation of alcohol-fixed tissue does not affect oocyte size significantly.

Species	t	df	p value
D. aff. *meteor*	0.4	446	0.68
V. flagellum	-0.78	434	0.43

Table S 0.2: Estimated coefficients for the analysis of each dependent variable in question. IOF: Immature Oocyte fecundity, ERF: Effective relative fecundity, LM: Linear model, GLM: Generalized linear model, SE: standard error.

Dependent variable	Independent variable	Analysis	Coefficient(s)	Estimate	SE	t value	p
Oocyte size	Species	LM	*D.* aff. *meteor*	166.74	4.52	36.84	$<2 \times 10^{-16}$
			V. flagellum	228.9	5.92	10.49	$<2 \times 10^{-16}$
IOF *D. meteor* 2010	Month	Poisson GLM	July	2.48	0.2	12.1	$<2\ 10^{-16}$
			September	1.45	0.2374	7.73	$1.23\ 10^{-5}$
			October	1.45	0.28	7.58	$6.14\ 10^{-6}$
IOF *D. meteor* 2011	Month	Poisson GLM	July	1.72	0.105	16.38	$<2\ 10^{-16}$
			September	0.29	0.51	13.49	0.004
			October	1.38	0.51	-15.64	0.504
			February	1.33	0.25	14.75	0.12
			May	1.54	0.28	15.65	0.512
			June	1.79	0.16	16.79	0.62
ERF *V. flagellum* 2011	Month	Poisson GLM	February	2.34	0.13	16.8	$<2\ 10^{-16}$
			May	1.67	0.21	13.66	0.001
IOF *V.flagellum* 2011	Month	Poisson GLM	February	1.87	0.2774	6.749	$1.49\ 10^{-11}$
			May	1.54	0.3852	5.88	0.38
			September	2.83	0.2920	10.06	0.0008
			October	2.57	0.2963	9.11	0.017

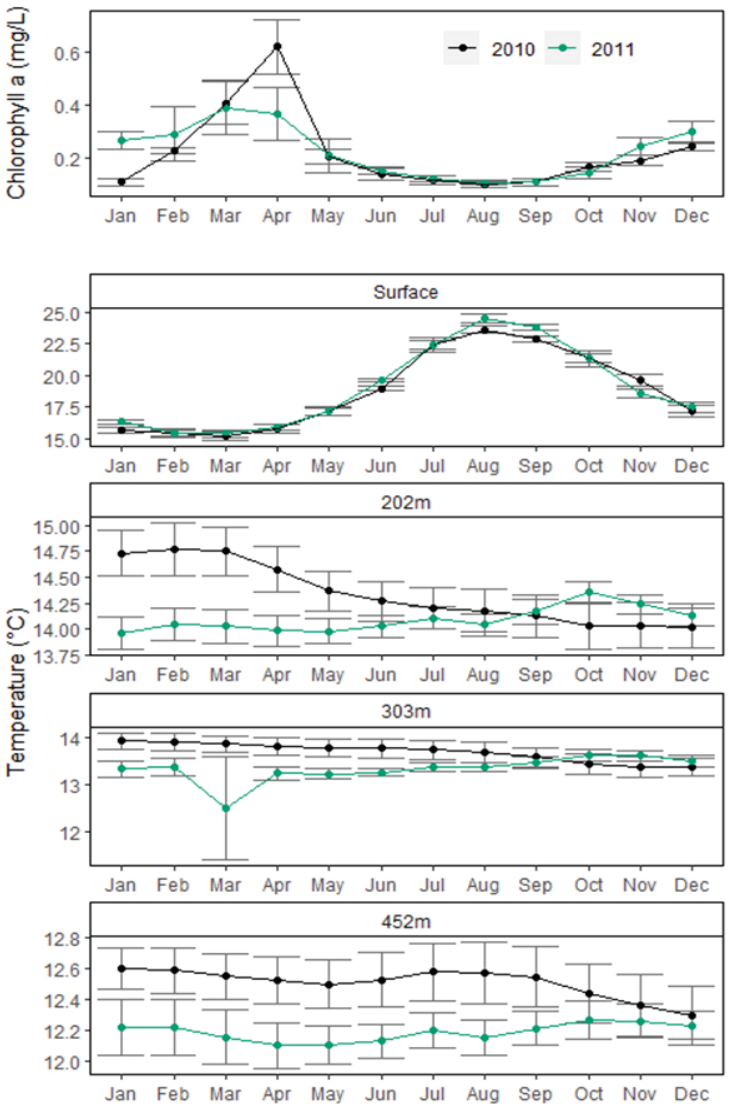

Figure S 0.2: Chlorophyll a, as well as temperature seasonality in four depth levels (surface, 202m, 303m, 452m) at the sampling locations. Values are provided as average ± standard deviation. Temperature data were compiled from the following datasets: ARC (AATSR Reprocessing for climate) dataset with Level 3 monthly sea surface temperature measurements (MacCallum, 2013), obtained by the NERC Earth Observation Data Centre; and Extended Reconstructed Sea Surface Temperature (ERSST) version 4 dataset (Huang et al., 2015) provided by NOAA. Chlorophyll a concentration data were compiled using the Chlorophyll concentration according to Color Index Algorithm (CHL-CIA) dataset (Hu et al., 2012) developed, validated, and distributed by ACRI-ST, France and available through the GlobColour project (http://globcolour.info).

