ENVIRONMENTAL ECOLOGY OF MARINE BRYOZOANS (Phylum Bryozoa) AND ASCIDIANS (Tunicata: Ascidiacea) UNDER MULTISTRESSOR SCENARIOS

VANESSA YEPES-NARVÁEZ

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A thesis submitted in partial fulfilment of the requirements of the Manchester Metropolitan University for the degree of DOCTOR OF PHILOSOPHY

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To Ana Luz and Juan Andrés for their unconditional love,
To my beautiful ocean blue, for constantly inspiring my days,
To Colombia

To all those with lophophores and branchial sacs that represent a reason in my life
"I will never do enough, but I will always let the world know about you"

Lokah Samastah Sukhino Bhavantu

CERTIFICATE

To certify that this thesis is an authentic record of the research work carried out by VANESSA YEPES NARVÁEZ, under our scientific supervision and guidance in the school of Natural Sciences of The Manchester Metropolitan University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy and no parts has been presented before the award of any other degree, diploma or associateship in any university.

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DECLARATION

I VANESSA YEPES NARVÁEZ, do hereby declare that this thesis entitled “ENVIRONMENTAL ECOLOGY OF MARINE BRYOZOANS (Phylum Bryozoa) AND ASCIDIANS (Class: Ascidiacea) UNDER MULTISTRESSOR SCENARIOS” is a genuine record of the research work done by me under the supervision of Professor Richard Preziosi and Doctor Hannah Mossman from the Department of Natural Sciences, and has not previously formed the basis of the award of any degree, diploma or associateship in any university.

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Vanessa Yepes-Narváez
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GENERAL ABSTRACT

Environmental stressors determine the extent of occurrence of marine species and modify their biology and ecology; understanding the tolerance range of marine fauna to disturbances is an important conservation tool that supports the development of better strategies to protect species and their ecosystem functions. Monitoring is key to understand the behaviour of species against current stressors, however, in order to analyse the species performance under predicted multi-stressor scenarios for the end of the century, controlled laboratory experiments also known as mesocosms are a good tool that allow us to modify environmental conditions and evaluate the degree of impact on the species biology and ecology. In this study, I collected and modelled environmental data and created mesocosms in order to evaluate the biological response of Bryozoans and Ascidians against current and future stressor scenarios. Firstly, I identified the diversity of bryozoans at different geographic and bathymetric ranges in the Colombian Caribbean with the purpose of complement the species record and to compare their distribution and ecology with the environmental variables in those areas. This allowed me to describe new species for science and their ecology in the Caribbean. I demonstrated that bryozoans modify their reproductive patterns and create habitat complexity in high disturbance areas in the Colombian Caribbean. Secondly, I developed controlled mesocosms in laboratory to test the effects of turbidity, ocean acidification, global warming and microplastic pollution on the biological functions of coastal ascidians. I discovered a novel behaviour in these Chordates of energetically expensive total evisceration after environmental stress, this behaviour allows the animals to create a new digestive system within two weeks but exposes them to further threats. In addition, I demonstrated that the ingestion of microplastic polymers in ascidians provokes severe gastrointestinal damage and a 60% increase in mortality. With these findings of the performance of bryozoans and ascidians against stressors, I discuss their consequences on the provision of ecosystem functions and suggest further research approaches for their conservation.

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Chapter 1: General Introduction

The marine benthic biodiversity is in constant interaction with environmental factors (Hillebrand et al., 2018). The main driver of biodiversity loss and disturbances in the ocean is climate change (Warren et al., 2013; Harley at al., 2006), because it modifies the conditions at which animals coexist and takes them outside their tolerance limits, creating a stress response (Beaugrand et al., 2015) that could be beneficial or threatening depending on the species (Jones & Cheung, 2015). Those responses include changes in the distribution along bathymetric or geographical ranges (Garcia-Molinos et al., 2016), modifications of the reproductive strategies (Pankhurst & Munday, 2011; Thompson & Ollason, 2001) and physiological and behavioural adaptations to prolonged fluctuations in food availability, temperature, salinity or pH (Wernberg, et al., 2011).

The analysis of the multi-stressor responses on the benthic fauna provides insights for the understanding of the factors that affect their biological functions that further lead to ecosystem services and support higher levels of multifunctionality within ecosystems (Lefcheck et al., 2016; Mooney, 2010). From a conservation perspective, marine systems are expected to be more vulnerable to disturbances caused by climate change, as several natural and anthropogenic (e.g. pollution) stressors are co-occurring compromising the life cycles (Woodward et al., 2010) and the primitive life forms of most invertebrates including bryozoans, ascidians, sponges and corals (Prather et al., 2013) threatening with local extinctions and changes in gene flow patterns (Wiens, 2016; Cahill, et al., 2012).

Monitoring the impacts of climate change scenarios in ecosystems that hold key species such as marine suspension-feeders, represent an efficient way to contribute to the protection and preservation of economical important species with lower levels of adaptation to environmental stressors such as coral reefs. In addition, predicting the effects of those scenarios on the performance of coastal organisms, also constitutes a cost-efficient mean of conservation as mitigating and restoration strategies could be implemented prior major affectations to the species and ecosystems.

Natural and anthropogenic multi-stressor scenarios on marine ecosystems

Single-driver stressors such as high temperature, high salinity and low pH, are considered abiotic variables that surpass their natural fluctuations and lead the organisms to a physiological response (Vinebrooke et al., 2004). The ecosystem's resilience to these stressors depend mostly on the ability of its species to mitigate and compensate those impacts on their biological functions (Boyd & Brown, 2015). However, when the ecological processes rely only on few species, lethal effects are observed on the biodiversity (Klug & Cottiham, 2001).

There is enough alarming evidence from climate modelling and time series monitoring about the increasing co-occurring multi-stressors on the marine fauna (Boyd et al., 2014) such as the interaction of two or more variables in a system, such as high temperature, low pH and high salinity (expected for the end of the century). The evaluation of the effects of multiple stressors on the biological functions of marine suspension feeders represent a more realistic scenario as it includes different synergistic and antagonistic effects that constantly interact in the natural systems. The analyses of the effect of each variable and their combinations on the organisms provide a better understanding of the animal's real-life performance under predicted stressors.

A common anthropogenic stressor for marine species is the increasing concentration of microplastic particles suspended or within the ocean's sediments. These polymers can accumulate in the animal's essential tissue, be involved in the food web or as demonstrated here, lead to lethal soft tissue damages for marine organisms, restricting them to perform their biological function within the ecosystems they inhabit and reducing their survival.

High turbidity

Turbidity is a measurement of the degree at which light can penetrate the water column. It reflects the concentration of suspended particulate material either organic or inorganic (Johannes, 1972); when these concentrations are higher than the saturation capacity of the water body, it precipitates and build up at the bottom, potentially affecting sessile fauna and slow-moving organisms such as snails and polychaetes (Loya, 1976). The main triggers for turbidity include natural factors such as runoff from land, the influence of river deltas, upwelling phenomena, strong wind currents, rainfall (in tropical shallow areas), seawater currents, tsunamis, sandstorms, etc. Animals that inhabit those areas have modified their ecology to adapt to the strong fluctuations (Moore, 1977). In the case of man-made origins for turbidity, this includes a poor coastal management, coastal excavations (to build marinas and ports), and inorganic injections of chemicals or minerals such as from coal mines as in the case of the Colombian Caribbean.

Ocean acidification

Ocean acidification is caused by the injection of atmospheric CO_2 into the water column that causes a reduction of the ocean's pH (Dickson, 2010). This reduction in the water acidity has indirect impacts on the physiology of some marine organisms such as gas exchange, feeding pattern, reproduction and larval survival. In addition, this stressor reduces the uptake of $CaCO_3$ by calcified organisms such as corals (Anthony et al., 2008), which under acidic environments reduce their skeletons as a consequence of corrosive circumstances (Beniash et al., 2010). As mentioned before, organisms with larval stages are likely to be affected by this stressor, as larvae shells are usually gelatinous and less calcified which makes them more vulnerable to be dissolved by the acidity in the water column (Byrne, 2011). Microalgae could perform well under acidified conditions, however, this may cause an increase in water turbidity outside the tolerance margins of filter-feeders, also, some microalgae are poisonous for some marine animals and the increase of toxic components in the water column could limit the survival of those organisms or transfer the toxins into the food webs.

Global Warming

The Intergovernmental Panel on Climate Change predicted an increase on the surface sea temperature for the end of the century of between 1.4°C to 5.8°C (IPCC, 2012; 2018; Hobday et al., 2015). Ocean warming as well as ocean acidification affect species distribution, metabolism and for instance ecosystem health (Hale et al., 2011). This increase in temperature could induce habitat loss and reduce the survival of species such as coral reefs by bleaching. In addition, animals exposed outside their thermic range would reduce their feeding strategies, reproduction and subsequently increase their mortality rate. In addition, some oceanographic processes depend on a stabilized sea water temperature, vertical mixing for example, which is the responsible for nutrient transportation from deep to coastal areas is highly affected by the reduction of the dissolved oxygen as a consequence of warming.

Marine pollution – Microplastics

Microplastics are one of the major concerns for marine conservation. It is impossible to calculate exactly the total number of microparticles (suspended or precipitated into the ocean. It is estimated the about 80% of marine fauna have been in contact or currently hold traces of microplastics. (Eriksen et al., 2014). From all types of microplastics in the sea, microplastic fibres are one of the most common (Lusher et al., 2014), and as demonstrated here, can provoke severe gastrointestinal damage and subsequently increase mortality (Watts et al., 2014). The ingestion of these particles for instance, can potentially affect non-selective filter-feeder organisms whose feeding strategy consist in the constant filtration of suspended organic matter causing deleterious affectations to their health (Wright et al., 2013; Lusher et al., 2013; 2014; Lusher, 2015).

Study groups

Environmental relationships of marine bryozoans (Phylum Bryozoa)

Bryozoans are colonial, suspension-feeding invertebrates that can inhabit both marine and freshwater environments and have a sessile life mode after a short stage as swimming larvae (Wendt, 2000; Zilman et al., 2013). Their exact position inside Metazoa has been widely discussed. To date they are placed within the clade Lophotrochozoa (Spiralia) belonging to Protostomia due to their genetic affinities (Nielsen, 2012).

These organisms are selective suspension feeders as they feed mainly on phytoplankton of specific sizes according to each species' mouth diameter (Bullivant 1968). Their surprisingly high water clearance rates (up to 8.8 ml per zooid/day) allow them to survive in upwelling environments where suspended turbidity is higher, playing a particular function as builders, providing habitat complexity and structure for the establishment of other substrate-dependent organisms and even other less calcified bryozoans, somehow replacing the absence of coral reefs.

For instance, are potential bio-constructors; their colonies can have different shapes, sizes and orientation and can be found from 20 cm to 8000 m depth around the world. Their morphology can provide important information about the physical and chemical conditions of their habitats. It is believed that some environmental factors in the developing zooid bud can contribute to the occurrence of polymorphisms in a bryozoan colony. For example, bryozoans with erect growing types present strict construction rules in regard to the position and distribution of polymorphic zooids according to the type of substrate, water currents or light exposure (Borg, 1926).

The distribution of bryozoans can depend on different environmental, oceanographic and physical factors such as bathymetry, dissolved nutrients, temperature, salinity, latitude, substrate, and marine currents. At the moment, many studies have tried to

explain to what extent the environment surrounding the colonies can modify or control the bryozoan life cycle (Winston, 1995).

In the Colombian Caribbean over 200 species of bryozoans have been registered in coastal and deep-sea environments from 1m to 3888m depth and correspond to 10% of the marine biodiversity of the country. Previous studies on this group have focused mainly in the description of species from 200 to 600mdepth and there has been little sampling of the shallow ecosystems.

For Colombia, the proportions of bryozoan species in different taxonomic groups is 60% Cheilostomata, 30% Ctenostomatida and 10% Cyclostomatida, which is similar to the rest of the great Caribbean region (Yepes-Narváez, 2013; Flórez et al., 2007). They have been mostly reported as part of the fouling community attached to seagrasses, submersed mangrove roots, seaweeds (*Sargassum* spp.), on subtidal rocks, on the available surface of bigger fauna (sponges, corals, mussels, etc.) and attached to artificial substrates such as human litter, artificial submersed reefs, marinas and ports (Gracia et al., 2018; Yepes-Narváez, 2013). Bryozoan richness in tropical areas like the Colombian Caribbean is high but with reduced abundance compared to temperate regions (Winston, 1995) and do not participate in the beach formation and are not reported as beach material. In shallow areas bryozoans present flexible-arborescent and membraniporid growth types and the encrusting forms are present in deeper environments (Yepes-Narváez, 2013).

In addition to depth, environmental variables rule most of the bryozoan life cycle as depending on the conditions they can adopt sexual or asexual reproduction. Conditions in the surrounding environment lead to environmental plasticity in some bryozoans and in the deployment of morphological structures; also, oceanographic conditions such as temperature, dissolved particulate material, dissolved carbon are related to the healthy settlement and growth of bryozoans.

Environmental relationships of Ascidians (Chordata: Ascidiacea)

Ascidians are marine filter feeders from the phylum Chordata. These organisms play important roles within the ecosystems they inhabit such as water clearance and nutrient recirculation (Ruppert et al., 2004). Also, they contribute to the complexity of the habitats they live in by creating available substrates for the establishment of other organisms and ascidians (Lambert, 2005). In addition, they have the ability to adapt to environmental disturbances and to survive in high energetic and euthrophic environments (Shenkar & Swalla, 2011). They can filter up to 1US gallon per day (Ruppert et al., 2004), but, as indicated earlier, turbidity above their tolerance ranges could limit the filtration activity and reduce their populations.

Due to their high tolerance to environmental fluctuations, ascidians can potentially become invasive (Lambert, 2005). Several ascidians are registered as introduced or invasive worldwide; and occasionally, they can create problems for coastal protection as their colonies can build up and create complex structures in marinas and ports (Minchin & Sides, 2006). In addition, those ascidians can displace native fauna or asphyxiate sessile organisms by overgrowing as is the case of cultured bivalves (Lengyel et al., 2009; Rocha et al., 2009; Tan et al., 2002).

Tropical ascidians reproduce all year and produce short-lived non feeding larvae (Millar, 1970), metamorphosis occurs around 12-hour after hatching and it is influenced by environmental conditions (Turon & Becerro, 1992). The colonization of new substrates depends on how fast the larva adapts to the new habitat and the growth rate post metamorphosis to reach sexual maturity (Morris & Carman, 2012; Valentine et al., 2007; 2009). Asexual reproduction is also triggered by the environment, but it is this type of growth which secures colonization success more than sexual reproduction which allows dispersal (Dijkstra et al. 2007; Minchin & Sides 2006). In this thesis we focused on the physiological and feeding behaviour of the Indo-Pacific *Polycarpa captiosa* under multi-stressors scenarios, its performance and resilience provided insights of their ecological adaptation to disturbances and evolutive success.

Thesis aims and chapters

The aims of this thesis are as follows,

1 To determine the environmental variables that contribute most to the composition, distribution and reproduction of marine bryozoans in the Colombian Caribbean from 1 to 3880m depth.

1.1 To perform taxonomic identification of deep-sea bryozoans in the Colombian Caribbean to complement the species records for the country.

1.2 To compare collected and modelled environmental variables with the reproduction strategy of *Bugula neritina* associated with contrasting coastal environments.

1.3 To compare the environmental information with the bathymetric and geographical distribution of bryozoans in the Colombian Caribbean.

2. To evaluate the environmental stressors that trigger evisceration in the tropical solitary ascidian *Polycarpa captiosa* in laboratory conditions.

2.1 To test predicted environmental multi-stressor scenarios (elevated turbidity, temperature and pCO2) for the end of the century on the biological functions of tropical ascidians. in controlled mesocosms in laboratory.

3) To evaluate the effects of microplastic pollution on the survival and biological functions of *Polycarpa captiosa* in laboratory conditions.

This thesis is composed of six data chapters:

In Chapter 2, I describe the systematics and diversity of deep-sea marine bryozoan species associated with deep-sea environments in the Colombian Caribbean from 70 to 3880m depth and register new species for science.

In Chapter 3, I examine the relationship between environmental variables and the bathymetric and geographical distribution of bryozoans in the Colombian Caribbean and identify the factors which have most influence on their distribution.

In Chapter 4, I compare contrasting localities across the Atlantic and Pacific Oceans to demonstrate that *Bugula neritina* can modify its reproduction strategy based on environmental conditions.

In Chapter 5, I Perform high turbidity mesocosms experiments to evaluate its effects on the filtration rates of *Polycarpa captiosa* and report novel data about evisceration and gut regeneration in Chordates.

In Chapter 6, I Design a high-quality ocean acidification and warming mesocosms to evaluate the biological impacts of low pH and high temperature on the resilience of *Polycarpa captiosa*.

In Chapter 7, I Design a cost-effective mesocosms to induce microplastic ingestion by the tropical ascidian *Polycarpa captiosa* and provide important data about bioaccumulation, evisceration and resilience in this species.

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Chapter 2: Systematics and diversity of deep-sea bryozoans (Phylum Bryozoa) from the Colombian Caribbean

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ABSTRACT

During recent deep-sea research expeditions from 72 to 3888m depth along the Colombian Caribbean, a large number of bryozoans were recovered. From the taxonomic revision of the bryozoan fragments collected, ninety-five species have been identified, from which nine are new, *Adeonellopsis avicularia* **sp. nov.**, *Adeonella coralina* **sp. nov.**, *Thalamoporella colombiana* **sp. nov.**, *Catenicella guajirensis* **n. sp.,** *Margaretta elongata* **sp. nov.**, *Bryopesanser dentata* **sp. nov.**, *Microporella granulata* **sp. nov.**, *Gemelliporina winstoniana* **sp. nov.**, and "*Incertae sedis".* Four represent new records for the Great Caribbean*, Laminopora* cf. *contorta, Adeonella* cf*. calveti, Adeonella* cf. *pallasii* and *Electra verticillata*. twelve are new records for Colombia, *Oncousoecia dilatans*, *Stomatopora* sp., *Semihaswellia sinuosa, Paralicornia sinuosa, Steginoporella connexa, Puellina smitti, Turbicellepora pourtalesi, Gemelliporina hastata, Gemellipora eburnea, Adeoneollopsis* aff. *Subsulcata, Plesiocleidochasma* sp. and *Trematoocia gemmea*. Additionally, 30% of the previously known species for the region were found in new geographic and bathymetric zones. Although the presence of bryozoans has been previously reported in regional-scale studies, this research contributes to their description, growth forms and distribution along higher bathymetric ranges and provides ecological insights on their adaptations to the unstable and highly sedimentary deep-sea environments. We suggests that bryozoans contribute to the habitat complexity that had historically been solely attributed to Anthozoa as structuring organisms. Here, we describe the nine new species and provide the total list of species found.

Keywords: Bryozoa, Deep-water, Colombia, New Species, PNNCP.