APPENDIX B

Supplementary Data-Chapter 3.1

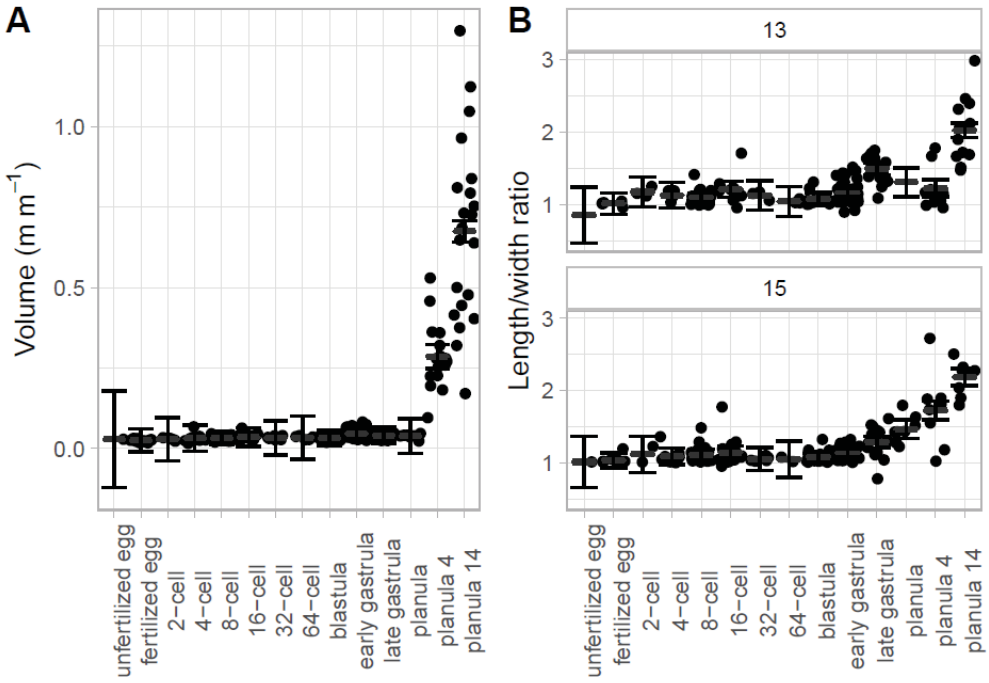

Figure S 1.1: Visualization of model effects constructed to compare embryo size (volume) and embryo length/width ratio among different developmental stages of the octocoral *Dentomuricea* aff. *meteor*. Lines represent model fit and 95% confidence intervals, while points represent model partial residuals.

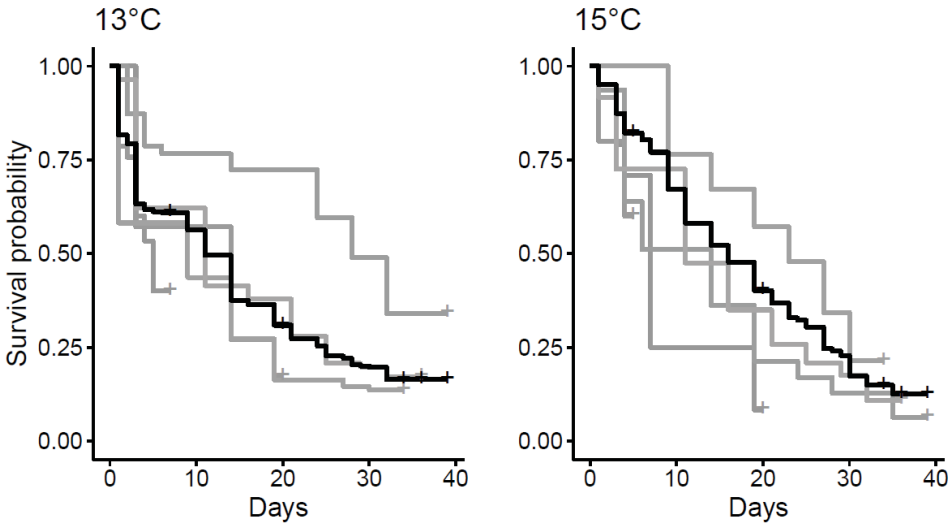

Figure S 1.2: Kaplan–Meier estimate of larvae survival probability of the species *Dentomuricea* aff. *meteor* under two temperatures. Grey lines represent estimates for separate batches while black lines represent estimates for all data pooled together.

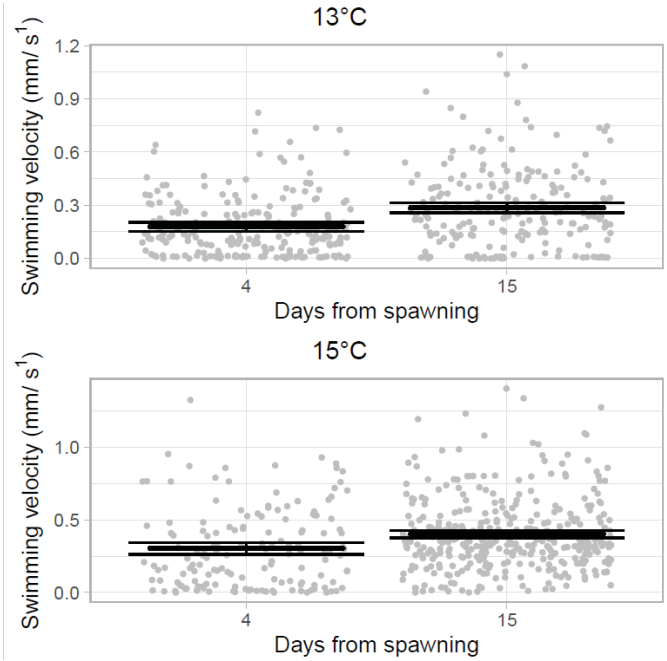

Figure S 1.3: Visualization of model effects constructed analyze the swimming velocity of larvae of *Dentomuricea* aff. *meteor* in time under two temperature regimes. Lines represent model fit and 95% confidence intervals, while points represent model partial residuals.

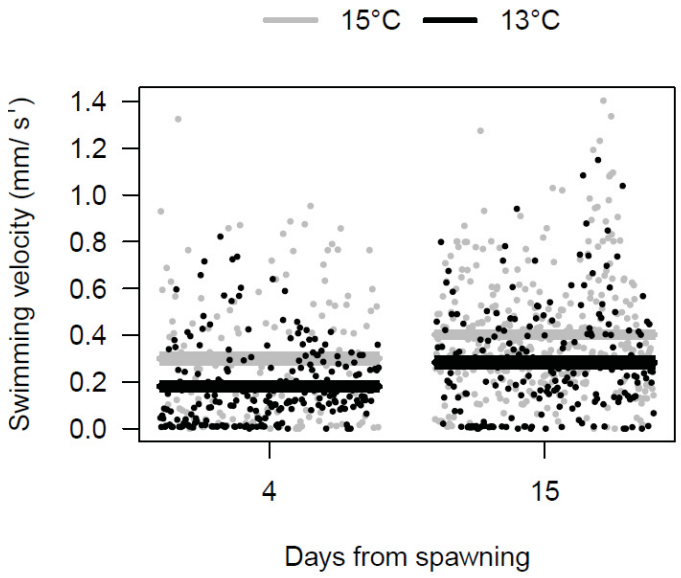

Figure S 1.4: Visualization of model effects constructed to compare the swimming velocity of larvae of *Dentomuricea* aff. *meteor* between two different temperature regimes. Lines represent model fit and 95% confidence intervals, while points represent model partial residuals.

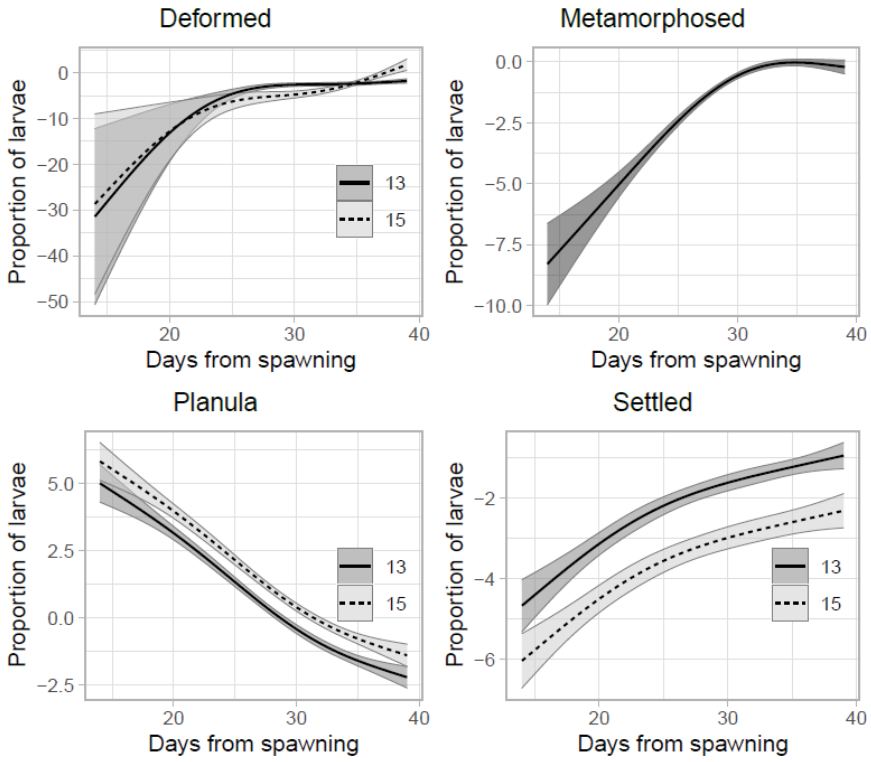

Figure S 1.5: Visualization of model effects constructed to analyze the proportion of larva of *Dentomuricea* aff. *meteor* at each larval stage (deformed, metamorphosed, planula settled), under two temperature regimes. Lines represent model fit and 95% confidence intervals.

APPENDIX C

Supplementary Data-Chapter 4

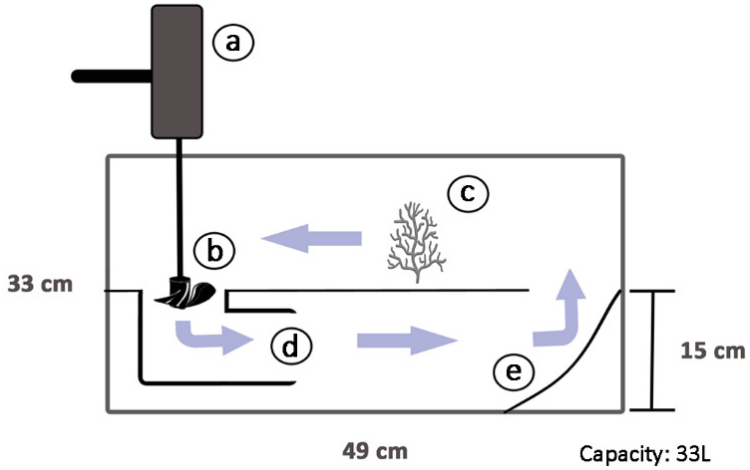

Figure S 2.1: Experimental flumes used to determine the ability of the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum* to exploit different food substrates. Flume volume 33L. (a) Heavy duty motor; (b) 8 cm marine propeller; (c) coral fragment; (d) 9cm tube to direct flow; (e) platform used to facilitate flow direction.

Table S 2.1: Coral colonies used in the feeding experiments, sampling locations and assignment of coral fragments to treatments and experimental aquaria

Species	Colony	Latitude	Longitude	Sampling depth (m)	Fragment	Treatment	Aquaria
D. aff. *meteor*	AC14	38.5406	-29.0541	190-230	AC14A	FAST	8
					AC14B	PHYTO	5
					AC14C	ZOO	7
					AC14D	ZOO	5
					AC14F	PHYTO	6
					AC14G	FAST	8
					AC14H	DOM	5
					AC14J	FAST	8
					AC14K	DOM	7
					AC14M	PHYTO	7
					AC14O	DOM	6
					AC14P	ZOO	6
	AC1	38.5770	-29.0180	230-240	AC1A	DOM	6
					AC1B	ZOO	5