INTRODUCTION

Colombia is one of the countries with the highest marine biodiversity in the world and has almost 50% of the national territory underwater (Diaz & Acero, 2003). Its deep Caribbean seascapes include hard and soft bottoms, seamounts, canyons, and channels that represent important 'conservation objects' for the region due to the great diversity attributed to them (Navas et al., 2010). For this reason, the country currently holds 43 deep 'significant areas for biodiversity' (ASB) and two submarine protected areas, in which several research efforts aiming to add knowledge and preserve the species assemblages and habitats have been taking place (Alonso et al., 2010; Cardique et al., 2016).

Deep-sea research has supported the management of potential hydrocarbon exploration and exploitation zones in the Colombian Caribbean for more than two decades. Most of this research has focused on areas inhabited by structuring organisms such as azooxanthellate corals because of their conservation status under CITES regulations and their ability to create habitat for other species; hence the recently recognized deep-sea corals National Park (PNNCP) (Alonso et al., 2015; Polanco et al., 2017). However, recent research has confirmed that there are other bio-constructing organisms supporting biodiversity and generating complex calcified structures along the Caribbean, including bryozoans, which often co-exist with them or that occupy high productivity areas in the absence of zooxanthellae corals (Flórez et al., 2007; Yepes-Narváez, 2013; Cedeño-Posso et al., 2017).

The diversity of bryozoans in the continental shelf and the upper slope is high with a marked reduction in species abundance from north to south Caribbean (Flórez & Montoya-Cadavid, 2004). However, this also corresponds with a lack of sampling effort and regional taxonomic expertise. Recent deep-sea exploration from 2013 to 2018, carried out by the Marine and Coastal Research Institute of Colombia (INVEMAR) in partnership with the Colombian Institute of Petroleum (ICP), the National Hydrocarbon Agency (ANH) and National Parks (PNN) has collected considerable bryozoan material belonging to Gymnolaemata and Stenolaemata from different offshore ecosystems using different techniques such as box-core,

ROV video recording and camera-assisted sampling (Invemar-ICP, 2013; Cedeño-Posso et al., 2017; Vides & Alonso, 2018).

Encrusting specimens attached to empty bivalve shells and rigid erect forms were commonly found along the areas sampled belonging to Cheilostomatida and Cyclostomatida. The most abundant families were Steginoporellidae, Adeonidae, Schizoporellidae, Cupuladriidae, Cleidochasmatidae, Candidae and Phidoloporidae, which correlates to existing knowledge of shallower areas in the rest of the Caribbean and suggesting connectivity between bathymetric zones. The abyssal zone was inhabited by low calcifying species belonging to Bugulidae and Catenicellidae characterized by flexible colonies and several polymorphisms. Stenolaemata were rare; the family Crisiidae wasthe most diverse with three species identified.

Here we describe nine new species associated with diverse substrates and depths and provide a list of the total identified species. This research contributes to the knowledge of those important habitat modifiers in the deep environments and to the species record database that is key for the establishment of management plans and conservation strategies for the subsystem of marine protected areas of Colombia.

METHODS

The bryozoan material was collected along with associated macro and meiofauna in 28 stations during five recent offshore expeditions along the Colombian Caribbean (Figure 1) between the 72 and 3888m depth. Surveys were on board modified research vessels hired through a contractor (SERPORT S.A) from 2012 to 2018 as part of baseline projects lead by Invemar in partnership with the Colombian Institute of Petroleum (ICP), the National Hydrocarbon Agency (ANH) and National Parks (PNN) (Table 1).

Study area

The Colombian Caribbean corresponds to the northernmost part of South America with a north to south linear extension of over 3500km (Figure 1). Its offshore margin extends over 157km from the nearest point in mainland (Vides & Alonso, 2018). The

area features a complex geography and geological origins with varied seascapes such as seamounts, canyons, channels, hills, fans and abyssal plains. This area supports vast resident biodiversity and hosts many species whose life histories are connected to shallower areas on the coastline. The area is a high biodiversity location for conservation (Invemar, 2010). Several oceanographic conditions modify the area and dictate the distribution of its fauna (Navas et al., 2010).

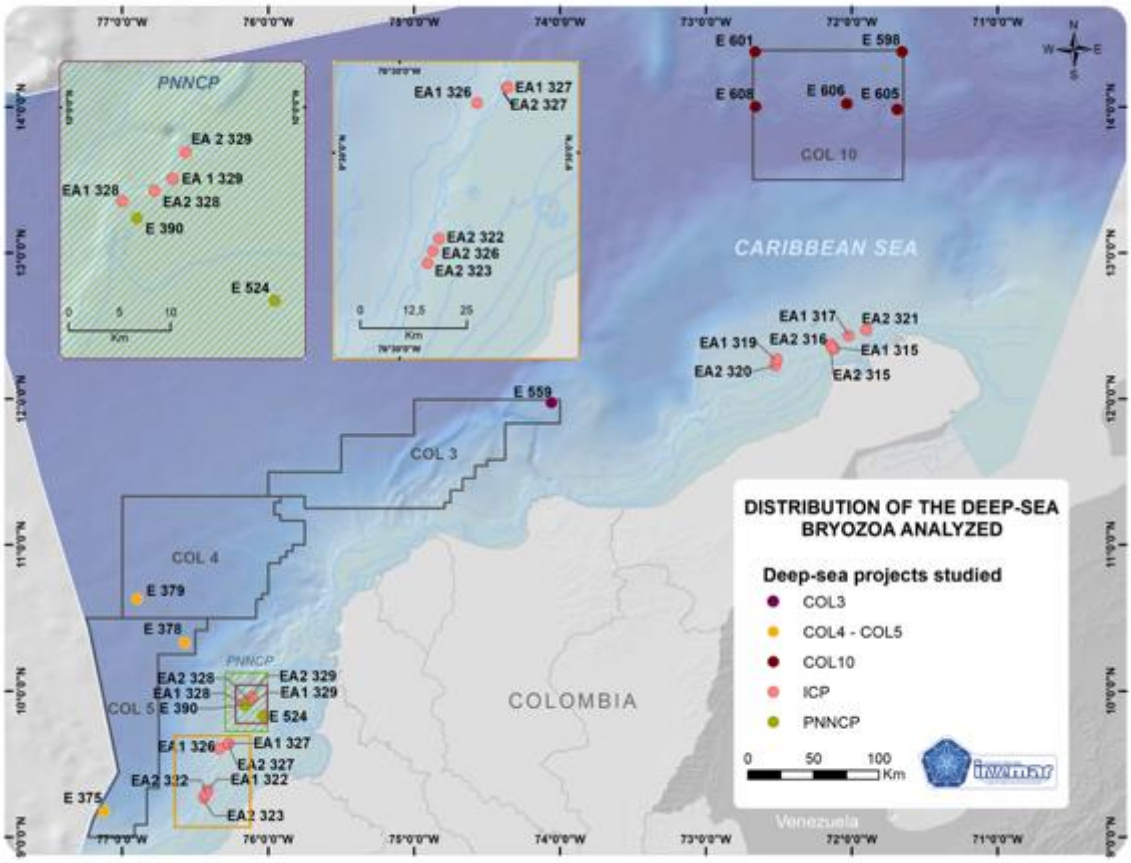

Figure 1. Sampling stations with deep-sea bryozoans collected in five recent expeditions in the Colombian Caribbean.

Most sampling stations belong to offshore exploration blocks assigned by the Environmental Ministry of Colombia to gas and oil corporations; these blocks are COL3, COL4, COL5, COL10, RC-11, RC-12, Fuerte Norte (FN) and Fuerte Sur (FS). Another two stations are located within the delimitations of the Deep-sea Corals National Park (PNNCP) in Sector 7 and Sector 8 (Table 1). The exploration campaigns aimed to contribute to the baseline knowledge of deep-sea environments associated with gas and oil exploration blocks and to the

management plan of the PNNCP. Information associated with the collections is stored in the Marine Environmental Information System-SIAM (Invemar-ICP, 2013; Garrido-Linares et al., 2014; Vides et al, 2017; Cedeño-Posso et al., 2017; Vides & Alonso, 2018).

Table 1. Stations of recent offshore expeditions which sampled deep-sea bryozoans.

Expedition	Station	Year	Latitude	Longitude	Ecoregion	Depth (m)
COL10	E 608	2018	14° 0' 9.8" N	72° 39' 35.1" W	Caribbean Offshore	3700
COL10	E 606	2018	14° 1' 19.4" N	72° 2' 5.9" W	Caribbean Offshore	3888
COL10	E 605	2018	13° 58' 58.6" N	71° 41' 24.5" W	Caribbean Offshore	3600
COL10	E 601	2018	14° 22' 46.9" N	72° 39' 46" W	Caribbean Offshore	3290
COL10	E 598	2018	14° 22' 42.7" N	71° 39' 19.1" W	Caribbean Offshore	2887
COL3	E 559	2017	11° 58' 31.6" N	74° 3' 33" W	Caribbean Offshore	1673
COL4 - 5	E 379	2014	10° 37' 47.4" N	76° 54' 4.1" W	Caribbean Offshore	1796
COL4 - 5	E 378	2014	10° 19' 46.9" N	76° 34' 32" W	Caribbean Offshore	2910
COL4 - 5	E 375	2014	9° 10' 37.5" N	77° 7' 34.2" W	Caribbean Offshore	3134
ICP(FN)	EA2 329	2013	9° 56' 14.1" N	76° 7' 26.8" W	Coraline Archipelagos	72
ICP(FN)	EA1 329	2013	9° 57' 38.7" N	76° 6' 43.3" W	Coraline Archipelagos	72
ICP(FN)	EA2 328	2013	9° 55' 36.4" N	76° 8' 23.8" W	Caribbean Offshore	73
ICP(FN)	EA1 328	2013	9° 55' 2.5" N	76° 10' 6.6" W	Caribbean Offshore	78
ICP(FN)	EA2 327	2013	9° 38' 15" N	76° 16' 26.8" W	Caribbean Offshore	82
ICP(FN)	EA1 327	2013	9° 38' 17.8" N	76° 16' 20.8" W	Coraline Archipelagos	95
ICP(FN)	EA1 326	2013	9° 36' 18" N	76° 20' 10.5" W	Coraline Archipelagos	98
ICP(FS)	EA2 323	2013	9° 16' 3.5" N	76° 26' 26.8" W	Caribbean Offshore	124
ICP(FS)	EA2 322	2013	9° 19' 7.2" N	76° 24' 59.1" W	Caribbean Offshore	125
ICP(FS)	EA1 322	2013	9° 17' 36.7" N	76° 25' 46.9" W	Caribbean Offshore	126
ICP(RC12)	EA2 321	2012	12° 28' 19.4" N	71° 54' 2.6" W	Guajira	128
ICP(RC11)	EA2 320	2012	12° 13' 55.2" N	72° 31' 37.7" W	Guajira	190
ICP(RC11)	EA1 319	2012	12° 16' 12.4" N	72° 30' 50.2" W	Guajira	220
ICP(RC12)	EA1 317	2012	12° 26' 1.4" N	72° 1' 21.4" W	Guajira	270
ICP(RC11)	EA2 316	2012	12° 22' 32.7" N	72° 8' 44.6" W	Guajira	240
ICP(RC11)	EA2 315	2012	12° 20' 44.8" N	72° 7' 21" W	Guajira	230
ICP(RC11)	EA1 315	2012	12° 20' 45.9" N	72° 7' 52.7" W	Guajira	250
PNNCP (S8)	E 524	2017	09°49'47.5" N	76°12'01.6" W	Coraline Archipelagos	180
PNNCP (S7)	E 390	2015	9°54'09,1" N	76°09'19,0" W	Coraline Archipelagos	110

Collection methods

Samples were collected between 72 and 3888m depth using several methodologies (Figure 2). RC11 expeditions deployed bottom-trawls 9.5m x 7.7m (Marinovich Trawl Co. Inc) in two stations at which 1km bottom epifauna was dragged during 10 minutes at 8knots. The other exploration blocks (RC12 FN, FS, COL3, 4, 5 and 10) sampled in 24 stations using a box-core ($1m^3$) in which a subsample was collected for epifauna analysis using a $0.5m^2$ quadrat. The exploration campaigns in PNNCP were carried out using video-assisted dredging using a driftcam (CADEM) and ROVs video transects in two stations (Figure 2). Due to the collection method, some bryozoan colonies were fractured, and when large rocks or individuals were found, immediate separation was performed to avoid additional damage. Once on deck, biological material was preserved in 96% Ethanol, labelled and transported to the Museum of Marine Natural History of Colombia-MAKURIWA, where samples were cleaned, sorted and separated by morphotypes.

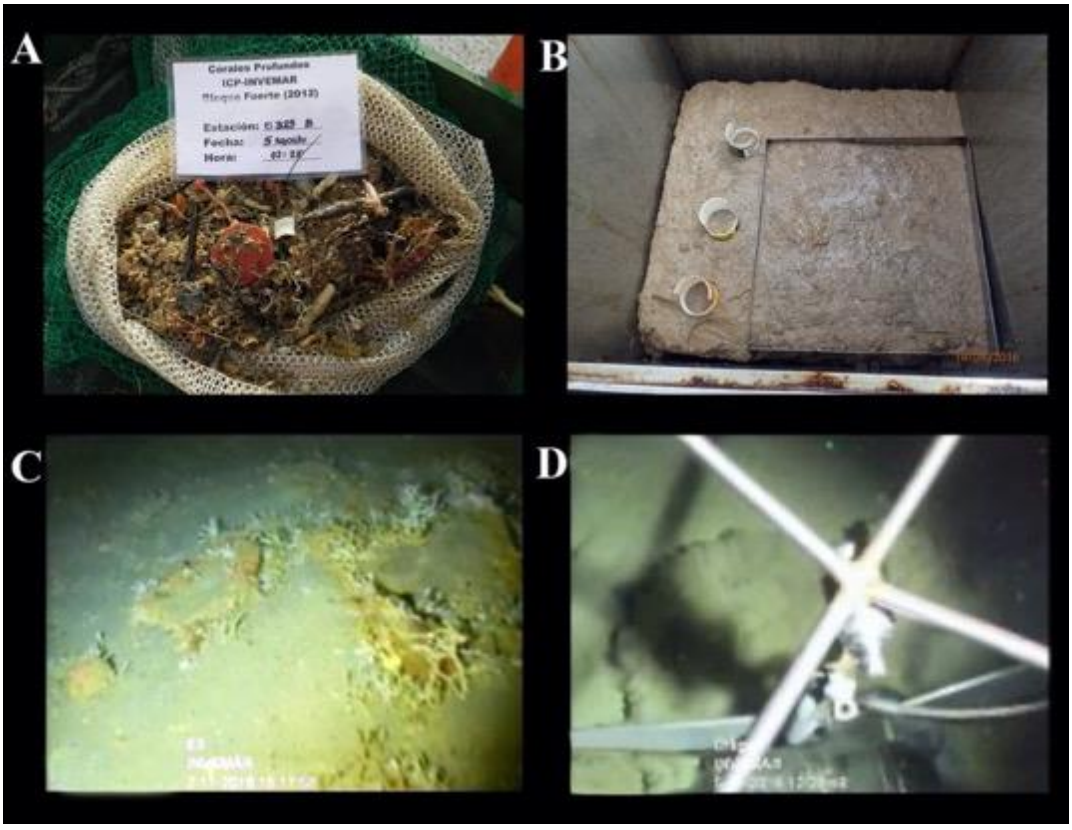

Figure 2. Different methods used to collect deep-sea bryozoans in the Colombian Caribbean. A) Bottom trawling; B) Box core with $0.5m^2$ quadrat; C) ROV video-transects; D) Video assisted-grab (CADEM). Image credits: Invemar.

To allow the observation of key taxonomic characters, some specimens were cleaned with a solution of Sodium Hypochlorite (10%) then washed with Hydrogen Peroxide (4%) and rinsed in distilled water in an ultrasonic cleaner to remove tissue residues and facilitate subsequent microscopic review, identification and photographic recording. Some specimens were more difficult to clean without damaging key features, so were slightly brushed. Taxonomic identification was performed based on Hayward and Ryland (1985) for Stenolaemata; Hayward (1985), Winston (1982) and Winston and Woollacoot (2009) for Ctenostomata; and for Cheilostomata, Canu and Bassler (1928a; b), Osburn (1940), Winston (1982; 1984; 1986; 2005; 2016) Winston & Håkansson (1986), Soule et al. (1995), Hayward and Ryland (1998, 1999); Flórez et al. (2007); Vieira et al. (2008, 2010a; 2010b; 2012) and Yepes-Narváez (2013). As a support for taxonomic description, three colonies of every species were selected from which 10 zooids of each were measured using ImageJ software 1.45s, Java 1.6 .0-20. Each species described here has a measurement table with number of zooids measured (n) mean, standard deviation (SD), minimum (min) and maximum (max). A list of the abbreviations used are in Table 2. In addition, the best colony of each species was photographed using Scanning Electron Microscopic (SEM) and illustrated here as identification reference.

Table 2. Measurement abbreviations used for Cyclostomatida and Cheilostomatida.

Abbreviation	Description	Abbreviation	Description	Abbreviation	Description
Lz	Zooid length	**Lovo**	Oocium orifice length	**Wgz**	Gonozooid width
Wz	Zooid width	**Wovo**	Oocium orifice width	**Lgzo**	Gonozooid orifice length
Lzoec	Zoeciule length	**Lgz**	Gonozooid length	**Wgzo**	Gonozooid orifice width
Wzoec	Zoeciule width	**LzB**	Zooid B length	**Lavz**	Avicularian zooid length
Lo	Orifice length	**WzB**	Zooid B width	**Wavz**	Avicularian zooid width
Wo	Orifice width	**Lrhz**	Rhizooid length	**Lav**	Avicularium length
Do	Orifice diameter	**Lstol**	Stolon length	**Wav**	Avicularium width
Lop	Opesia length	**Lintd**	Internode length	**Lavm**	Avicularian mandible length
Wop	Opesia width	**Lsc**	Scutum length	**Opl**	Opesia inclination
Lov	Ooecium length	**Wsc**	Scutum width	**Lcol**	Colony length
Wov	Ooecium width	**Lsp**	Spine length	**Dcol**	Colony diameter

To calculate the estimated species richness in the area, accumulated richness observed (SOBs) and estimated (Jacknife 1and Jacknife 2) were used based on the extent of occurrence of the species in the sampled area (Table 12) using the packages SpadeR and Vegan from the software R. The identified material was deposited into the Bryozoan reference collection at the Museum of Marine Natural History of Colombia – MAKURIWA and related information to the Marine Biodiversity Information System database - SIBM.

RESULTS

Ninety-five encrusting and erect species of bryozoans were identified inhabiting the deep-sea environments of the Colombian Caribbean between 72 and 3880m depth. In total, 47 families and 63 genera belonging to Gymnolaemata (Cheilostomatida (84spp), Ctenostomatida (3spp)) and Stenolaemata (Cyclostomatida (8spp)). Cheilostomatida was the order with greatest number of families (41 [88%] of the species identified. The most conspicuous families were Adeonidae (9spp) and Phidoloporidae (6spp), followed by Schizoporellidae (4spp), Cupuladriidae (4spp), Candidae (4spp), Steginoporellidae (3spp), and Cleidochasmatidae (3spp). All of them were characterized for being found alive in the majority of the stations sampled. Abyssal zone was mostly inhabited by low calcifying species from the family Bugulidae (3spp). Nine species are new, *Adeonellopsis.avicularia* **sp. nov***, Adeonella***.** *Coralina* **sp. nov.***, Thalamoporella colombiana* **sp. nov.**, *Catenicella guajirensis* **sp. nov.**, *Margaretta elongate* **sp. nov.**, "*Incertae sedis"*, *Bryopesanser dentata* **sp. nov.**, *Microporella granulata* **sp. nov.** and *Gemelliporina winstoniana* **sp. nov.** Four represent new records for the Great Caribbean*, Laminopora* cf. *contorta, Adeonella* cf*. calveti, Adeonella* cf. *pallasii* and *Electra verticillata*. Twelve are new records for Colombia, *Oncousoecia dilatans*, *Stomatopora* sp., *Semihaswellia sinuosa, Paralicornia sinuosa, Steginoporella connexa, Puellina smitti, Turbicellepora pourtalesi, Gemelliporina hastata, Gemellipora eburnea, Adeoneollopsis* aff. *Subsulcata, Plesiocleidochasma* sp. and *Trematoocia gemme*. These results also provide an update of the geographic and bathymetric records for species previously described. The species list is provided in table 12 and here we describe the new species. The Supplementary 1 contains the plates of the new records for the Great Caribbean and Supplementary 2 the plates of the new records for Colombia.

Systematic account

Class Gymnolaemata

Order Cheilostomatida Busk, 1852

Suborder Thalamoporellina

Genus *Thalamoporella* Hincks, 1887

***Thalamoporella colombiana* sp.nov.**
(Figure 3; Table 3)

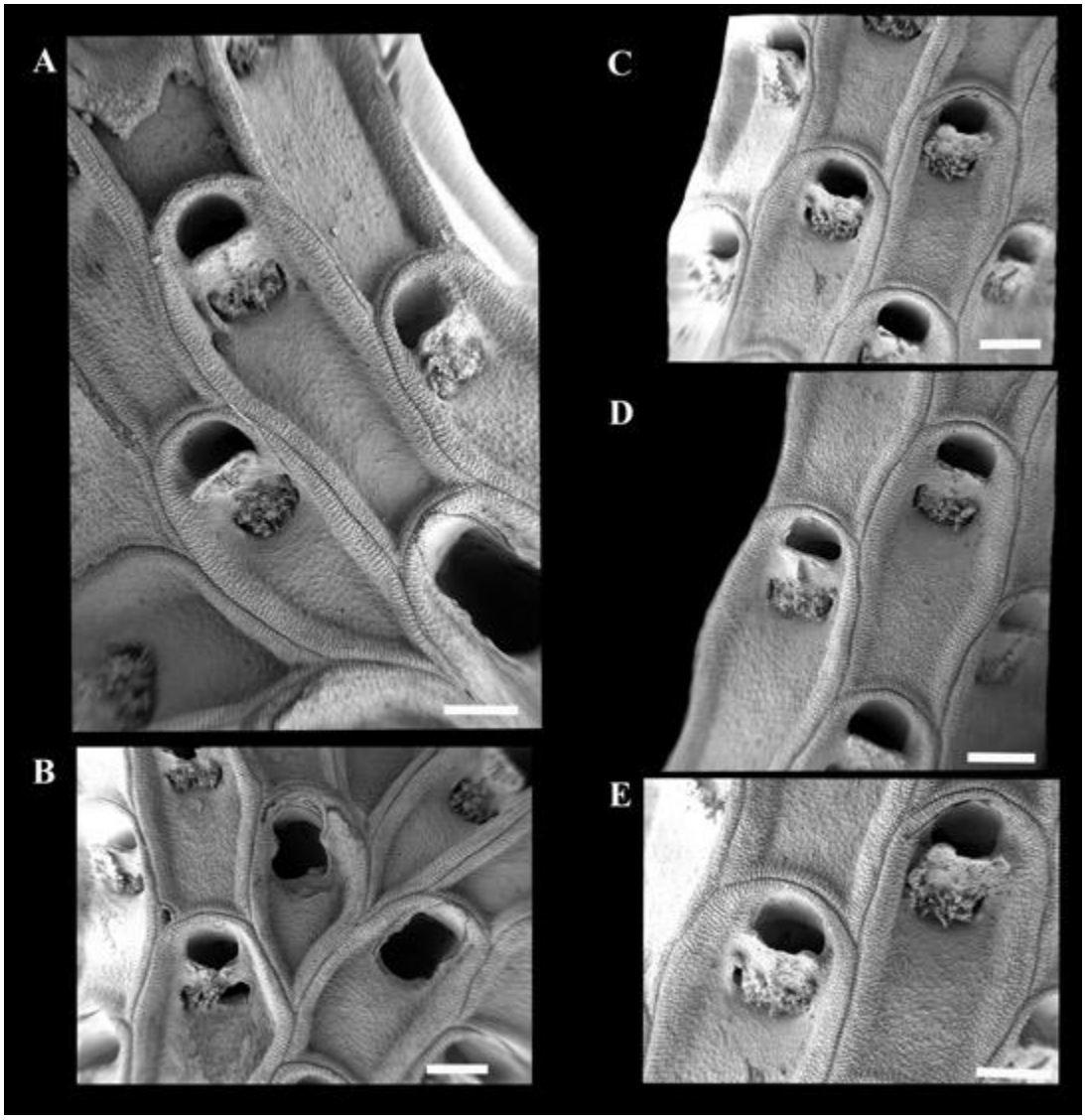

Figure 3. *Thalamoporella colombiana* **sp. nov**.*:* A. Fragment of an erect colony showing zooid shape, scale bar: 100µm B. Different zooids sizes and shapes at the bifurcation section, scale bar: 100µm C. Group of zooids with granulated cryptocyst, scale bar: 100µm D. Detail of longest zooids found in the colony, scale bar: 100µm E. Spinous projected lip surrounding the very small opesiules, detail of the orifice, scale bar: 100µm.

Type material. *Holotype*: INVBRY 2069, PNNCP 110m, Colombia 9°54’09,1” N, 76°09’19,0” W; along with coral rubble. *Paratypes*: INVBRY 2070, same data as Holotype; INVBRY 2074, 98m depth in Coraline Archipelagos 9° 36' 18" N, 76° 20' 10.5" W.

Table 3. Measurements in mm of *Thalamoporella colombiana* **sp. nov.**

	Lz	**Wz**	**Lo**	**Wo**	**Lop**	**Wop**
N	10	10	10	10	10	10
Mean	1.33	0.72	0.28	0.36	1.15	0.60
SD	0.51	0.022	0.015	0.04	0.53	0.44
Min	1.10	0.63	0.22	0.31	0.87	0.59
Max	1.61	0.84	0.31	0.39	1.57	0.68

Lz zooid length; Wz zooid width; Lo length orifice; Wo width orifice; Lop length opesia; Wop width opesia

Diagnosis. Erect-rigid *Thalamoporella* with very depressed granular cryptocyst, highly calcified and granular interzooidal margin. The cryptocyst extends towards the base of the orifice in which forms a shoe-tongue lip projection proximally sharp bearing heavy spination complex almost covering the opesiules. Zooids are considerably elongated and usually rectangular shape, except on the bifurcation zone in which zooids are modified and present different forms and sizes. Opesiules are very small and narrow to each side of the lip. Orifice is narrow and wide and has short and rounded condyles to each side. projected adventitious avicularia, no ovicells observed.

Description. Colony erect, bifurcated. Zooids are rectangular, arranged in linear rows. Very depressed granular cryptocyst with few pseudopores (Fig. 3A); paired narrow and small opesiules are placed at the end of the cryptocyst at the basis of the oral orifice inserted down a projected shoe-tongue lip (Fig. 3E), baring a complex spination that covers 40% of them (Table 3). Orifice is short and wide (Fig. 3B). The interzooidal rim is highly calcified and granular (Fig. 3C). Avicularia is small and elongated rostrum is pointed. Opesia semi-rectangular (Fig. 3D). No ovicells are observed in the holotype and paratypes.

Etymology. The epithet *colombiana* refers to the occurrence of this species in the Colombian Caribbean.

Remarks. The genus *Thalamoporella* Hincks, 1887, consist of 89 accepted species (Soule et al., 1999) from which four are recent species registered for Florida and the Western Atlantic *T. winstonae, T. mayori, T. floridana,* and *T. evelinae* (Marcus, 1939; Osburn, 1940; Winston, 1982; Soule, Soule & Chaney, 1999), after the

morphological revision of the Atlantic specimens we were not able to place this specimen closely related to them. Soule et al. (1999) described several *Thalamoporella* species in which we notice a slight similarity of our specimen with *T. spinosa* Chaney, Soule & Soule, 1989 and *T. labiata* (Levinsen, 1909) in regards the presence of spines around the opesiules, however, those species do not present the spinous complex and the projected shoe-tongue lip at the basis of the oral orifice, as well as the narrow and almost covered in spines opesiules which is a key feature of *T. colombiana* sp. nov**.** We also compared our specimen with *T. harmelini* Soule, Soule & Chaney, 1999 but it does not meet the descriptive taxonomical features specially in the zooid size, orifice shape, opesiules shape and size and the very characteristic spines at the base of the oral lip. We did not observe any ovicells in our samples or a substrate that it was attached to, possibly due to the collection method used (dredges and box core).

Distribution. *Thalamoporella colombiana* sp. nov. is found in deep waters of the Colombian Caribbean in corallinous environments of the PNNCP and La Guajira at 98 and 110m depth.

Superfamily Catenicelloidea Busk, 1852

Family Catenicellidae Busk, 1852

Genus *Catenicella* de Blainville, 1830

***Catenicella guajirensis* sp. nov.**
(Figure 4, Table 4)

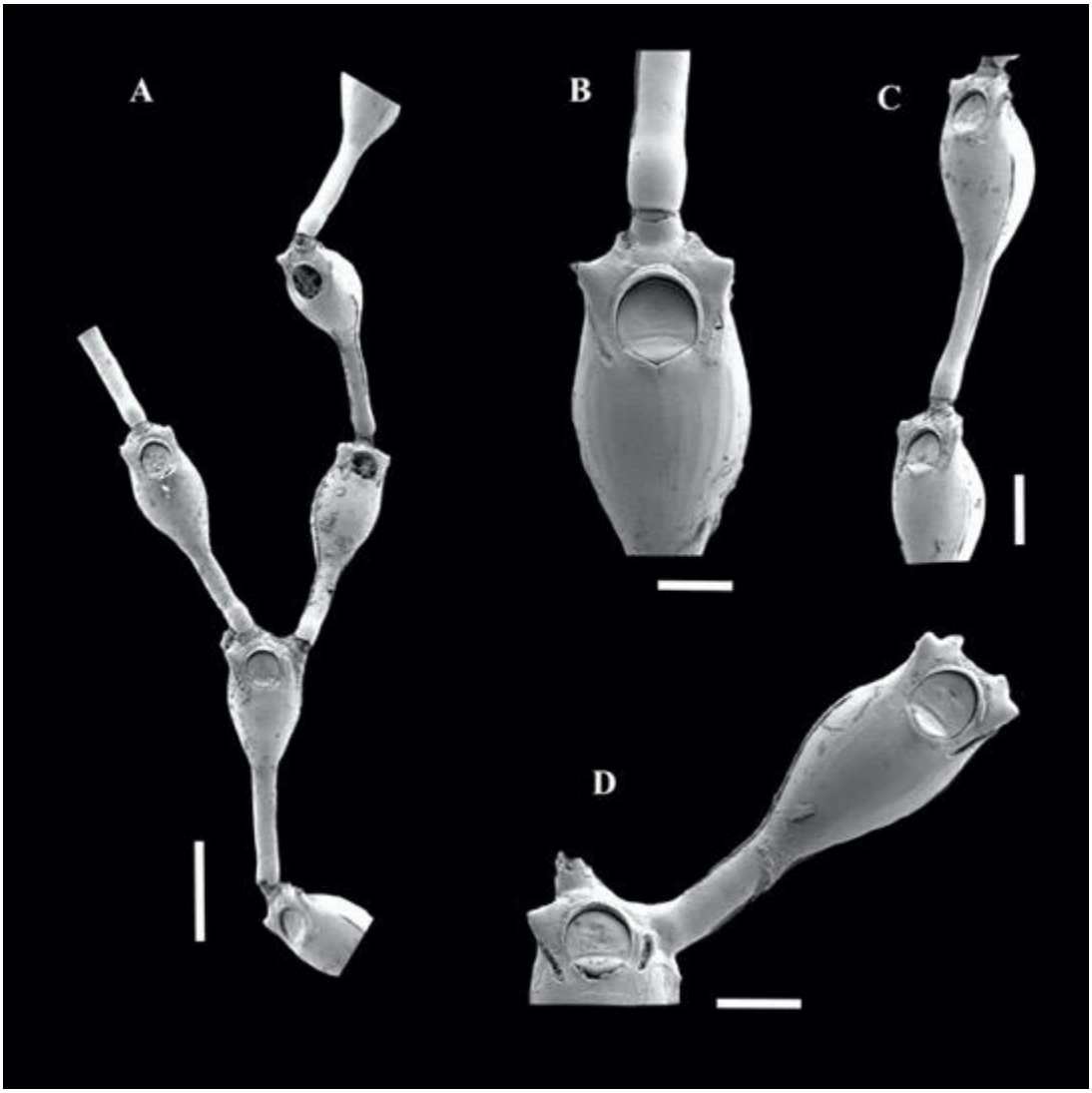

Figure 4. *Catenicella guajirensis* **sp. nov**.*:* A. General view of the erect colony, scale bar: 200µm B. Detail of zooid showing orifice, scale bar: 50µm C. Detail of lateral zooids and distance between them, scale bar: 100µm D. Branched colony detail showing vittae, scale bar: 50µm.

Type material. *Holotype*: INVBRY 2078, La Guajira 270m, Colombia 12° 26' 1.4" N 72° 1' 21.4" W along with coral rubble. *Paratypes*: INVBRY 2079, same data as Holotype.

Table 4. Measurements in mm of *Catenicella guajirensis* **sp. nov.**

	Lz	**Wz**	**Lav**	**Wav**	**Lo**	**Wo**
N	10	10	10	10	10	10
Mean	0.98	0.38	0.10	0.04	0.15	0.16
SD	0.05	0.11	0.03	0.03	0.05	0.03
Min	0.87	0.26	0.07	0.02	0.12	0.13
Max	1.26	0.52	0.15	0.07	0.22	0.20

Lz zooid length; Wz zooid width Lav length avicularia; Wav width avicularia; Lo length orifice, Wo width orifice

Diagnosis. *Catenicella* species with very long unizooidal segments, distally elongated, connected to each other with a chitinous articulation. Long and narrow vittae on each side of the zooid.

Description. Colony erect, branched and delicate (Fig. 4A), with one elongated zooid per chitinous internod (Fig. 4B), each zooid is tubular at the base and globular at the proximal end near the orifice. Basal zooids connect the branches proximally (Fig. 4D). Orifice is semi-circular with a small V-shaped sinus (Fig. 4B). Frontal wall is smooth and imperforated. Zooids have one long and narrow vittae on each side (Fig. 4C). Zooids have a small and narrow avicularium located proximolateral to each side of the orifice facing towards the scapular chamber (Fig. 4D). No ovicells were observed.

Etymology. The epithet *guajirensis* refers to the occurrence of this species in La Guajira, Colombian Caribbean.

Remarks. *Catenicella guajirensis* sp.nov is very similar to *C. paradoxa* described by Rosso (2009), in regards the zooid distal elongation and organization within the colony, specially at the bifurcation zone, zooids are connected to a basal zooid through a very narrow tubular section of the daughter zooid, which is also a key characteristic of the Taiwan species *C. marceli* (Gluhak, Lewis and Popijac, 2007), however, this last one does not present the distal processes of *C. guajirensis*. Our species has longer zooids and smaller vittae to each side of the body than *C. paradoxa and C. marceli*. This last feature makes *C. guajirensis* distinctive from other Atlantic species. The lateral distal processes are similar to *C. uberrima,* described for Florida (Winston, 1982) and Brazil (Ramalho et al., 2014), however, the shape and length of the lateral vittae and the zooid size differs. Also, the orifice shape is similar to *C. contei* from Florida (Winston, 1982) but the rest of key

taxonomic features including the avicularia and spiny projecting distal processes are completely different from *C. guajirensis*. Finally, after the morphological revision of *Catenicella* specimens, we were not able to place this morphotype closely related to any and we suggest it correspond to a new species.