Species	Colony	Latitude	Longitude	Sampling depth (m)	Fragment	Treatment	Aquaria
					AC1D	PHYTO	5
					AC1E	PHYTO	6
					AC1F	DOM	5
					AC1H	FAST	8
					AC1I	ZOO	7
					AC1J	DOM	7
					AC1K	FAST	8
					AC1L	ZOO	6
					AC1O	FAST	8
					AC1P	PHYTO	7
	AC26	38.5406	-29.0541	190-230	AC26A	PHYTO	5
					AC26AB	ZOO	5
					AC26AH	DOM	7
					AC26AJ	DOM	6
					AC26AL	FAST	8
					AC26AM	ZOO	6
					AC26D	ZOO	7
					AC26K	PHYTO	6
					AC26L	FAST	8
					AC26P	PHYTO	7
					AC26Y	FAST	8
					AC26Z	DOM	5
	AC7A	38.5770	-29.0180	230-240	AC7A	FAST	8
					AC7AD	ZOO	7
					AC7E	FAST	8
					AC7F	PHYTO	5
					AC7G	PHYTO	7
					AC7I	DOM	5
					AC7L	ZOO	5
					AC7P	FAST	8
					AC7T	DOM	6
					AC7U	PHYTO	6
					AC7W	ZOO	6
					AC7X	DOM	7
	AC8A	38.5770	-29.0180	230-240	AC8A	ZOO	5
					AC8B	DOM	7
					AC8C	FAST	8
					AC8D	PHYTO	6
					AC8F	DOM	5
					AC8K	FAST	8

Species	Colony	Latitude	Longitude	Sampling depth (m)	Fragment	Treatment	Aquaria
					AC8L	ZOO	6
					AC8M	PHYTO	7
					AC8N	DOM	6
					AC8O	ZOO	7
					AC8P	FAST	8
					AC8R	PHYTO	5
V. flagellum	AC10	38.5396	-29.0399	190-210	AC10C	FAST	6
					AC10D	PHYTO	7
					AC10L	ZOO	5
					AC10M	DOM	8
	AC11	38.5396	-29.0399	190-210	AC11A	DOM	5
					AC11B	FAST	6
					AC11C	PHYTO	7
					AC11G	ZOO	8
	AC12	38.5396	-29.0399	190-210	AC12A	PHYTO	7
					AC12C	FAST	5
					AC12E	DOM	8
					AC12F	ZOO	6
	AC13	38.5770	-29.0180	230-240	AC13A	FAST	8
					AC13B	PHYTO	6
					AC13C	ZOO	5
					AC13D	DOM	7
	AC15	38.5770	-29.0180	230-240	AC15A	PHYTO	6
					AC15B	DOM	7
					AC15C	ZOO	8
					AC15D	FAST	5
	AC16	38.5396	-29.0399	190-210	AC16A	DOM	8
					AC16D	ZOO	6
					AC16E	PHYTO	5
					AC16I	FAST	7
	AC17	38.5396	-29.0399	190-210	AC17A	PHYTO	8
					AC17D	ZOO	5
					AC17F	FAST	6
					AC17G	DOM	7
	AC27	37.6012	-25.8824	250-255	AC27A	DOM	5
					AC27B	PHYTO	8
					AC27D	ZOO	6
					AC27H	FAST	7
	AC29	37.6012	-25.8824	250-255	AC29A	PHYTO	5
					AC29D	FAST	7

Species	Colony	Latitude	Longitude	Sampling depth (m)	Fragment	Treatment	Aquaria
					AC29F	ZOO	8
					AC29H	DOM	6
	AC30	37.6012	-25.8824	250-255	AC30A	PHYTO	5
					AC30B	DOM	6
					AC30C	FAST	8
					AC30D	ZOO	7
	AC9	38.5396	-29.0399	190-210	AC9A	DOM	6
					AC9B	ZOO	7
					AC9C	PHYTO	8
					AC9D	FAST	5
	AV9	38.5770	-29.0180	230-240	AV9A	PHYTO	6
					AV9B	ZOO	7
					AV9C	DOM	5
					AV9D	FAST	8

Table S 2.2: Characteristics of coral fragments of the octocoral species *Dentomuricea* aff. *meteor* and *Viminella flagellum* used in the different experimental treatments.

	D.* aff. *meteor				***V.flagellum***			
	FAST	**PHYTO**	**DOM**	**ZOO**	**FAST**	**PHYTO**	**DOM**	**ZOO**
Dry weight (g)	0.28 ± 0.15	0.30 ± 0.16	0.29 ± 0.17	0.35 ± 0.17	1.58 ± 0.87	1.74 ± 1.14	1.44 ± 1.03	1.63 ± 0.93
Skeletal weight (g)	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.03	1.24 ± 0.79	1.41 ± 1.05	1.0 ± 0.87	1.31 ± 0.84
Org. C content (%)	7.09 ± 2.18	6.96 ± 1.96	7.03 ± 1.7	7.29 ± 1.25	7.36 ± 1.6	8.00 ± 1.7	8.22 ± 1.11	8.41 ± 1.40

APPENDIX D

Supplementary Data-Chapter 5

Table S 3.1: Results from Maximum Likelihood Ratio tests including the Akaike Criterion (AIC) values, residual deviance (RD), degrees of freedom (df), deviance and p value, for all constructed models to test the effect of pCO_2 and food availability scenarios on physiological variables of the deep-sea octocoral *Viminela flagellum*. Best models are shaded with gray.