Distribution. This species was found attached to rocks and mollusc shells in La Guajira, North Colombian Caribbean at 270m depth. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Superfamily Adeonoidea Busk, 1884

Family Adeonidae Busk, 1884

Genus *Adeonellopsis* MacGillivray, 1886

***Adeonellopsis avicularia* sp. nov.**
(Figure 5; Table 5)

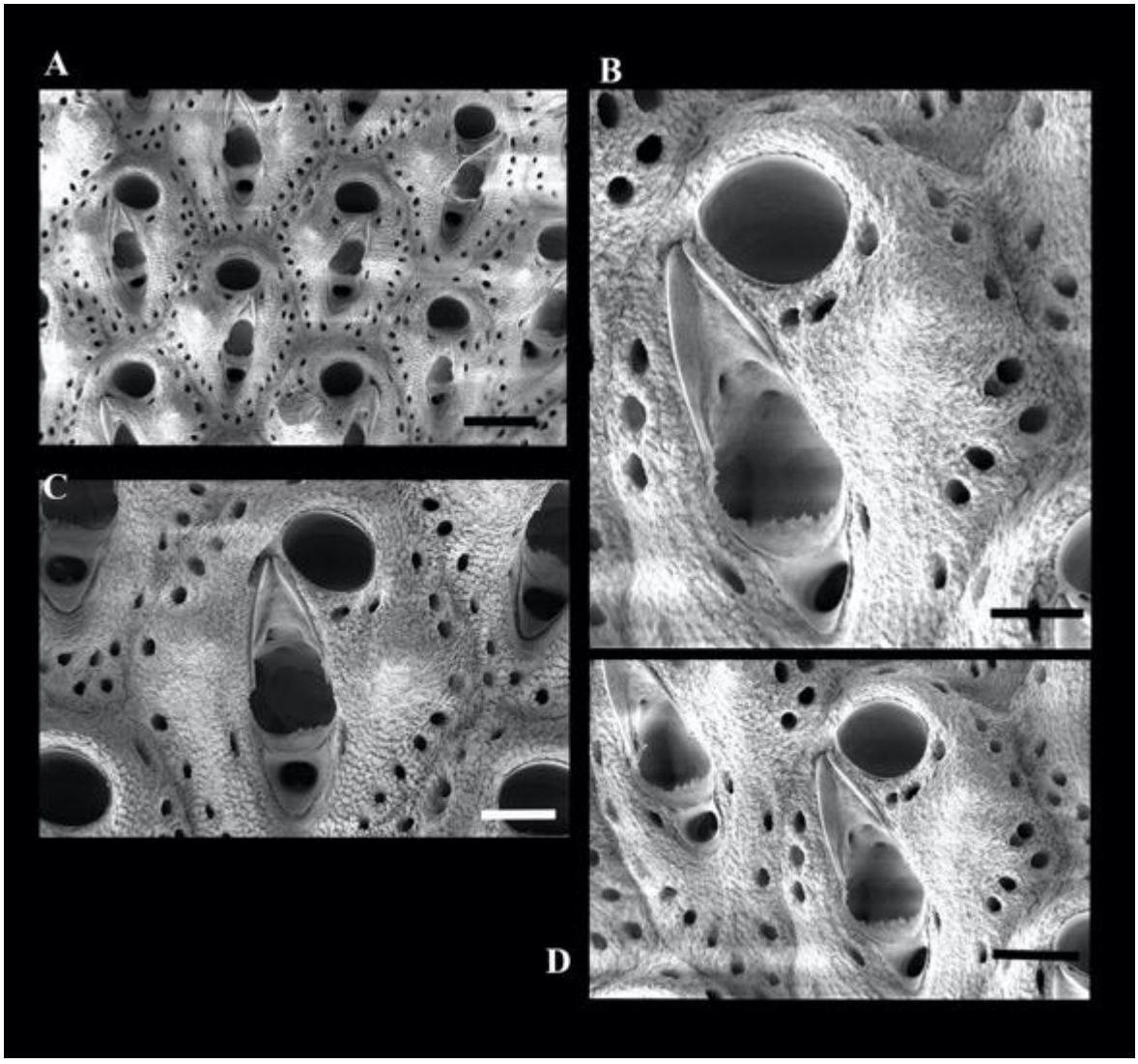

Figure 5. *Adeonellopsis avicularia* **sp. nov.**: A. General view of the colony, scale bar: 200µm B. Detail of zooid and prominent avicularia, scale bar: 50µm C. Detail of zooid, avicularia and marginal pores, scale bar: 50µm D. Close up detail of zooids, avicularia and marginal pores, scale bar: 50µm.

Type material. *Holotype*: INVBRY 2084, PNNCP at 110m depth, Colombia, 9°54'09,1" N 76°09'19,0" W. *Paratype*: INVBRY 2085 same information as Holotype.

Table 5. Measurements in mm of *Adeonellopsis avicularia* **sp. nov**

	Lz	**Wz**	**Lav**	**Wav**	**Lo**	**Wo**
N	10	10	10	10	10	10
Mean	0.45	0.34	0.37	0.55	0.51	0.72
SD	0.15	0.12	0.15	0.05	0.04	0.15
Min	0.42	0.32	0.32	0.53	0.48	0.58
Max	0.47	0.36	0.33	0.60	0.53	0.81

Lz zooid length; Wz zooid width Lav length avicularia; Wav width avicularia; Lo length orifice, Wo width orifice

Diagnosis. *Adeonellopsis* species with a large and prominent suboral avicularium of around 80% of the total zooid length with triangular rostrum slightly inclined pointing latero-proximal from the orifice.

Description.

Colony bilaminar, erect, branched and rigid. Semi-rhomboidal or hexagonal zooids with a large and pointed suboral avicularium of around 80% of the zooid length (Fig. 5B). This avicularium has a triangular rostrum proximally directed and it is placed above the circular spiramen (Fig. 5D). The opening of the avicularium has a spiny irregular ornamentation at the distal base (Fig. 5B). Frontal wall is slightly granulated with two prominent tubercles to each side of the avicularium (Fig. 5C). The interzooidal margin is ornamented with pores evenly separated to each other (Fig. 5A). The orifice is circular to oval with suboral pores to one side of the suboral avicularium (Fig. 5B). No ovicells or vicarious avicularia were observed.

Etymology. The epithet *avicularia* refer to the presence of a large suboral avicularium in this species.

Remarks. This finding increases the species record for this genus in the Great Caribbean in where *A.subsulcata* represented the only previously known species. Our species resembles the description of *A. arculifera* (Canu & Bassler, 1929) but the occurrence range of this description correspond to another marine system in the Indo-pacific with no direct migration route to the Colombian deep-sea. Four fragments of this species were found without live tissue which we hypothesize as belonging to nearby locations from the point of collection. The main differences between *A. arculifera* and *A. avicularia* sp. nov. are the zooid size as Colombian

samples are wider, the suboral avicularia is also longer than the descriptions for Holocene Indonesian specimens by Di Martino & Taylor (2018). Also, no additional avicularia from the already suboral mentioned or vicarious avicularia were found in the fragments analysed. Finally, after the revision of *Adenollopsis* specimens in different museum collections, were not able to place this specimen within any known identification and we suggest it correspond to a new species.

Distribution. This species was found nearby coral habitats in mix bottom sampling in the PNNCP Colombian Caribbean at 110m depth. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Genus *Adeonella* Busk, 1884

***Adeonella coralina* sp. nov.**
(Figure 6; Table 6)

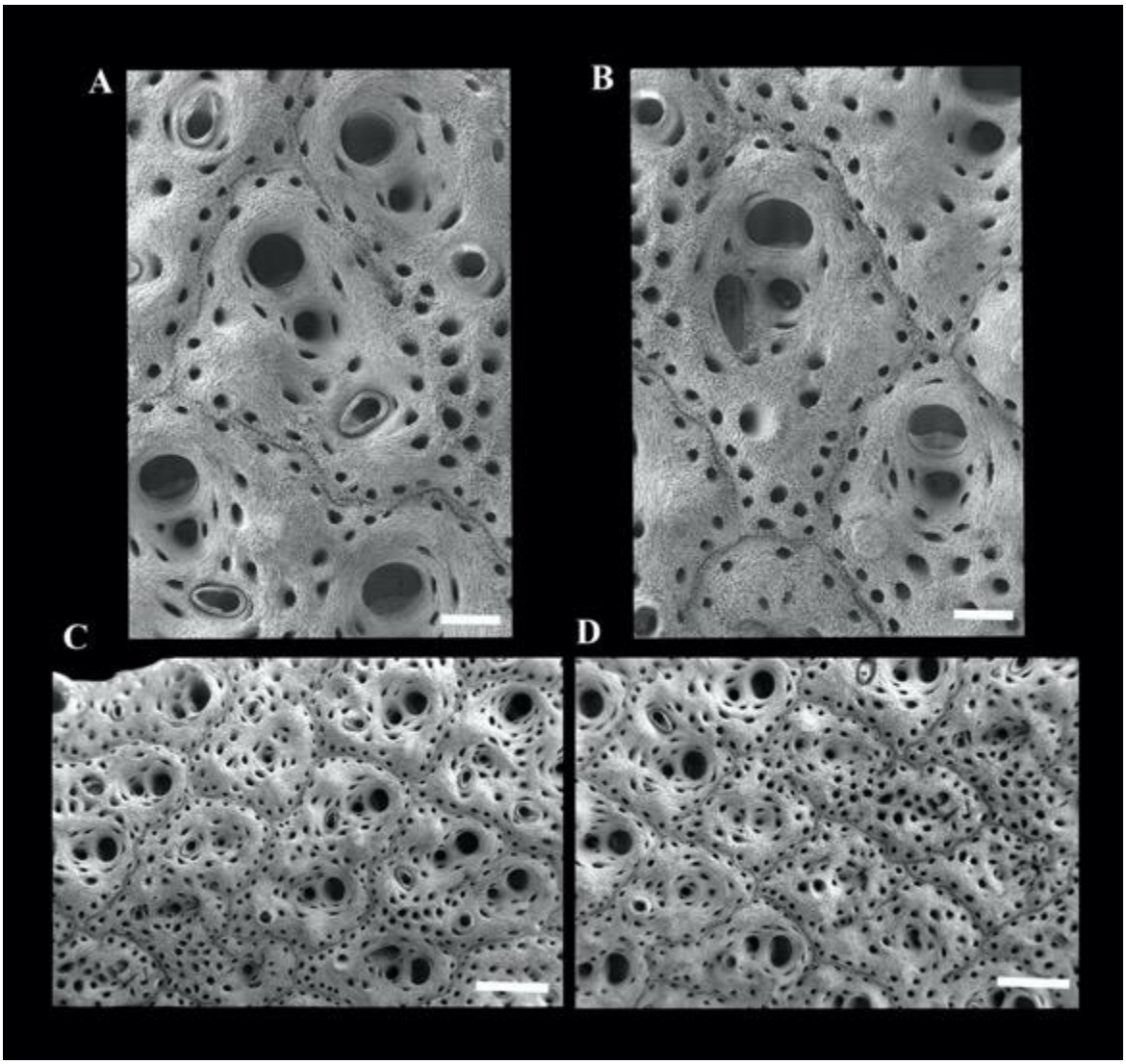

Figure 6. *Adeonella coralina* **sp. nov**.: A. Detail of zooid, avicularia and marginal pores, scale bar: 50µm. B. Detail of a rhomboid zooid and avicularia, scale bar: 50µm. C. General view of the colony, scale bar: 100µm D. Sealed zooids with calcified processes, scale bar: 10µm.

Type material. *Holotype*: INVBRY 2091 PNNCP at 180m depth, Colombia 09°49'47.5" N 76°12'01.6" W. *Paratype*: INVBRY 2096 same information as Holotype.

Table 6. Measurements in mm of *Adeonella coralina* **sp. nov.**

	Lz	**Wz**	**Lavf**	**Wavf**	**Lavs**	**Wavs**	**Lo**	**Wo**
N	10	10	10	10	5	5	10	10
Mean	0.46	0.36	0.09	0.05	0.11	0.07	0.50	0.69
SD	0.05	0.12	0.05	0.02	0.05	0.04	0.05	0.04
Min	0.41	0.24	0.07	0.04	0.10	0.06	0.46	0.56
Max	0.56	0.58	0.11	0.06	0.13	0.09	0.59	0.85

Lz zooid length; Wz zooid width Lavf length frontal avicularia; Wavf width frontal avicularia; Lavs length suboral avicularia; Wavs width suboral avicularia Lo length orifice, Wo width orifice

Diagnosis. *Adeonella* species with perforated frontal wall and small frontal avicularium and one latero-proximal suboral avicularium with triangular rostrum.

Description. Colony bilaminar, erect, branched and rigid. Semi-rhomboidal or irregular-shaped zooids with a suboral rounded spiramen and interzooidal furrows with areolar pores (Fig. 6A-B). Frontal wall perforated and slightly convex with 1-2 tubercles at each side of the zooid in the mid region (Fig. 6C). There are smaller pores around the orifice, spiramen and avicularia (Fig. 6B). The orifice is rounded to oval. Small frontal avicularium below the spiramen and in some zooids an elongated suboral avicularium is placed next to spiramen pointing the distal region of the zooid (Fi. 6A). Some zooids from the base of the colony had their orifice sealed with calcified processes, these zooids were totally perforated with pores and some presented frontal avicularia (Fig. 6D). No ovicells or adventitious avicularia were observed

Etymology. The epithet *coralina* refers to the occurrence of this species in the recently recognized Deep-sea Corals Natural National Park (PNNCP) in the Colombian Caribbean.

Remarks. This finding represents the first record of *Adeonella* in the Colombian Caribbean. Two fragments of colony of *Adeonella coralina* sp. nov. were found but no mature zooids were observed, this makes challenging to identify it taxonomically, however, we did an extensive revision of *Adeonella* type material in several museum collections and literature and to our criteria it slightly resembles *A. lichenoides* (Lamarck, 1816) described by Hayward (1988; figure 3D but not figure 1-2) for the Indian ocean in regard the frontal avicularia and zooid shape, however, our samples did not present adventitious avicularia or more than one frontal avicularia as the described there. Also, the zooid size and shape were similar to the descriptions of

Adeonella cf *lichenoides* by Di Martino & Taylor (2018: figure 37 but not figure 35-36 or 38-44) for Indonesia, but our samples presented a lateral tubercle not distal as described there. In addition, *A. coralina* sp. nov. does not present a peristome or a densely granular frontal wall. In order to unfold whether or not our specimen relate to *A. lichenoides* descriptions we also compared it to other author's descriptions and samples, and it is totally different from the descriptions of *A.* cf *lichenoides* by Hirose (2016; figure 2-3) for Japanese waters. It is also different from Australian specimens (Harmer, 1957). We also compared these specimens to other *Adeonella* descriptions and to our concern *A. coralina* does not resemble completely to any description or type material for the Western Atlantic or the Tropical indopacific ocean. Some authors have hypothesized a high phenotypic plasticity in this species or intraspecific variability in regards the zooid shape and avicularia shape in the *Adeonella platalea* complex, which includes *A. lichenoides* and *A. intricaria* (Busk, 1884). However, the original descriptions of this species do not correspond to the found in the Colombian samples. For instance, we suggest it correspond to a new species.

Distribution. This species was found nearby coral habitats in mix bottom sampling in the PNNCP Colombian Caribbean at 180m depth. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Superfamily Lepraliellоidea Vigneaux, 1949

Family Margarettidae Harmer, 1957

Genus *Margaretta* Gray, 1843

***Margaretta elongata* sp. nov.**
(Figure 7; Table 7)

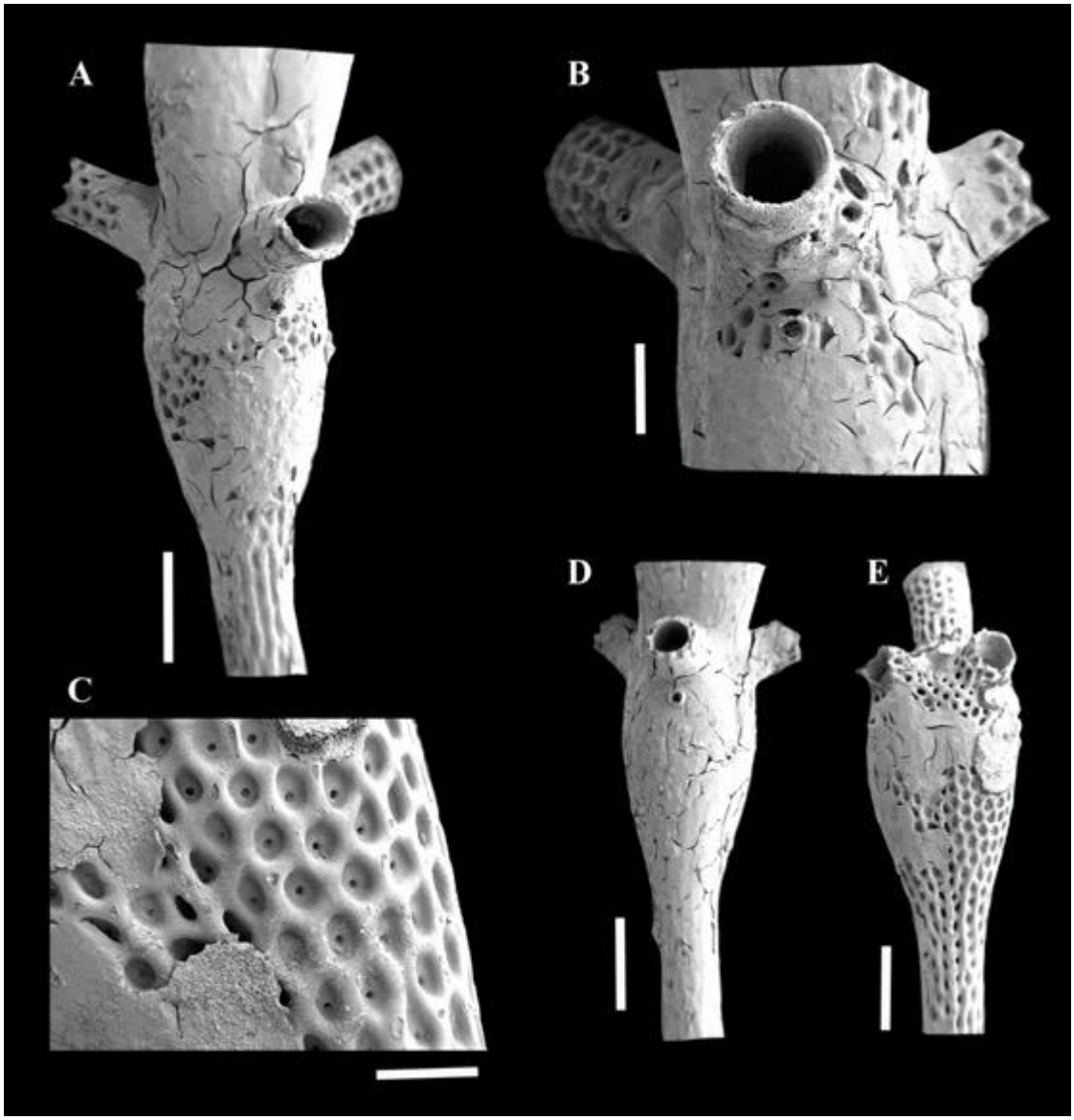

Figure 7. *Margaretta elongata* **sp. nov**.*:* A. Detail of zooid, and pointed long peristome, scale bar: 200µm. B. Detail of orifice, ascopore and peristome, scale bar: 50µm. C. Detail of zooid ornamentation under the skin, scale bar: 50µm D. Front and D. side view of the colony showing long tubular section in between zooid groups, scale bar: 200µm.

Type material. *Holotype*: INVBRY 2108, La Guajira, 220m depth, Colombia, 12° 16' 12.4" N 72° 30' 50.2" W. *Paratype*: INVBRY 2110, same information that Holotype, INVBRY 2129 La Guajira, 190m depth 12° 13' 55.2" N 72° 31' 37.7" W.

Table 7. Measurements in mm of *Margaretta elongata* **sp. nov.**

	Lz	**Wz**	**Lp**	**Wp**	**Las**	**Was**
N	10	10	10	10	10	10
Mean	1.06	0.43	0.20	0.11	0.06	0.05
SD	0.15	0.06	0.25	0.05	0.02	0.02
Min	1.01	0.41	0.18	0.09	0.05	0.04
Max	1.12	0.49	0.27	0.13	0.08	0.07

Lz zooid length; Wz zooid width Lp length peristome; Wp width peristome; Las length ascopore, Was width ascopore

Diagnosis. *Margaretta* species with one or two zooid groups (3 zooids) per internode which connects through a very long tubular section. Pseudopores within a crate-like basin. The region in between each zooidal group is narrower distally and enhanced proximally. Long peristomes pointing frontally with a small rounded ascopore right below.