Dependent variable	Independent variable	AIC	RD	df	Deviance	p
Feeding activity	null	1896.05	1894.10			
	DW	1889.96	1886.00	1	8.09	0.004
	DW + pCO_2	1879.43	1873.40	1	12.5	0.0004
	DW + pCO_2 + Food Availability	1735.47	1725.50	2	147.9	2.20×10^{-16}
	DW x Food Availability + pCO_2	1735.03	1721.00	2	4.436	0.10
	DW x pCO_2 + Food Availability	1737.02	1725.50	1	0.012	0.91
	DW x pCO_2 x Food Availability	1712.76	1688.80	6	32.25	1.69×10^{-6}
Prey capture	null	1475.40	2692004.00			
	DW	1456.90	2248353.00	1	443651	1.61×10^{-8}
	DW + pCO_2	1457.90	2228357.00	1	19996	0.23
	DW + pCO_2 + Food Availability	1436.60	1817533.00	1	410824	5.40×10^{-8}
	DW x Food Availability + pCO_2	1421.20	1559773.00	1	257760	1.65×10^{-5}
	DW x pCO_2 + Food Availability	1421.50	1536855.00	1	22918	0.20
	DW x pCO_2 x Food Availability	1420.70	1473552.00	2	63303	0.10
Tracer C incorporation, organic fraction	null	319.30	713760.00			
	log(DW)	316.60	586986.00	1	126774	0.03
	log(DW) + pCO_2	318.40	581614.00	1	5372	0.65
	log(DW) + pCO_2 + Food Availability	318.80	545048.00	1	36566	0.24
	log(DW) x Food Availability + pCO_2	321.30	512947.00	2	32101	0.55
	log(DW) x pCO_2 + Food Availability	321.09	466295.00	1	46652	0.19
	log(DW) x pCO_2 x Food Availability	322.25	414247.00	2	52048	0.38
Tracer C incorporation, inorganic fraction	null	253.50	46024.00			
	log(DW)	249.30	35621.00	1	10402.8	0.02
	log(DW) + pCO_2	251.10	35291.00	1	330	0.68
	log(DW) + pCO_2 + Food Availability	252.10	33865.00	1	1426.2	0.39

Dependent variable	Independent variable	AIC	RD	df	Deviance	p
	log(DW) x Food Availability + pCO_2	255.00	32289.00	2	1575.8	0.66
	log(DW) x pCO_2 + Food Availability	255.70	30611.00	1	1677.4	0.35
	log(DW) x pCO_2 x Food Availability	258.10	28691.00	2	1920.5	0.61
Tracer N incorporation	null	267.90	83862.00			
	log(DW)	265.90	70965.00	1	12896.4	0.09
	log(DW) + pCO_2	267.90	70952.00	1	13	0.96
	log(DW) + pCO_2 + Food Availability	269.90	70951.00	1	1.8	0.98
	log(DW) x Food Availability + pCO_2	273.80	70833.00	2	118	0.99
	log(DW) x pCO_2 + Food Availability	275.50	69839.00	1	993.6	0.64
	log(DW) x pCO_2 x Food Availability	278.70	65571.00	2	2268.45	0.78
Orcanic C content, tissue	null	139.07	115.80			
	DW	137.39	103.50	1	12.22	0.04
	DW + pCO_2	139.39	103.56	1	0.0139	0.94
	DW + pCO_2 + Food Availability	135.90	82.73	2	20.82	0.03
	DW x Food Availability + pCO_2	136.70	75.06	2	7.66	0.27
	DW x pCO_2 + Food Availability	138.10	73.61	1	1.45	0.48
	DW x pCO_2 x Food Availability	140.70	62.47	4	11.13	0.44
Nitrogen content, tissue	Null	45.05	6.70			
	DW	41.51	5.66	1	1.03	0.01
	DW + pCO_2	43.44	5.65	1	0.01	0.79
	DW + pCO_2 + Food Availability	39.24	4.41	2	1.24	0.02
	DW x Food Availability + pCO_2	39.99	3.99	2	0.41	0.30
	DW x pCO_2 + Food Availability	41.03	3.88	1	0.11	0.41
	DW x pCO_2 x Food Availability	47.03	3.65	4	0.22	0.85
C content, tissue	Null	109.81	47.70			
	DW	107.40	41.70	1	5.99	0.03
	DW + pCO_2	109.40	41.70	1	0	0.99
	DW + pCO_2 + Food Availability	104.70	32.08	2	9.67	0.02
	DW x Food Availability + pCO_2	105.80	29.37	2	2.71	0.34
	DW x pCO_2 + Food Availability	106.70	28.40	1	0.96	0.38
	DW x pCO_2 x Food Availability	112.90	26.92	4	1.47	0.88
C/N ratio, tissue	Null	120.27	65.50			
	DW	116.93	55.71	1	9.79	0.01
	DW + pCO_2	118.79	55.47	1	0.23	0.69
	DW + pCO_2 + Food Availability	115.12	43.90	2	11.5	0.02
	DW x Food Availability + pCO_2	113.45	37.03	2	6.94	0.10

Dependent variable	Independent variable	AIC	RD	df	Deviance	p
	DW x pCO_2 + Food Availability	114.20	35.65	1	1.37	0.34
	DW x pCO_2 x Food Availability	118.36	31.70	4	3.91	0.62
Organic C content, axis	Null	86.20	39.20			
	DW	88.20	39.20	1	0	0.99
	DW + pCO_2	88.90	37.35	1	1.86	0.33
	DW + pCO_2 + Food Availability	92.14	36.11	2	1.23	0.73
	DW x Food Availability + pCO_2	94.21	33.43	2	2.68	0.51
	DW x pCO_2 + Food Availability	94.10	30.70	1	2.7	0.24
	DW x pCO_2 x Food Availability	97.70	30.35	2	0.37	0.91
N content, axis	Null	28.77	3.94			
	DW	30.72	3.93	1	0.007	0.84
	DW + pCO_2	31.02	3.67	1	0.25	0.25
	DW + pCO_2 + Food Availability	34.32	3.57	2	0.1	0.77
	DW x Food Availability + pCO_2	36.16	3.27	2	0.29	0.47
	DW x pCO_2 + Food Availability	36.17	3.02	1	0.25	0.26
	DW x pCO_2 x Food Availability	39.86	2.99	2	0.03	0.91
C content, axis	Null	63.86	16.05			
	DW	65.61	15.88	1	0.16	0.66
	DW + pCO_2	66.05	14.92	1	0.96	0.28
	DW + pCO_2 + Food Availability	69.35	14.51	2	0.41	0.78
	DW x Food Availability + pCO_2	71.87	13.67	2	0.83	0.6
	DW x pCO_2 + Food Availability	71.94	12.66	1	1.01	0.27
	DW x pCO_2 x Food Availability	75.65	12.51	2	0.14	0.91
C/N ratio, axis	Null	47.17	8.23			
	DW	49.14	8.22	1	0.009	0.88
	DW + pCO_2	49.36	7.65	1	0.56	0.24
	DW + pCO_2 + Food Availability	52.95	7.53	2	0.12	0.86
	DW x Food Availability + pCO_2	54.72	6.88	2	0.64	0.46
	DW x pCO_2 + Food Availability	54.76	6.37	1	0.51	0.26
	DW x pCO_2 x Food Availability	58.48	6.30	2	0.07	0.92
Oxygen consumption	Null	-24.86	149.82			
	DW	-79.30	97.50	1	52.28	2.2×10^{-16}
	DW + pCO_2	-77.90	97.10	1	0.43	0.26
	DW + pCO_2 + Food Availability	-125.80	64.20	2	32.8	2.2×10^{-16}
	DW x Food Availability + pCO_2	-139.00	55.80	2	8.41	3.28×10^{-6}
	DW x pCO_2 + Food Availability	-144.90	82.33	1	3.49	1.10×10^{-3}
	DW x pCO_2 x Food Availability	-154.40	45.30	4	7.03	2.00×10^{-4}

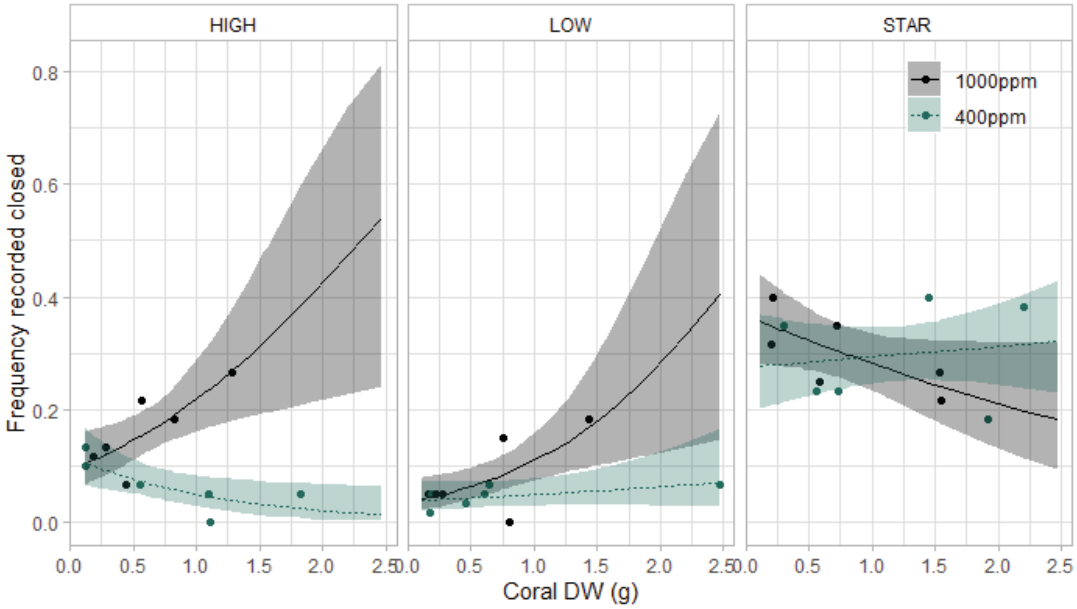

Figure S 3.1: Polyp activity of the deep-sea octocoral *Viminella flagellum* in respect to coral dry weight (DW), under two acidification and food availability scenarios. Acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and starved conditions (STAR) with no prey provision. Provided in the graph, raw data (points), predictions of constructed models (lines) and 95% confidence intervals (shaded areas).

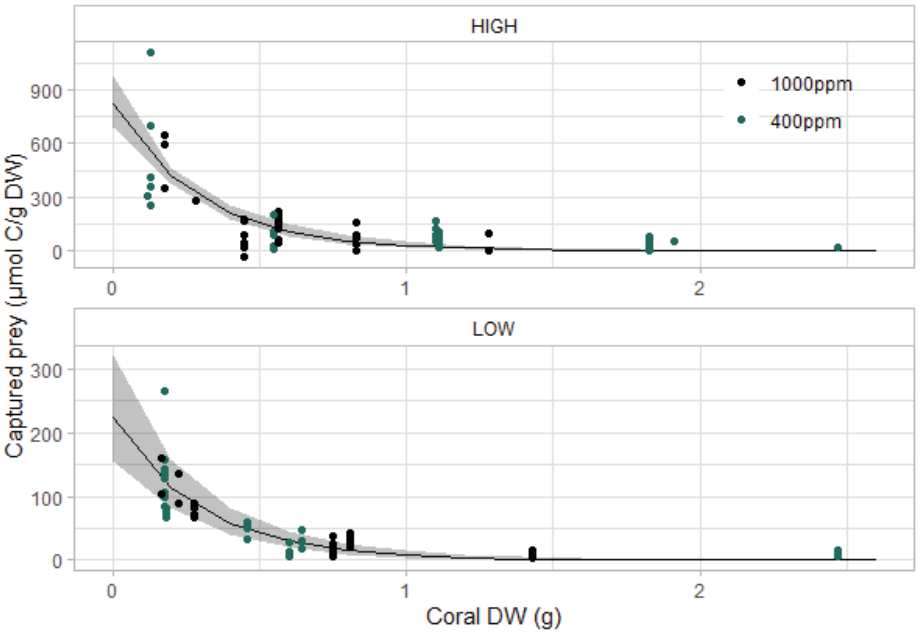

Figure S 3.2: Capture of live zooplankton prey in respect to coral dry weight (DW), under two acidification and food availability scenarios. Acidification scenarios correspond to ambient (400ppm) and increased

(1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and starved conditions (STAR) with no prey provision. Provided in the graph, raw data (points), predictions of constructed models (lines) and 95% confidence intervals (shaded areas).