Description. Colony erect, delicate and articulated with chitinous Internodes. Zooids convex and oval with marginal furrows and arranged in one or two triplets' groups (Fig. 7A), connected through a tubular section at the base of the group (Fig. 7D). The colony wall is densely perforated by small pseudopores each of them at the centre of a deep crate-like basin (Fig. 7C, E). There is a small rounded and projected ascopore at the base of the peristome, this last one is long and frontally projected (Fig. 7B). No ovicells or brooding observed.

Etymology. The epithet *elongata* refers to the morphological feature of this species of an elongated peristome and distal end of the zooid's triplet.

Remarks. *Margaretta elongata* sp. nov. constitutes the third Margaretta species reported for the Colombian Caribbean to date. Previously only *M. cereoides* and *M. buski* had been registered. The identification of this species also represented a challenge due to the very delicate and slightly calcified colonies which did not allow us to perform a stronger bleaching procedure to eliminate skin. We found six colonies of around 1 cm long each associated to rocks. These specimens resemble

to the *M. tenuis* Harmer, 1957 descriptions by Di Martino & Taylor (2018; fig. 124 but not 125-126) in regards the colony shape, zooids triplets organization but differs in the shape and arrangement of the pseudopores as the Indonesian species present shallower crate-like basins, in which pseudopores are located. Also, the shape and size of the peristomes shape at the base are different to the found in *M. elongata* sp. nov. as our specimens did not have a peristome valley or ridge and also did not have a denticulate oral rim, in addition we did not observe a D-shaped primary orifice as the described in *M. tenuis* by Di Martino &Taylor (2018). In order to assess the resemblance of this species to *M. tenuis* we reviewed several descriptions of this species around the world, it has been described for the Queensland coast of Australia (Weaver et al., 2018) and also differs in zooid arrangement and size. In addition, we compared our specimens to the registers for the rest of the Atlantic and we could not place our descriptions with the previously known species, for instance, we suggest it correspond to a new species.

Distribution. This species was found in La Guajira, North Colombian Caribbean at 190m and 220m depth on rocks in a mix bottom sampling. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Family Escharinidae Tilbrook, 2006
Genus *Bryopesanser* Tilbrook, 2006

***Bryopesanser dentata* sp. nov.**

(Figure 8; Table 8)

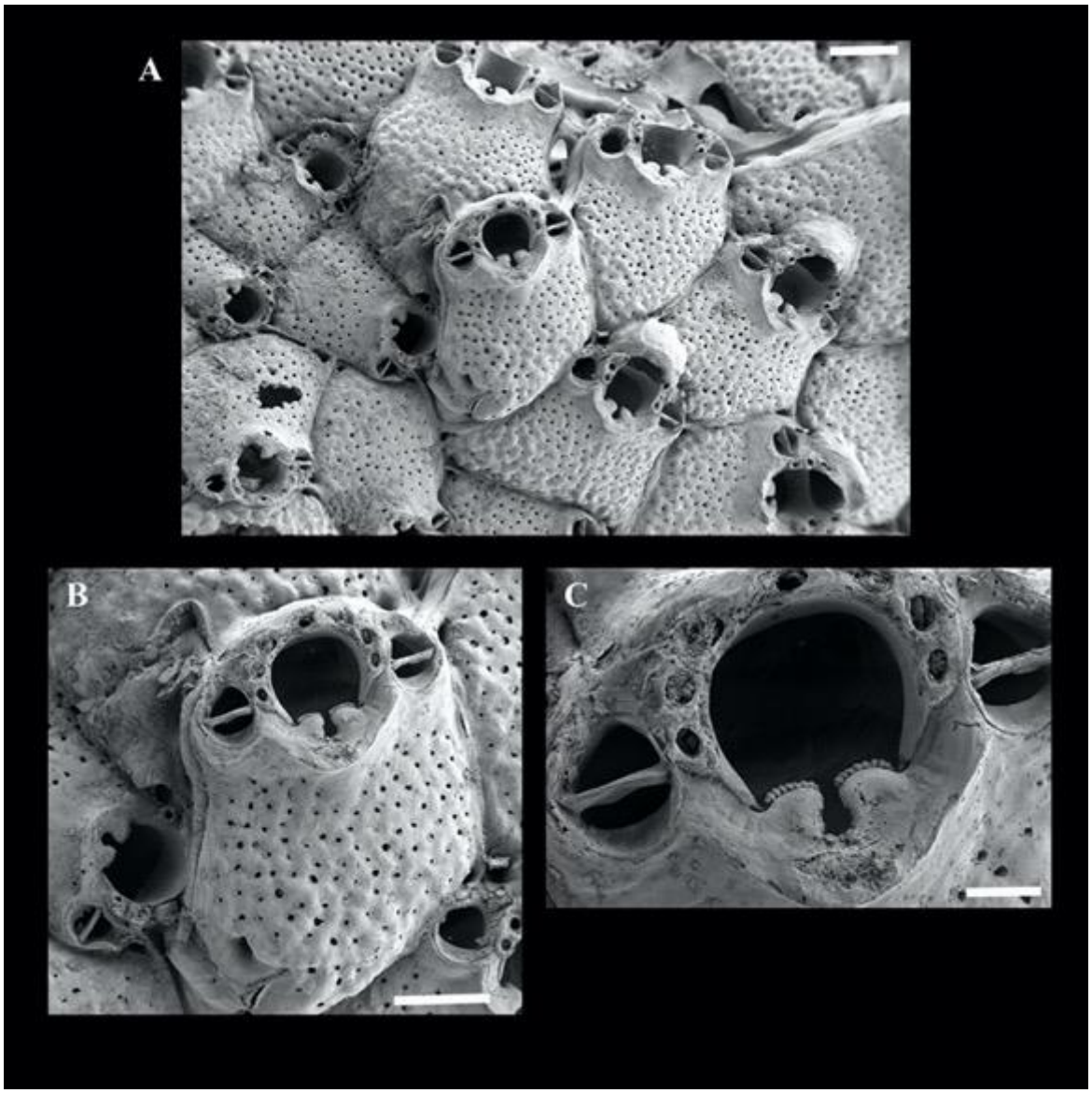

Figure 8. *Bryopesanser dentata* **sp. nov.:** A. General view of the colony and zooid orientation, scale bar: 100µm. B. Detail of orifice zooid shape, scale bar: 50µm. C. Detail of orifice and condyles, scale bar: 20µm

Type material. *Holotype*: INVBRY 2117, at 98m depth, Coraline archipelagos, Colombia, 9° 36’ 18” N 76° 20’ 10.5” W. *Paratype*: INVBRY 2120 same information as Holotype, INVBRY 2181 at 124m Caribbean offshore depth 9° 16' 3.5" N 76° 26' 26.8" W.

Table 8. Measurements in mm of *Bryopesanser dentata* **sp. nov.**

	Lz	**Wz**	**Lav**	**Wav**	**Lo**	**Wo**
N	10	10	10	10	10	10
Mean	0.78	0.65	0.17	0.15	0.22	0.18
SD	0.12	0.25	0.20	0.05	0.05	0.05
Min	0.71	0.60	0.15	0.13	0.20	0.16
Max	0.93	0.71	0.20	0.16	0.25	0.22

Lz zooid length; Wz zooid width; Lav length avicularium; Wav width avicularium; Lo length orifice, Wo width orifice

Diagnosis. *Bryopesanser* Species with tonsil-like oral condyles ornamented with 10-15 teeth-like proximal margin.

Description. Encrusting colony. Zooids polygonal or with irregular shape separated by a deep marginal rim (Fig. 8A). The frontal wall irregular and evenly perforated with small pores. Orifice is surrounded by a slightly prominent peristome with 6-7 hollow spines; the orifice shape is rounded proximally with two prominent and tonsil-shaped condyles with 10-15 teeth-like ornaments at the proximal edge (Fig. 8C). Condyles form a pronounced drop-shaped sinus rounded at the base. Two small and rounded avicularia are at the proximo-lateral side of the orifice with a complete cross bar and spatulated rostrum facing proximally and a duck-feet like chitinous mandible (Fig. 8B). Some zooids present a small hyperstomial imperforate ovicell.

Etymology. The epithet *dentata* refers to the presence of dental-like ornaments at the proximal end of the oral condyles.

Remarks. *Bryopesanser dentata* sp. nov. is characterised by presenting 10-15 teeth-like ornaments at the proximal margin of the oral condyles. This is the second *Bryopesanser* species registered for the Colombian Caribbean. The taxonomical characters of this species resemble to *Bryopesanser tonsillorum* (Tilbrook, 2012) found in the Indian and Pacific oceans. Although in general, our specimens match with most of the described characters for *B tonsillorum*, it differs slightly in regards the number of “teeth” at the proximal margin of the oral condyles, the shape and size or vicarious avicularia, shape of the chitinous mandible and zooid size. The previously known Caribbean species *B. pesanseris* is also different from our specimens in regards the condyle shape, granular frontal wall and it does not present the ornamentation on the condyle margin. The original description of *B.*

tonsillorum included the revision of a Pacific sample nearby the coastal Gorgona Island originally described by Hastings (1930) and even though correspond to a species record for the country it does not have direct connectivity to the Northern Caribbean coast in which our specimen was found, leading us to the questioning of whether or not our differences are a case of phenotypic plasticity or an actual new species. The rest of the localities at which this species was reported by Tilbrook (2012) also do not hold a natural species interchange known to date with the Colombian Caribbean. Further molecular analysis could unfold the state of this species in the Colombian marine territory.

Distribution. If our records remain different from the *B. tonsillorum* specimens, *B. dentata* sp. nov. is distributed from middle to North Colombian Caribbean from 98m to 124m depth associated to bivalve shells in mix bottoms.

Family Microporellidae Hincks, 1879

Genus *Microporella* Hincks, 1877

***Microporella granulata* sp. nov.**

(Figure 9; Table 9)

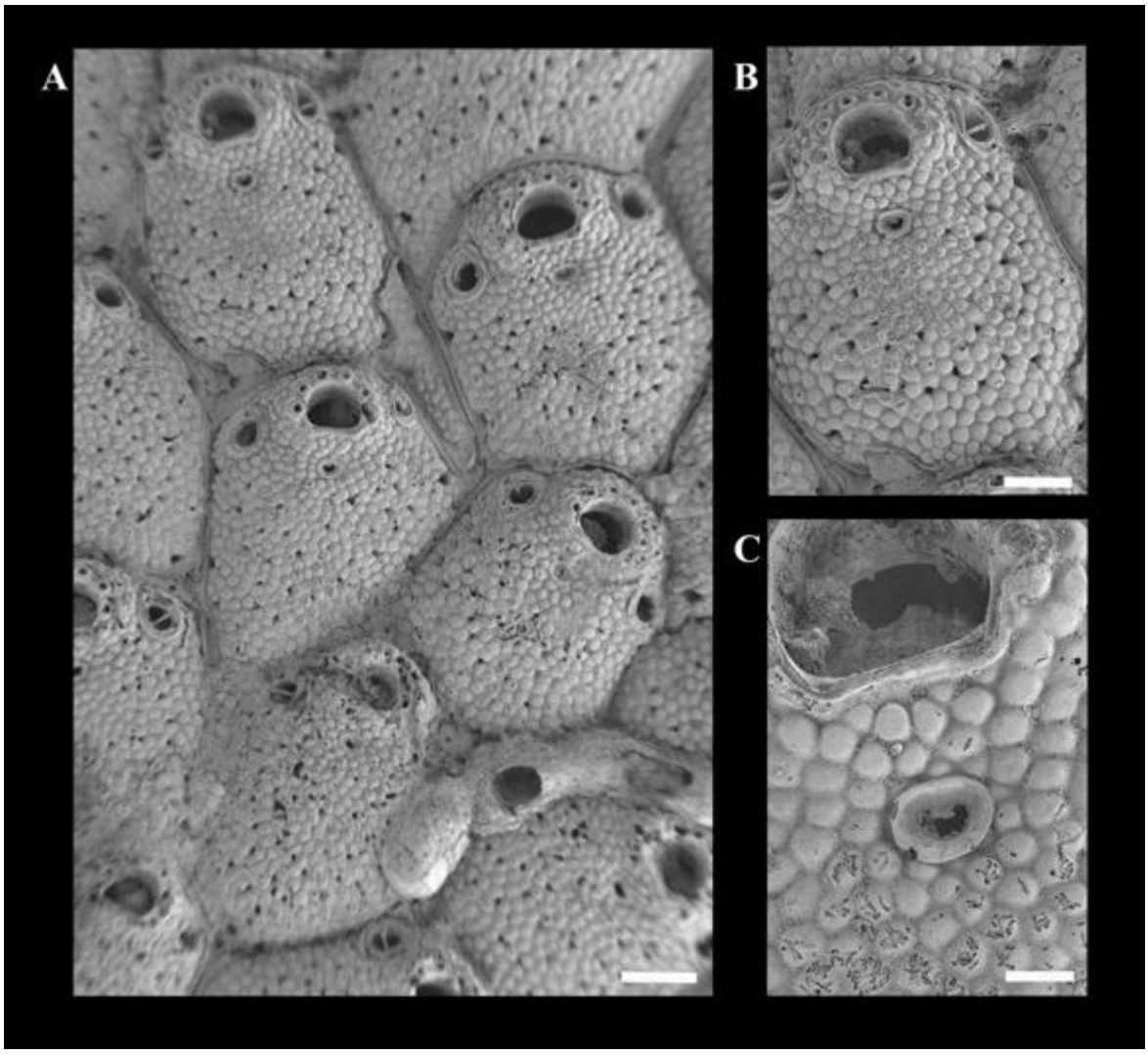

Figure 9. *Microporella granulata* **sp. nov.**: A. General view of a group of zooids, scale bar: 50µm B. Detail of zooid and frontal wall, scale bar: 50µm C. Detail of ascopore, scale bar: 20µm.

Type material. *Holotype*: INVBRY 2118, at 180m depth, PNNCP, Colombia, 09°49'47.5" N 76°12'01.6" W. *Paratype*: INVBRY 2121 same information as Holotype.

Table 9. Measurements in mm of *Microporella granulata* **sp. nov.**

	Lz	**Wz**	**Lav**	**Wav**	**Lo**	**Wo**
N	10	10	10	10	10	10
Mean	0.65	0.48	0.18	0.08	0.10	0.12
SD	0.22	0.25	0.05	0.05	0.05	0.03
Min	0.61	0.41	0.16	0.06	0.08	0.10
Max	0.69	0.53	0.20	0.10	0.11	0.15

Lz zooid length; Wz zooid width; Lav length avicularium; Wav width avicularium; Lo length orifice, Wo width orifice

Diagnosis. Zooids with a slightly porous and bead-like granulated frontal wall, especially dense at the margin of the orifice. The zooids also have a shallow U-shaped ascopore below the orifice with internal spiny ornamentation. Two proximo-lateral vicarious avicularia, one at each side of the orifice.

Description. Colony encrusting and highly calcified (Fig. 9A); Zooids are polygonal to irregular in shape (Fig. 9B) with a granulated with bead-like granules and slightly perforated with small pseudopores (Fig. 9B). The orifice is rounded with a flat distal margin and six hollow oral spines at the proximal border of the orifice. Two vicarious triangular avicularia with complete crossbar are located proximolateral to the orifice and projecting proximally. Below the orifice there is a shallow u-shaped ascopore with a spiny internal ornamentation (Fig. 9C). No ovicells were observed in the specimens analysed.

Etymology. The epithet *granulata* refers to the granule-like beads that characterize the frontal shield of this species.

Remarks. *Microporella granulata* sp. nov. constitutes the second *Microporella* species registered for the Colombian Caribbean. Our specimen resembles to *M. mayensis* (Winston 1984) in regards the beaded surface of the frontal wall, the type of ascopore and the six hollow oral spines, however, *M. mayensis* only has one vicarious avicularia pointing distally per zooid while *M granulata* sp. nov has two latero-proximal triangular avicularia. we compared our specimens to the registers for the rest of the Atlantic and we could not place our descriptions with the previously known species, for instance, we suggest it correspond to a new species.

Distribution. This species was found in deep-sea coral environments in the mid-Colombian Caribbean at 180m depth on empty bivalve shells in a mix bottom sampling. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Superfamily Mamilloporoidea Canu & Bassler, 1927

Family Cleidochasmatidae Cheetham & Sandberg, 1964

Genus *Gemelliporina* Canu & Bassler, 1926

***Gemelliporina winstoniana* sp. nov.**
(Figure 10; Table 10)

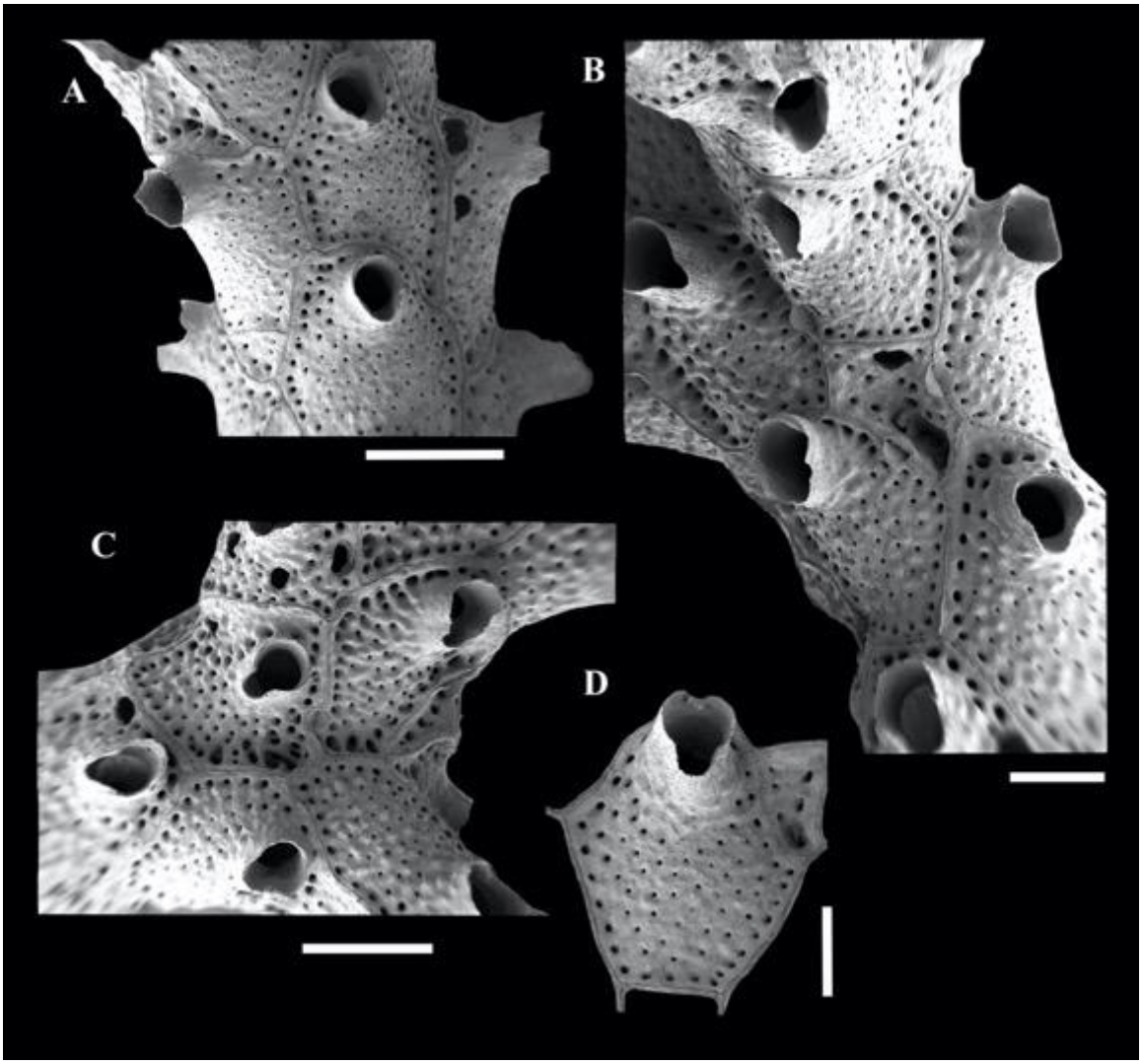

Figure 10. *Gemelliporina winstoniana* **sp. nov.** A. General view of a group pf zooids, scale bar: 100µm B. General view of the colony, scale bar: 100µm C. Detail of irregular zooids, scale bar: 100µm. D. Detail of a single zooid and its projected peristome, scale bar: 50 µm.