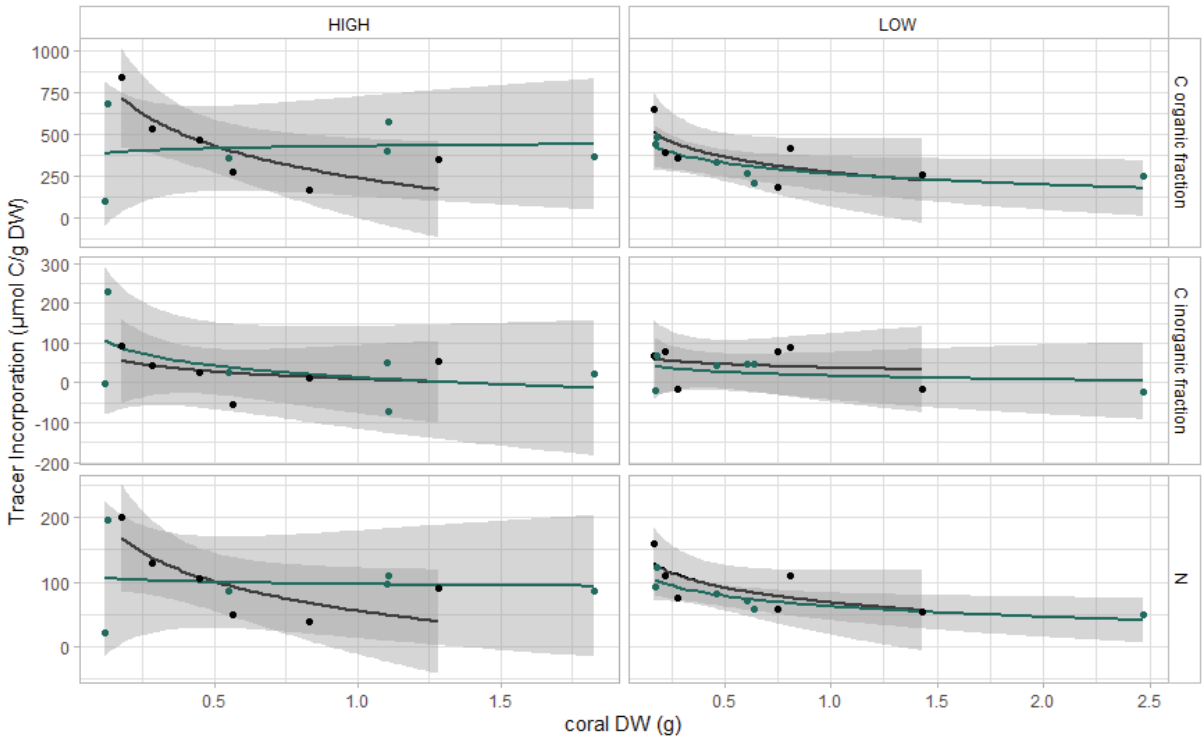

Figure S 3.3: Incorporation of C and N tracers in the coral coenenchyme in respect to coral dry weight (DW) under two acidification and food availability scenarios. Acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and starved conditions (STAR) with no prey provision. Provided in the graph, raw data (points), predictions of constructed models (lines) and 95% confidence intervals (shaded areas).

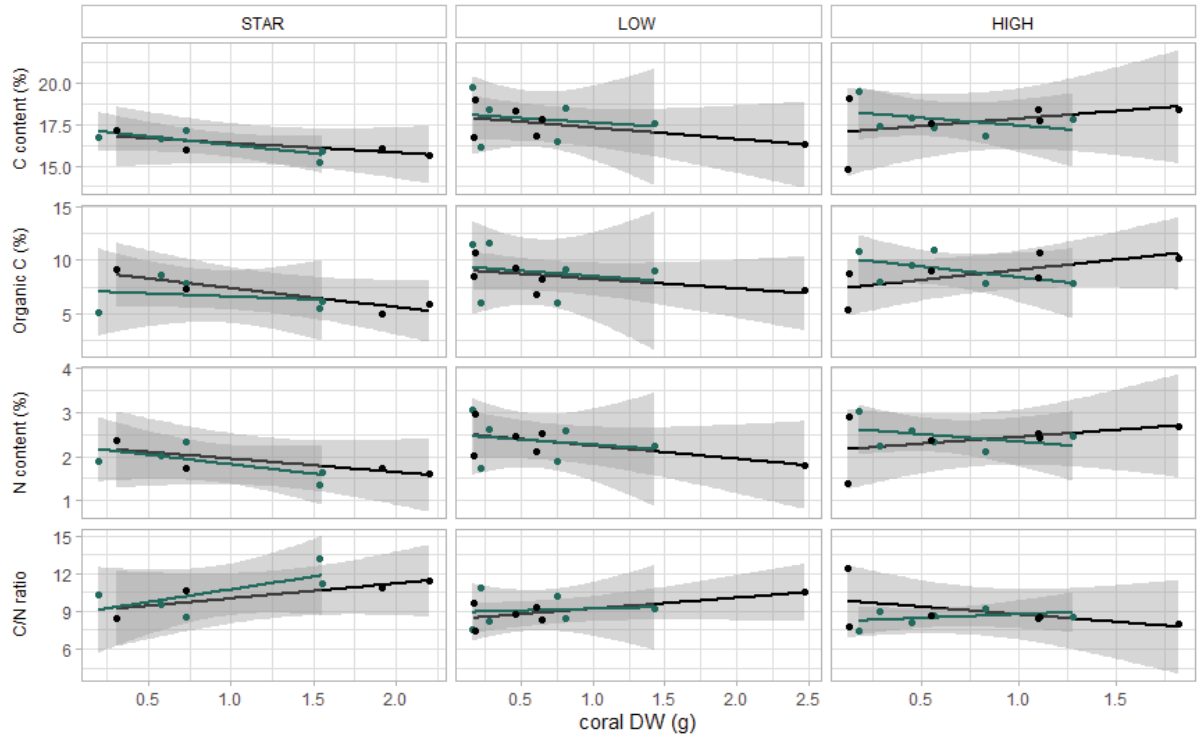

Figure S 3.4: State of the coral coenenchyme of the deep-sea octocoral *Viminella flagellum*, including carbon (C) content, organic C content, nitrogen (N) content and C/N ratio, in respect to coral dry weight (DW) under two acidification and food availability scenarios. Acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and starved conditions (STAR) with no prey provision. Lines represent model predictions and shaded areas 95% confidence intervals. Provided in the graph, raw data (points), predictions of constructed models (lines) and 95% confidence intervals (shaded areas).