Type material. *Holotype*: INVBRY 2126, PNNCP at 110m depth 9°54'09,1" N 76°09'19,0" W. *Paratype*: INVBRY 2128, same information as Holotype.

Table 10. Measurements in mm of *Gemelliporina winstoniana* **sp. nov.**

	Lz	**Wz**	**Lp**	**Wp**	**Lo**	**Wo**
N	10	10	10	10	10	10
Mean	1.21	0.81	0.16	0.12	0.21	0.13
SD	0.13	0.25	0.05	0.02	0.15	0.05
Min	1.19	0.76	0.13	0.09	0.18	0.12
Max	1.26	0.89	0.19	0.15	0.26	0.15

Lz zooid length; Wz zooid width; Lp length peristome; Wav width peristome; Lo length orifice, Wo width orifice

Diagnosis. *Gemelliporina* species with perforated frontal shield and interzooidal areolate pores and marginal rim. Peristome projected to the front and a soft U-shaped sinus.

Description. Colony erect, rigid and branched (Fig. 10B). Zooids polygonal to irregular with perforated frontal shield and marginal areolate pores in the interzooidal margin (Fig. 10C). Zooid also are separated from each other with a marginal rim (Fig 10A). Peristome is smooth and projected to the front of the colony with a u-shaped distal sinus (Fig. 10D). No ovicells or avicularia were observed in the samples analysed.

Etymology. The epithet *winstoniana* refers to J.E Winston in recognition for her life-long contribution to the knowledge of bryozoans in the American continent but specially in the Western central and the Caribbean.

Remarks. *Gemelliporina winstoniana* sp. nov. is the third Gemelliporina species registered for the Colombian Caribbean after *G. glabra* and *G. hastata*. This species resembles slightly to *G. hastata* Winston & Woollacott, 2009 in the colony shape and organization, the presence of a peristome and marginal areolate pores, however, this species does not present a perforated frontal shield as *G. winstoniana*, also, presents a small avicularium at the base of the peristome that is not present in our specimens.

Distribution. This species was found in deep-sea coral environments in the mid-Colombian Caribbean at 110m depth on a mix bottom sampling. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Incertae sedis
(Figure 11; Table 11)

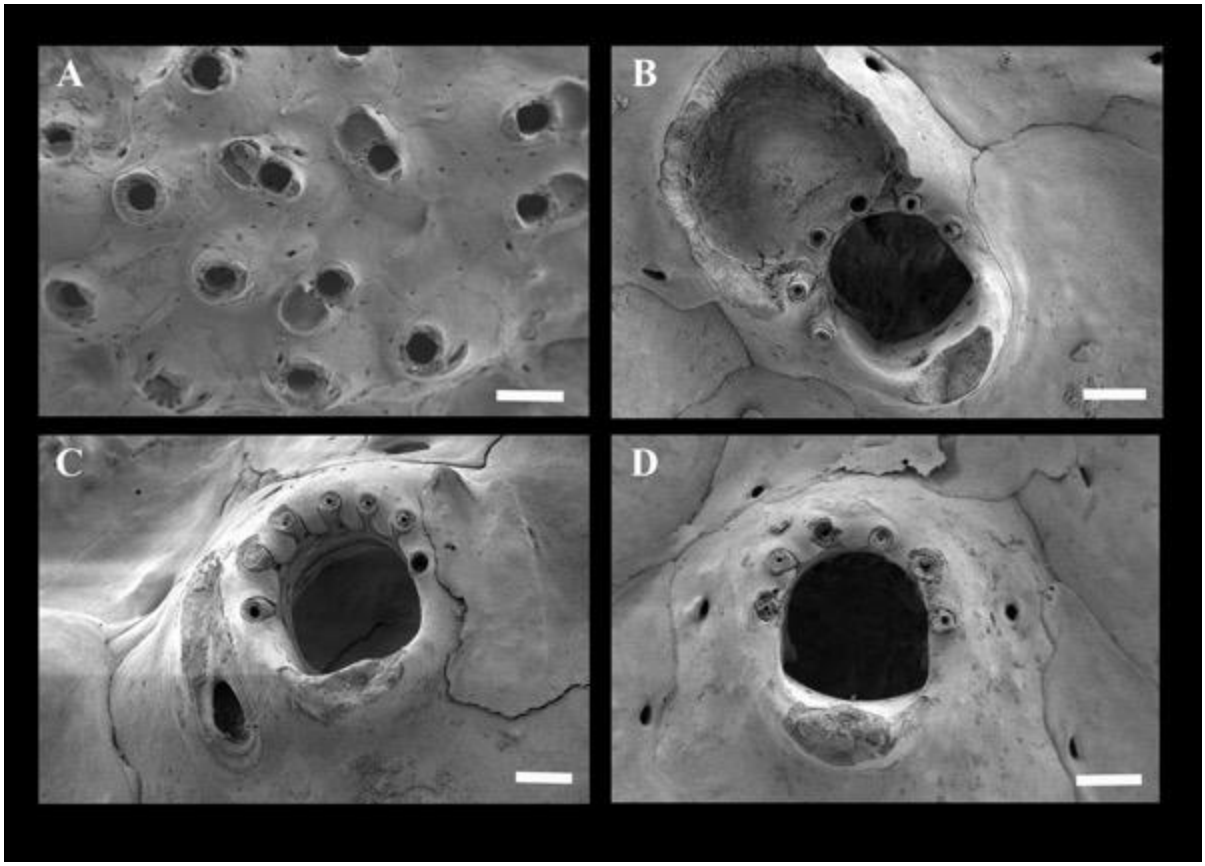

Figure 11. *Incertae sedis*. A. General view of the colony, scale bar: 100µm B. Detail of ovicells and oral spines, scale bar: 50µm C. Detail of vicarious avicularium, scale bar: 100µm. D. Detail of suboral tubercle, scale bar: 20 µm

Type material. *Holotype*: INVBRY 2140, PNNCP at 110m depth 9°54'09,1" N 76°09'19,0" W.

Table 11. Measurements in mm of *Incertae sedis.*

	Lz	**Wz**	**Lav**	**Wav**	**Lo**	**Wo**	**Lov**	**Wov**
N	10	10	5	5	10	10	10	10
Mean	0.81	0.72	0.10	0.05	0.15	0.10	0.18	0.19
SD	0.20	0.11	0.05	0.02	0.05	0.02	0.06	0.04
Min	0.73	0.65	0.08	0.03	0.13	0.08	0.10	0.17
Max	0.86	0.78	0.12	0.08	0.19	0.14	0.21	0.22

Lz zooid length; Wz zooid width; Lav length avicularium; Wav width avicularium; Lo length orifice, Wo width orifice; Lov Length ovicell; Wov Width ovicell

Description. Colony encrusting, highly calcified (Fig. 11A); Zooids are flat with irregular shape and smooth frontal shield and differentiated by a marginal rim and scarcely distributed pores (Fig. 11A). Orifice is rounded with a straight distal margin and occupy about the 20% of the zooid length with six hollow spines at the proximal margin (Fig. 11B). Some zooids have a narrow vicarious triangular avicularium proximo-lateral to the orifice pointed proximally (Fig. 11C). Also, some zooids have a projected and thick tubercle at the suboral region (Fig. 11D). Ovicells are globular and hyperstomial. The samples analysed presented broken ovicells (Fig. 11B)

Remarks. The taxonomic identification of this species has been challenging because we have not been able to place this species within a known genus for the Colombian Caribbean or the great Caribbean and further analysis should be used to clarify the taxonomic state of this species.

Distribution. This species was found in deep-sea coral environments in the mid-Colombian Caribbean at 110m depth on a mix bottom sampling. Further exploration of the Colombian submarine bottoms should be performed in order to determine the distribution of this species along bathymetric transects.

Table 12. List of recent deep-sea Bryozoan species identified in the Colombian Caribbean

Class	Order	species	Author	Station	New species	New Register Caribbean	New register CC	Knowledge increase	
								Bathymetric	Geographic
Stenolaemata	Cyclostomatida	*Crisia denticulata*	(Lamarck, 1816)	EA2 329					X
		Crisia sp.		EA2 323					
		Filicrisia sp.		EA2 322					X
		Mecynoecia delicatula	(Busk, 1875)	EA1 322					
		Proboscina sp.		EA2 329					
		Oncousoecia dilatans	(Johnston, 1847)	EA2 315			X		
		Stomatopora sp.		EA2 323					
		Tubulipora sp.		EA2 322					
Gymnolaemata	Ctenostomatida	*Amathia vidovici*	(Heller, 1867)	EA1 326					
		Amathia distans	Busk, 1886	EA2 329					
		Amathia maxima	(Winston, 1982)	EA2 328				X	X
	Cheilostomatida	*Electra verticillata*	(Ellis & Solander, 1786)	EA2 329		X			
		Electra pilosa	(Linnaeus, 1767)	EA2 323				X	
		Biflustra arborescens	(Canu & Bassler, 1928b)	EA1 322					X
		Aetea angina	(Linnaeus, 1758)	EA2 329					
		Aetea truncata	(Landsborough, 1852)	EA2 327					X
		Steginoporella magnilabris	(Busk, 1854)	E 524					
		Steginoporella connexa	Harmer, 1900	E 524			X		
		Siphonoporella dumonti	Canu & Bassler, 1928a	EA2 327				X	X
		Thalamoporella n. sp. *Colombiana*	Yepes & Preziosi, 2020	E 524	X				
		Parellisina curvirostris	Osburn, 1940	EA1 326					
		Cupuladria surinamensis	Cadée, 1975	EA1 326					X
		Cupuladria panamensis	Herrera-Cubilla, Dick, Sanner & Jackson, 2006	EA2 315				X	X
		Discoporella depressa	(Conrad, 1841)	EA1 317					
		Discoporella sp.		E 375					
		Akatopora leucocypha	(Marcus, 1937)	EA1 315					
		Nellia tenella	(Lamarck, 1816)	EA2 320					X
		Paralicornia pusilla	(Smitt, 1872)	EA1 315				X	X
		Paralicornia sinuosa	(Canu & Bassler, 1927)	EA1 326			X		
		Licornia jolloisii	(Audouin, 1826)	EA2 320				X	X
		Canda simplex	Busk, 1884	EA1 328					X
		Halophila antillaea	Winston, 2005	EA1 317				X	X
		Bugulidae 1		E 606		X	X		
		Bugulidae 2		E 601					
		Micropora acuminata	Winston, 2005	EA1 315					X
		Floridina antiqua	(Smitt, 1873)	EA2 322					
		Smittipora levinseni	(Canu & Bassler, 1917)	EA2 323					
		Puellina smitti	Winston, 2005	EA1 322					X
		Puellina radiata	(Moll, 1803)	EA1 327					X
		Catenicella n. sp *guajirensis*	Yepes and Preziosi, 2020	EA1 317	X				
		Savignyella lafontii	(Audouin, 1826)	EA2 322					
		Gemellipora eburnea	Smitt, 1873	EA1 315			X		
		Pasythea tulipifera	(Ellis & Solander, 1786)	EA2 321					
		Hipothoa flagellum	Manzoni, 1870	EA2 323				X	
		Trypostega striatula	(Smitt, 1873)	EA2 321					X
		Poricella mucronata	(Smitt, 1873)	EA2 329					
		Laminopora cf. *contorta*	Michelin, 1842	EA1 327		X			
		Adeonellopsis af *subsulcata*	Yepes and Preziosi, 2020	E 390	X				

	Adeoneollopsis subsulcata	(Smitt, 1873)	EA1 315					
	Adeonellopsis n.sp. avicularia	Yepes and Preziosi, 2020	E 390	X				
	Adeonella cf. calveti	Canu and Bassler, 1930	EA2 322		X			
	Adeonella cf. pallasii	(Heller, 1867)	EA2 322		X			
	Adeonella n.sp. coralina	Yepes and Preziosi, 2020	E 524	X				
	Reptadeonella bipartita	(Canu & Bassler, 1928b)	EA1 319				X	
	Reptadeonella hastingsae	Cheetham & Sandberg, 1964	EA2 321					X
	Celleporaria albirostris	(Smitt, 1873)	EA1 326					X
	Celleporaria magnifica	(Osburn, 1914)	EA2 329				X	X
	Stylopoma projecta	Canu & Bassler, 1923	EA2 316					
	Stylopoma smitti	Winston, 2005	EA2 323				X	X
	Stylopoma sp.		EA2 328					
	Schizoporella unicornis	(Johnston & Wood, 1844)	EA2 329					
	Margaretta tenuis	Harmer, 1957	EA2 322					X
	Margaretta n.sp. *elongata*	Yepes and Preziosi, 2020	EA1 319	X				
	Margaretta cereoides	(Ellis & Solander, 1786)	EA1 326				X	
	Petraliella bisinuata	(Smitt, 1873)	EA2 316					
	Bryopesanser pesanseris	(Smitt, 1873)	EA1 326				X	X
	Bryopesanser n.sp. *dentata*	Yepes and Preziosi, 2020	EA1 326	X				
	Microporella n.sp. *granulata*	Yepes and Preziosi, 2020	E 524	X				
	Microporella protea	Winston, 2005	EA2 315					
	Hippaliosina rostrigera	(Smitt, 1873)	EA2 328					
	Semihaswellia sinuosa	Canu & Bassler, 1928a	EA1 326			X		X
	Cosciniopsis violacea	(Canu & Bassler, 1928a)	EA2 320				X	
	Lagenicella spinulosa	(Hincks, 1884)	EA1 319					X
	Marcusadorea tubulosa	(Canu & Bassler, 1928b)	EA2 320					
	Rogicka biserialis	(Hincks, 1885)	EA1 329					
	Gemelliporina hastata	Winston and Woollacoot, 2009	E 524					X
	Gemelliporina n.sp. *winstoniana*	Yepes and Preziosi, 2020	E 524	X				
	Gemelliporina glabra	(Smitt, 1873)	EA1 317					
	Mamillopora cupula	Smitt, 1873	EA2 329					
	Pleurocodonellina signata	(Waters, 1889)	EA1 326					
	Parasmittina areolata	(Canu & Bassler, 1927)	EA1 317				X	
	Hippoporina caribaea	Winston, 2005	EA2 328					
	Trematooecia gemmea	(Winston & Woollacott, 2009)	EA1 326			X		
	Cigclisula turrita	(Smitt, 1873)	EA1 319					
	Plesiocleidochasma cleidostomum	(Smitt, 1873)	E 390				X	X
	Plesiocleidochasma porcellanum	(Busk, 1860)	EA2 316					
	Plesiocleidochasma sp.		E 524		X			
	Rhynchozoon spicatum	Osburn, 1952	EA1 317					
	Reteporellina marsupiata	(Smitt, 1873)	E 524				X	
	Turbicellepora pourtalesi	Winston, 2005	EA1 326			X		
	Pourtalesella rugosa	(Osburn, 1940)	E 524				X	
	Hippoporella pusilla	(Smitt, 1873)	EA2 323				X	
	Incertae sedis 1	Yepes and Preziosi, 2020	E 390		X	X		
	Incertae sedis 2	Yepes and Preziosi, 2020	E598		X	X		
	Incertae sedis 3	Yepes and Preziosi, 2020	E378		X	X		
Total	**94**			**9**	**9**	**11**	**19**	**27**

DISCUSSION

A great deep-sea bryozoan species diversity was found across 28 stations offshore in the Colombian Caribbean from 72m to 3888m depth in soft and mix bottoms. The biodiversity estimators SOBs, Jack 1 and Jack 2 indicate that the extent of occurrence of species found in this study show a high representativeness of 85% of the sampling effort (Jack 1: 120 spp; Jack 2: 150 spp) however, no stabilization of the curve was observed indicating that more species are likely to be discovered for the Colombian deep-sea. It is important to highlight that the species composition of a given location may vary over time (Adler & Lauenroth, 2003) because distribution ranges are not stable, and species can expand or reduce their distribution based on environmental changes. It is expected that subsequent studies in the area will find rare or new species. This study is a significant contribution to the bryozoan knowledge of Colombia. In total 95 species were described of which nine represent new species, twelve new records for Colombia, and four are new records for the Great Caribbean basin.

Deep-sea coral reef, where most of our new species were found, constitute one of the least explored areas in the Colombian Caribbean. Sampling effort in the past has scarcely sampled the significant biodiversity associated with azooxanthellate reef patches and used invasive methodologies such as demersal trawling. Nowadays, the sampling methods are less invasive due to new regulations intended to protect this ecosystem, but these methods provide fewer captures of bryozoans. Being able to identify new species regardless of the sampling methods, represents a success and indicates a need to continue exploration research in this important marine protected area. It might also suggest that there is a high endemism in the area and a possible connectivity with other deeper areas.

Interestingly, we found species with a wider geographic and bathymetric range, such as the species closely similar to the Mediterranean and Indo-pacific fauna with no apparent connectivity. We hypothesize that due to the position of the Colombian Caribbean, exposed to deep-sea current patterns from the North Atlantic, a possible species interchange could be taking place, either through a physical breakage and transport of colonies by the strong currents or the environmental conditions are naturally enhancing the larvae distribution to these areas for colonization. Our

findings also contribute to the understanding of the marine deep-sea systems of Colombia.

The majority of the bryozoan species found in the Colombian deep sea is similar to the fauna associated with other deep-sea corals in Florida (Winston, 2016), but differed in the number of species found and the new record of species. One surprising and rare finding was the new register of *Electra verticillata*, a species that had been previously known for the North Atlantic and the Western coast of Africa. Here we found it on rocks in mixed bottom ecosystems in La Guajira, revealing a broader geographical distribution for this species.

The deep-sea bryozoan richness described here, contrary to previous knowledge (Navas et al., 2010), did not decrease from north to south. In contrast, our findings show a consistent high diversity in La Guajira and the PNNCP mainly containing representatives of the families Steginoporellidae, Adeonidae, Schizoporellidae, Cupuladriidae, Cleidochasmatidae, Candidae and Phidoloporidae, which resembles the high abundance found in the shallower upwelling coastal zone of La Guajira, and suggesting a possible species connectivity between those bathymetric zones. The abyssal zone (3888m depth) was mainly inhabited by delicate calcifying species of the families Bugulidae and Catenicellidae, however, sampling effort in those areas is less representative than the shallower stations due to the complications of the sampling methodologies at those depths.

Recording several species belonging to Adeonidae was a rare finding here, as for the Caribbean only one species *Adeonellopsis subsulcata* had been previously described. Here we found fragments of *Laminopora* cf *contorta* a very rare species found in the Mediterranean, certainly never recorded before for Colombia, although, the deep conditions in La Guajira are the home of other widespread species such as the azooxanthellate coral *Lophelia pertusa*, of which to date only dead fragments have been collected, reflecting the scarce sampling in that vast area. In addition, *Adeonella* cf. *calvetti* and *Adeonella* sp. also represent rare findings, these colonies were also dead at the moment of collection and their taxonomic identification has challenged our bryozoan knowledge. Firstly, we support Rosso and Novosel's (2010) suggestion that this species has been misidentified and currently belongs to

a species complex, and secondly the distribution of this species at the deepest areas sampled in the deep-sea corals national park (PNNCP) indicates that surrounding areas might be holding large, rare bryozoan patches with higher diversity.

Finally, the similarities of the Colombian deep-sea species with the found in other marine systems such as the Pacific and Indian Oceans is interesting, the geographical distance between those populations does not correlate with possible natural gene flow, and several morphological differences between them allowed us to propose them as new species for science. For example, *Margaretta elongata* sp.nov. resembles *M. tenuis* in Indonesia but differs in few taxonomic traits; also, *Bryopesanser dentata* sp. nov. is very similar to *B. tonsillorum* (Tilbrook, 2012) found in the Pacific and Indian Ocean but differs in a few morphometric features resulting in a new species. Similarly, *Microporella granulata* sp. nov. is somewhat similar to the descriptions of *M. mayensis* (Winston, 1984) but differs in few morphological differences such as the shape, number and position of frontal avicularia and zooid size which allow us to nominate it as a new species. Further taxonomic and molecular inspection is necessary to clarify taxonomic issues with the deep-sea Colombian bryozoan species.

Conclusions

Most of the species found in this study present morphological features that differ from shallower species, although some are shared between bathymetric zones along the Colombian Caribbean. The high number of new species records for the deep-sea corals national park (PNNCP) indicates that this area must be holding a greater diversity and that could be connected to nearby zones with rare species hence the finding of dead branching fragments of Adeonids. Some encrusting bryozoans were colonizing different substrates including empty bivalve shells and deceased fragments of the azooxanthellate coral *Madracis Myriaster*.

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Supplementary material I: New species register for the Great Caribbean

Class Gymnolaemata
Order Cheilostomatida Busk, 1852
Suborder Membraniporina
Superfamily Membraniporoidea Busk, 1854
Family Electridae d'Orbigny, 1851
Genus *Electra* Lamouroux, 1816

***Electra* aff*. verticillata* (Ellis & Solander, 1786)**
(Fig. 1)

Flustra verticillata Ellis & Solander, 1786.
Electra verticillata: Lamouroux (1816; 1821); Gmelin (1789); Bosc (1802); Nikulina et al. (2013).

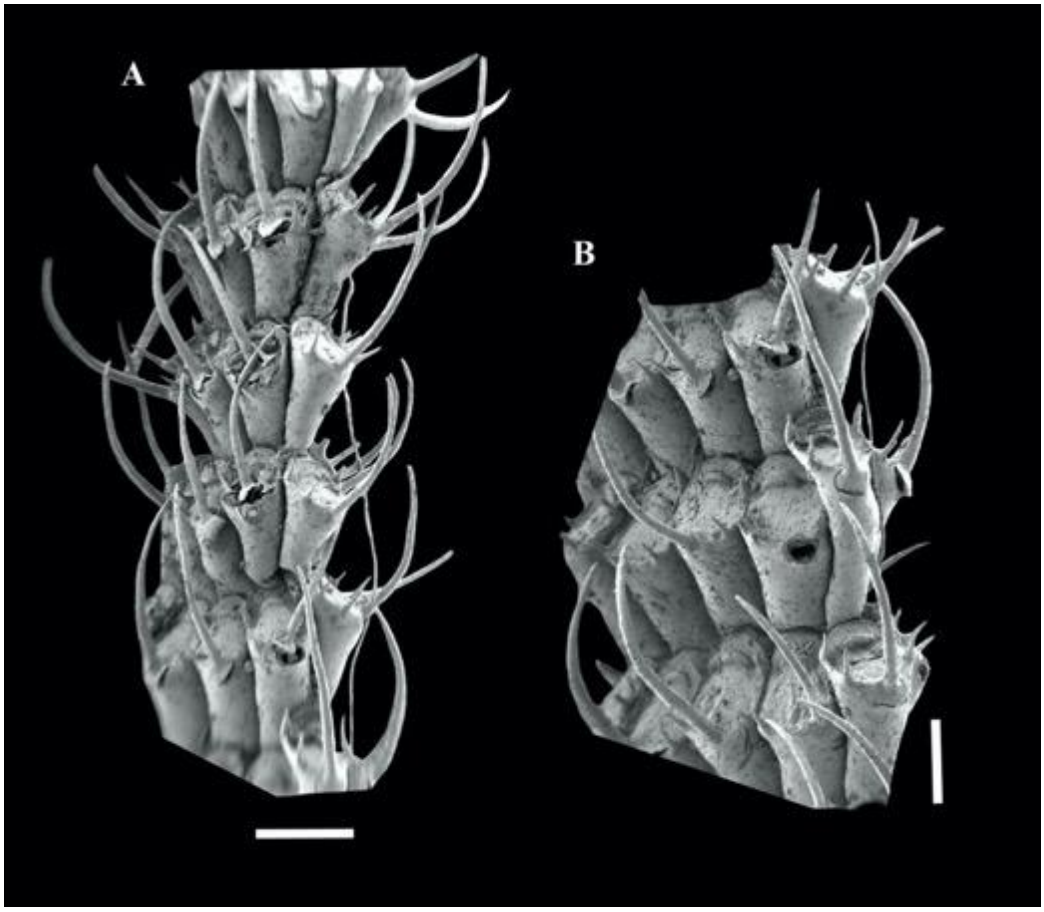

Figure 1. *Electra* aff. *verticillata* (Ellis & Solander, 1786): A. Colony fragment showing zooid organization and detail of branch structure, scale bar: 200 µm B. Group of zooids showing spines at the edge of the opesia and a large proximal spine, scale bar: 100 µm.

Material examined. INVBRY 2050; INVBRY 2058.

Superfamily Adeonoidea Busk, 1884
Family Adeonidae Busk, 1884
Genus *Laminopora* Michelin, 1842

***Laminopora* cf *contorta* Michelin, 1842**
(Fig. 2)

Adeonella (Laminopora) contorta Waters, 1912, Pl. X, fig 1, 6.

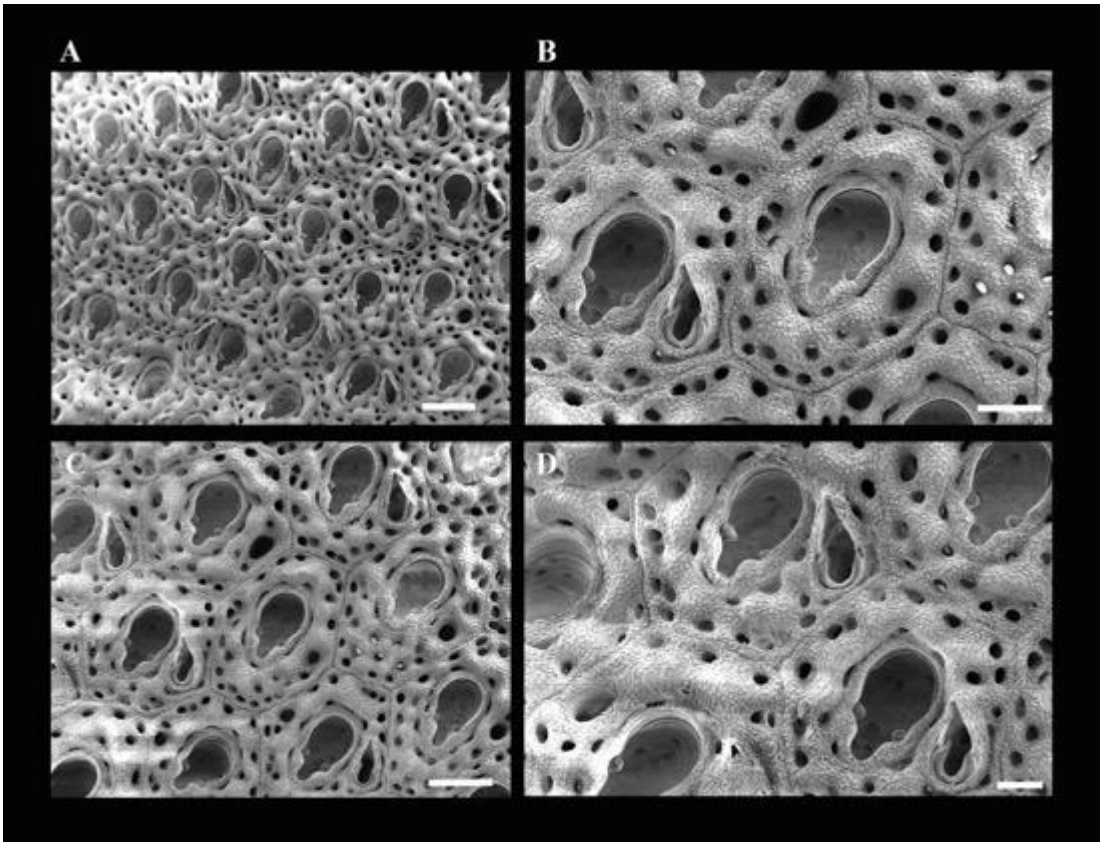

Figure 2. *Laminopora cf contorta* Michelin, 1842: A. General view of the colony, scale bar: 100µm B. Detail of zooids, sinuses, condyles and avicularia, scale bar: 100µm C. Detail of zooids shape with pores, scale bar: 100µm D. Detail of zooids and avicularia, scale bar: 20µm.

Material examined. INVBRY 2087, INVBRY 2089.

Genus *Adeonella* Busk, 1884

***Adeonella* cf. *calvetti* Canu & Bassler, 1930**
(Fig. 3)

Adeonella calveti Canu & Bassler, 1930: 68, pl. 10, figs. 1–4.
Adeonella pectinata f*. africana* Calvet, 1903: 33, pl. 2, fig. 4.
Adeonella polystomella (Reuss, 1848): Poluzzi and Rosso, 1988: 91, pl. 3, fig. 2; Pouyet and Moissette, 1992: 73, pl. 11, fig. 6.
Adeonella calvetti: Rosso & Novosel, 2010:4, Fig 1A-D, 2, 3, 4A.

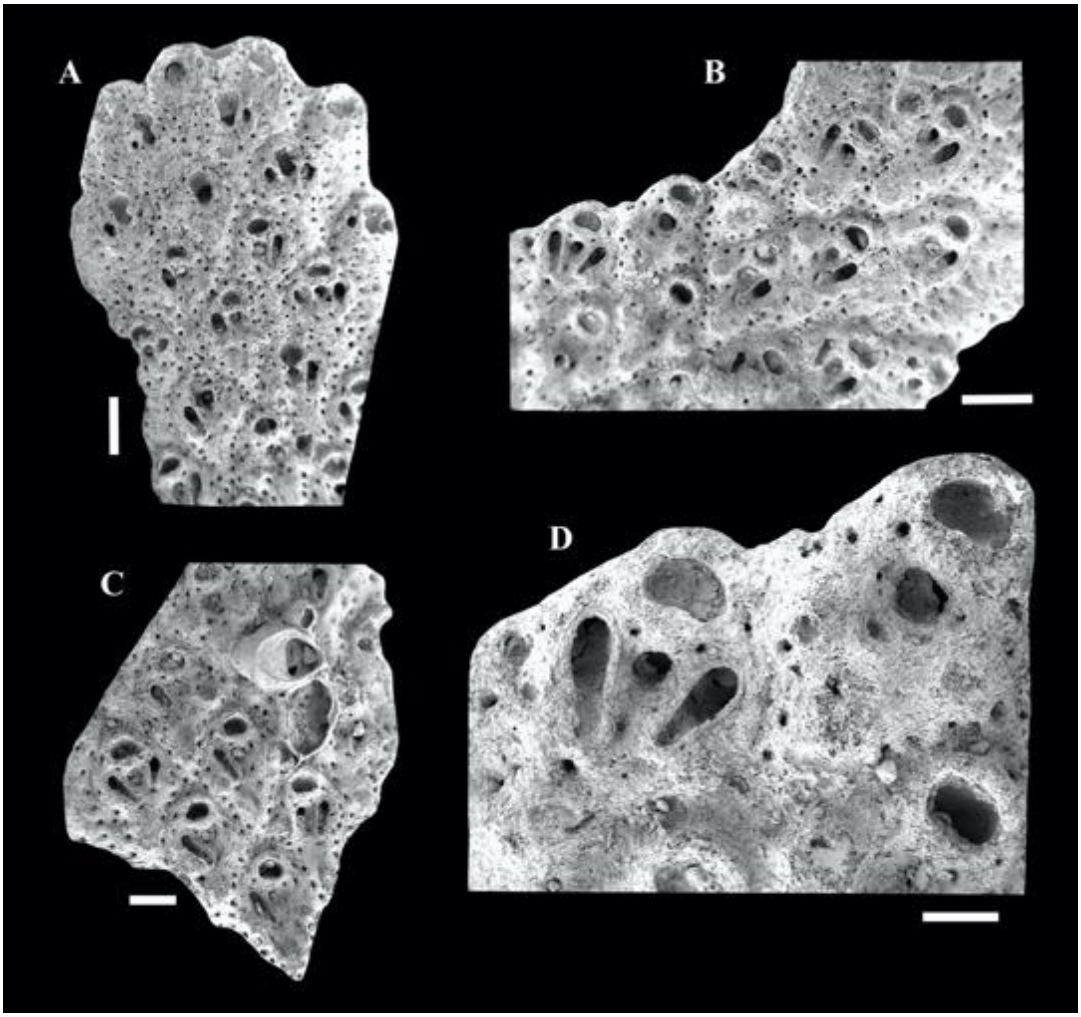

Figure 3. *Adeonella cf. calvetti?* A. General view of the colony, scale bar: 100µm, B. Detail of zooids and avicularia, scale bar: 100µm C. Detail of zooids shape, scale bar: 100µm D. Detail of avicularia, scale bar: 20µm.

Material examined. INVBRY 2099, INVBRY 2100.

***Adeonella* sp.**
(Fig. 4)

Adeonella cf. *pallasii* Rosso & Novosel, 2010 fig 6G.

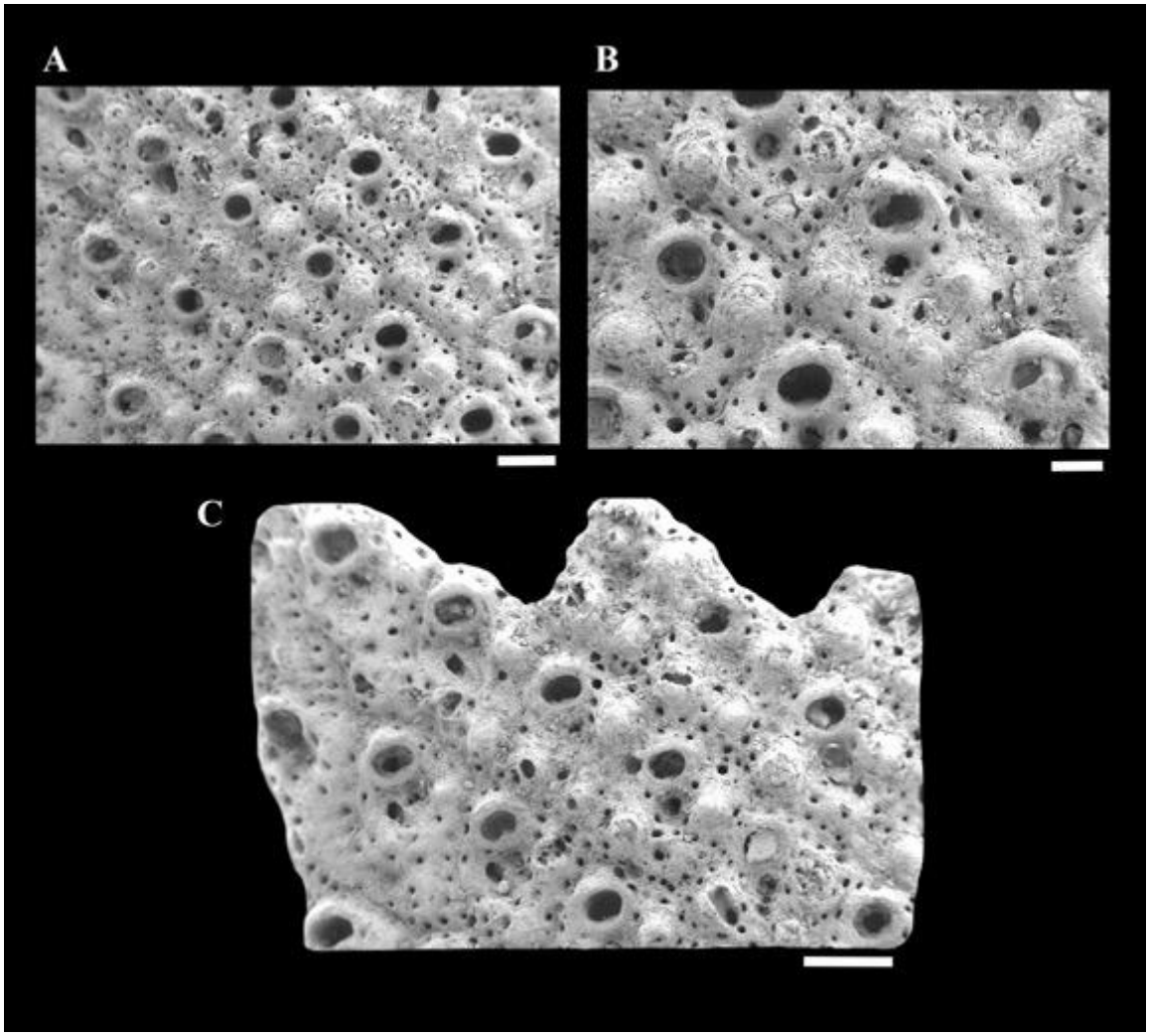

Figure 4. *Adeonella* sp. A. General view of the colony, scale bar: 80µm B. Detail of zooids shape, scale bar: 50µm C. Detail of zooids and colony shape, scale bar: 100µm.