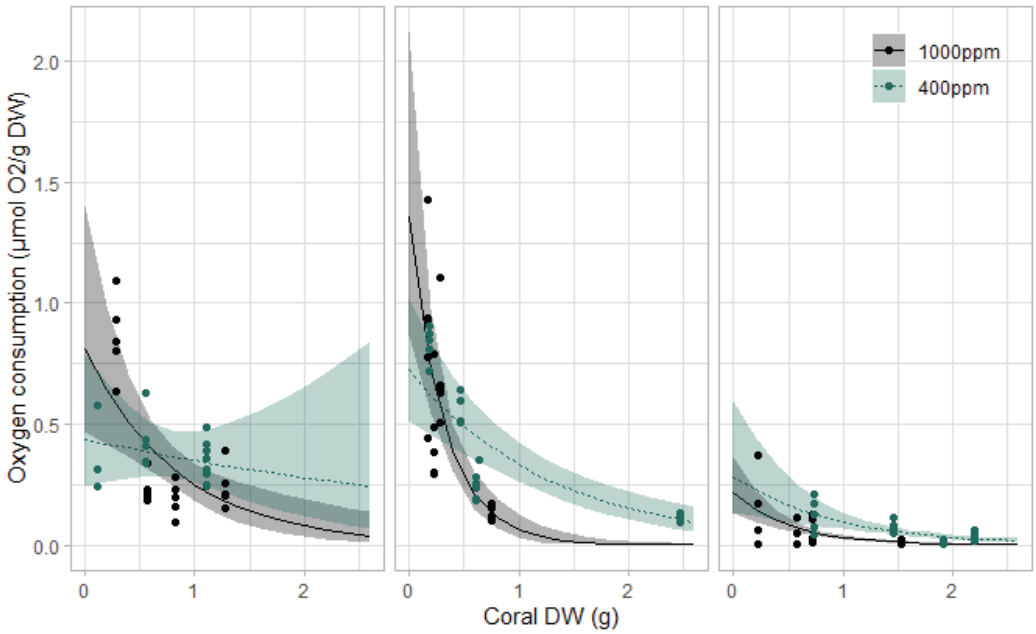

Figure S 3.5: Oxygen consumption of the deep-sea octocoral *Viminella flagellum* under different acidification and food availability scenarios. Acidification scenarios correspond to ambient (400ppm) and increased (1000ppm) pCO2 levels, while food availabilities correspond to increased (HIGH) prey concentration equivalent to 10.12 µmol C/L, low (LOW) prey concentration of 1.6 µmol C/L and starved conditions (STAR) with no prey provision. Lines represent model predictions and shaded areas 95% confidence intervals. Provided in the graph, raw data (points), predictions of constructed models (lines) and 95% confidence intervals (shaded areas).

ANNEX 1: Material published during the preparation of the thesis

Main publications

Chapter 2: Rakka M, Sampaio Í, Colaço A, Carreiro-Silva M. 2021. Reproductive biology of two deep-sea octocorals in the Azores Archipelago. Deep Sea Research Part I: Oceanographic Research Papers 175:103587.
DOI: 10.1016/J.DSR.2021.103587.

Contribution: MR performed the histological analysis and interpretation of histological slides, carried out the statistical analysis and compiled the draft manuscript integrating suggestions from co-authors.

Chapter 3.1: Rakka M., Godinho A., Orejas C., Carreiro-Silva M. In press. Embryo and larval biology of the deep-sea octocoral *Dentomurice*a aff. *meteor* under different temperature regimes. Peer J

Contribution: MR planned and prepared the larval rearing process, performed observations and daily monitoring of embryos and larvae, carried out the statistical analysis and prepared the draft manuscript integrating suggestions from co-authors.

Chapter 4: Rakka M, Maier SR, Van Oevelen D, Godinho A, Bilan M, Orejas C, Carreiro-Silva M. 2021. Contrasting metabolic strategies of two co-occurring deep-sea octocorals. Scientific Reports 11:10633.
DOI: 10.1038/s41598-021-90134-5.

Contribution: MR prepared the experimental design in collaboration with co-authors, performed aquaria experiments and subsequent preparation of coral samples, analysed isotope data, carried out statistical analysis and interpretation and compiled the draft manuscript integrating suggestions from all co-authors.

Manuscripts in preparation

Chapter 3.2: Rakka M., Bilan M., Godinho A., Carreiro-Silva M. Larval biology of the deep-sea octocoral *Viminella flagellum.* In preparation

Contribution: MR collected embryos and larvae, performed observations and daily monitoring, carried out the statistical analysis and prepared the draft manuscript in collaboration with co-authors.

Chapter 5: Rakka M, van Oevelen D, Maier S, Puerta P, Godinho A, Bilan M, Martins I, Orejas C, Hennige S, Wolff G, Carreiro-Silva M. Metabolism of the deep-sea octocoral *Viminella flagellum* under acidification and variable food availability. In preparation

Contribution: MR prepared the experimental design and experimental set-up in collaboration with co-authors, performed aquaria experiments, analysis of water samples and preparation of coral samples, analysed isotope data, carried out statistical analysis and compiled the draft manuscript integrating suggestions from co-authors.

Other publications

Rakka, M., Orejas, C., Maier, S. R., Van Oevelen, D., Godinho, A., Bilan, M., & Carreiro-Silva, M. (2020). Feeding biology of a habitat-forming antipatharian in the Azores Archipelago. Coral Reefs, 39(5), 1469-1482.

Rakka M., Bilan M., Godinho A., Movilla J., Orejas C., Carreiro-Silva M. (2019) First description of polyp bailout in cold-water octocorals under aquaria maintenance. Coral Reefs, 38, 15–20.

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Cold-water corals in a changing world

Maria Rakka