Material examined. INVBRY 2093, INVBRY 2095.

Supplementary material II: New species register for the Colombian Caribbean

Suborder Thalamoporellina
Superfamily Thalamoporelloidea Levinsen, 1902
Family Steginoporellidae Hincks, 1884
Genus *Steginoporella*

***Steginoporella connexa* Harmer, 1900**
(Fig. 1)

Steganoporella connexa Harmer, 1900: 254, pl. 12, fig. 6; pl. 13, fig.18.
Steginoporella connexa: Winston & Woollacott, 2009: 257, fig. 12.

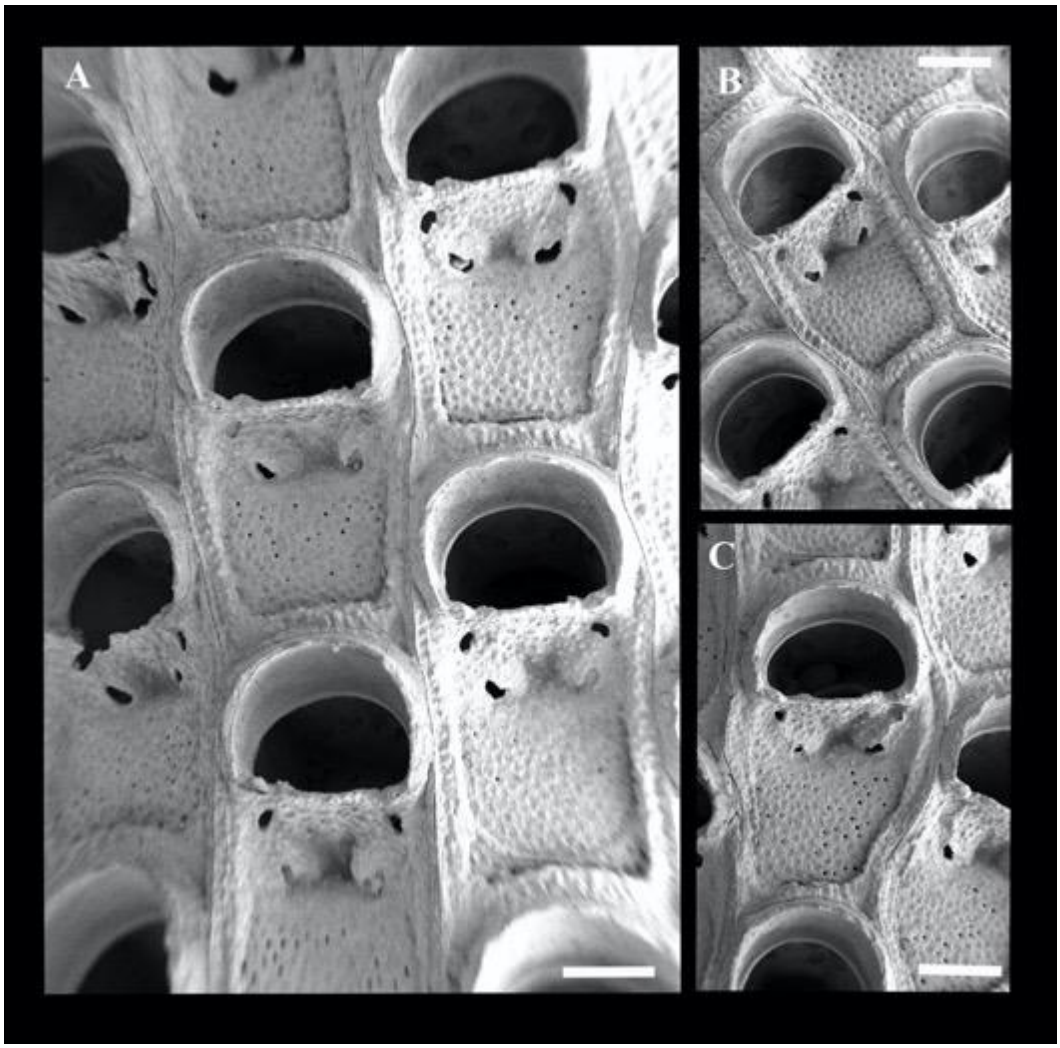

Figure 1. *Steginoporella connexa* Harmer, 1900: A. Fragment of colony showing zooid polygonal shape and organization, scale bar: 100 µm B. A-zooid with two pairs of opesiules perforating the proximal region of the cryptocyst, scale bar: 100 µm. C. A-zooid and detail of curved proximal rim, granular and slightly perforated cryptocyst, scale bar: 100 µm.

Material examined. INVBRY 2048; INVBRY 2051, INVBRY 2053.

Superfamily Buguloidea Gray, 1848
Family Candidae d'Orbigny, 1851
Genus *Paralicornia* Vieira, Spencer Jones, Winston, Migotto & Marques, 2014

***Paralicornia sinuosa* (Canu & Bassler, 1927)**
(Fig. 2)

Scrupocellaria sinuosa Canu & Bassler, 1927: 4, pl. 1, figs 4–5.
Scrupocellaria spatulata: Ryland & Hayward, 1992: 237, fig. 9.
Scrupocellaria sinuosa: Tilbrook & Vieira, 2012: 39, fig 7-8.

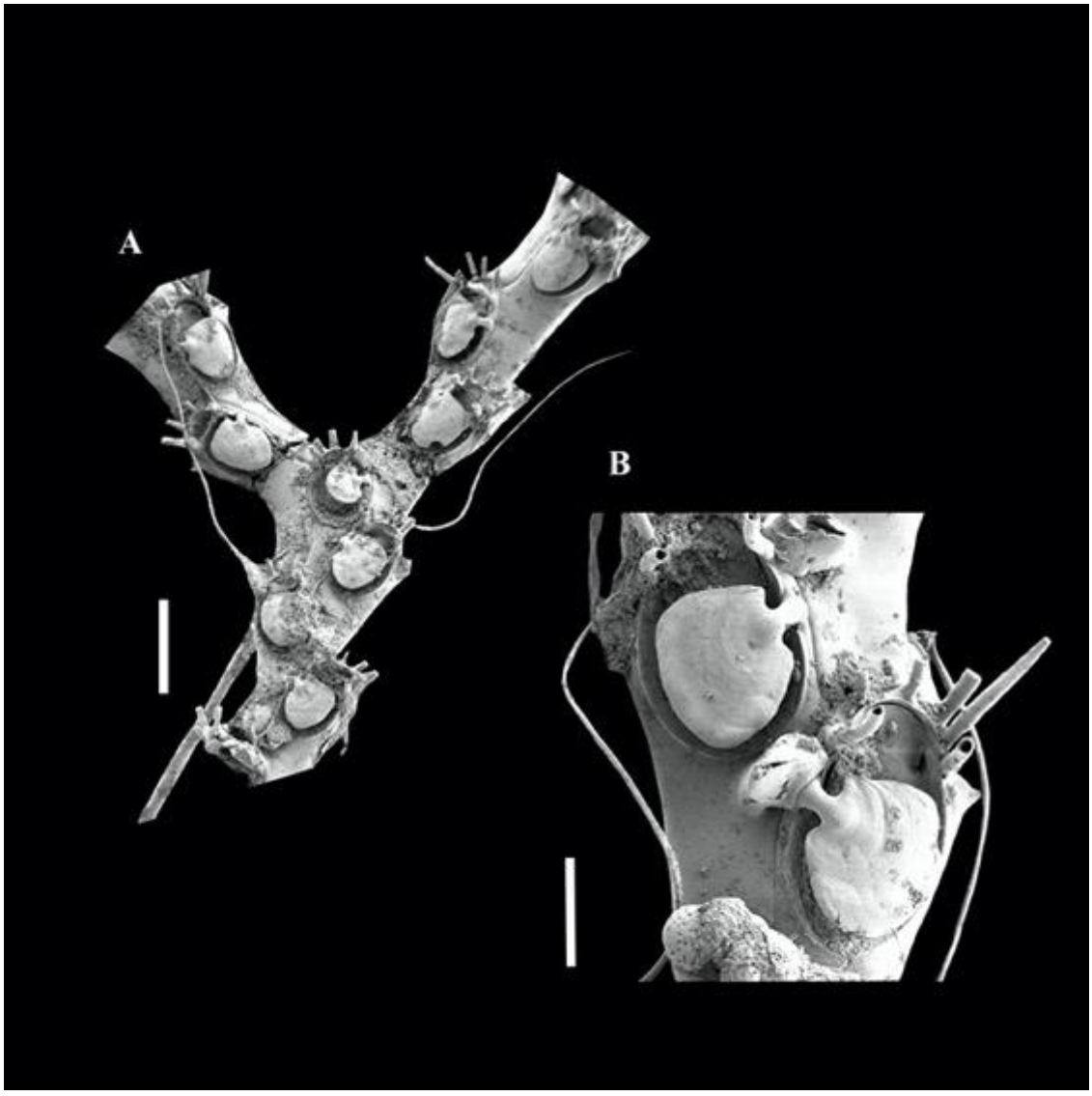

Figure 2. *Paralicornia sinuosa* (Canu & Bassler, 1927)*:* A. Fragment of the erect and branched colony showing zooids shape and organization, scale bar: 200µm B. Detail of two zooids, showing basal vibraculum, frontal wall, spines and scutum, scale bar: 50µm.

Material examined. INVBRY 2063, INVBRY 2067.

Superfamily Cribrilinoidea Hincks, 1879
Family Cribrilinidae Hincks, 1879
Genus *Puellina* Jullien, 1886

***Puellina smitti* Winston, 2005**
(Fig.3)

Cribrilina radiata: Smitt 1873: 22 (part).
Puellina smitti Winston, 2005: 34, figs 89–93.
Puellina smitti: Winston, 2016: 30, fig 16 table 15.

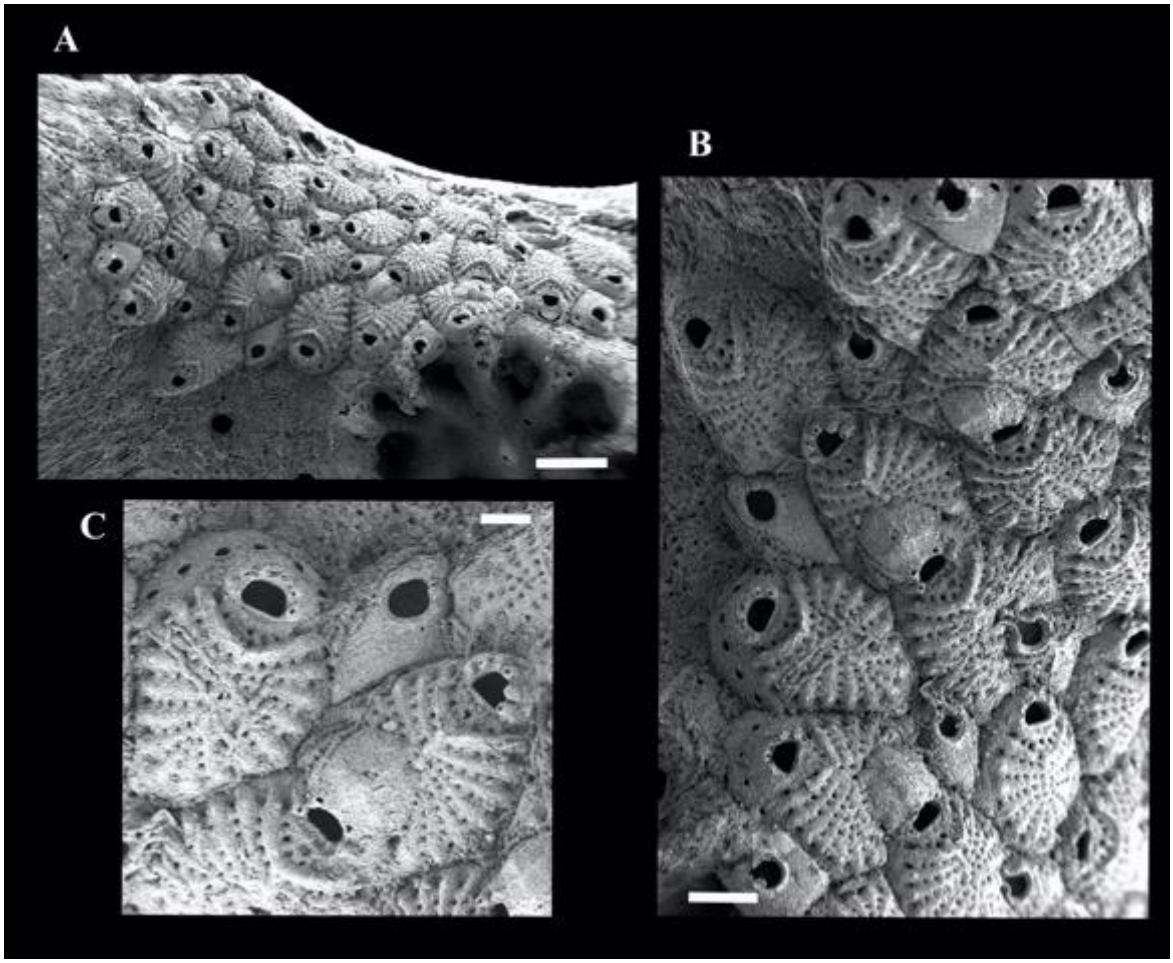

Figure 3. *Puellina smitti* Winston, 2005*:* A. Encrusting colony on *Madracis myriaster* dead fragment, scale bar: 200µm B. Detail of zooid arrangement and avicularia, scale bar: 100µm C. Detail of zooids, spines, ovicells and avicularia, scale bar: 50µm.

Material examined. INVBRY 2075.

Superfamily Hippothooidea Busk, 1859

Family Pasytheidae Davis, 1934

Genus *Gemellipora* Smitt, 1873

***Gemellipora eburnea* Smitt, 1873**
(Fig. 4)

Gemellipora eburnea Smitt, 1873: 35 in part, pl. VII, fig 152-156.
Pasythea eburnea: Smitt, 1873; Busk, 1885: 5, pl. 34, fig 1.
Gemellipora eburnea: Osburn, 1940: 463, pl. 9, figs. 73-74; Winston, 2005:41, fig. 104-106.

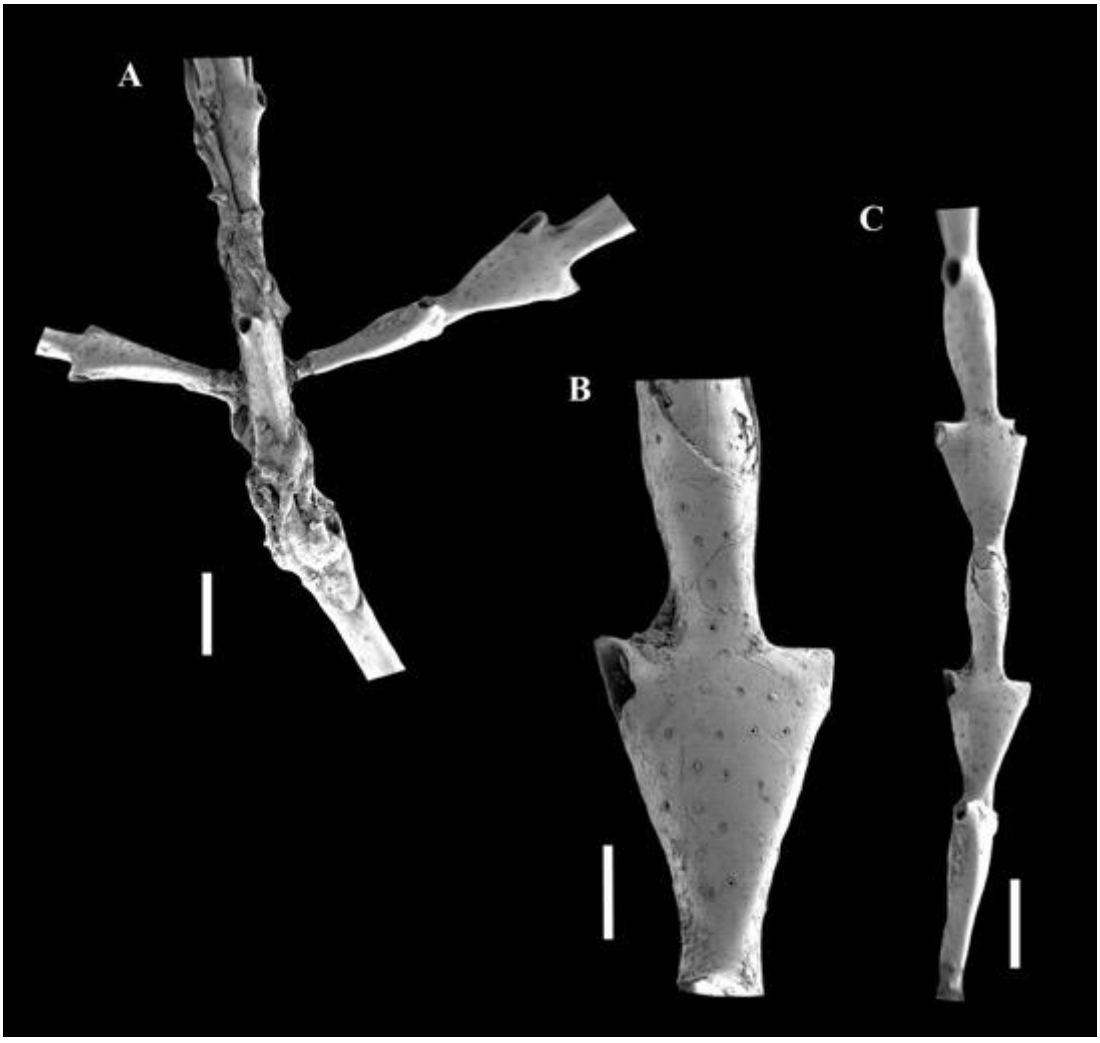

Figure 4. *Gemellipora eburnea* Smitt, 1873: A. View of a branching colony, scale bar: 100µm B. Detail of zooids shape and pores, scale bar: 50µm C. Detail of zooid organization in the colony, scale bar: 100µm.

Material examined. INVBRY 2080, INVBRY 2082, INVBRY 2086, INVBRY 2090.

Superfamily Adeonoidea Busk, 1884

Family Adeonidae Busk, 1884

Genus *Adeonellopsis* MacGillivray, 1886

***Adeonellopsis* af *subsulcata* (Smitt, 1873)**
(Fig. 5)

Bracebridgia subsulcata Canu & Bassler, 1928b: 127, pl. 233, figs. 1-3, 25; Osburn, 1940:446; Osburn, 1947: 38; Cook, 1973: 253, pl. 2, fig. 4-6; Winston, 2005: 44, fig. 113-120.

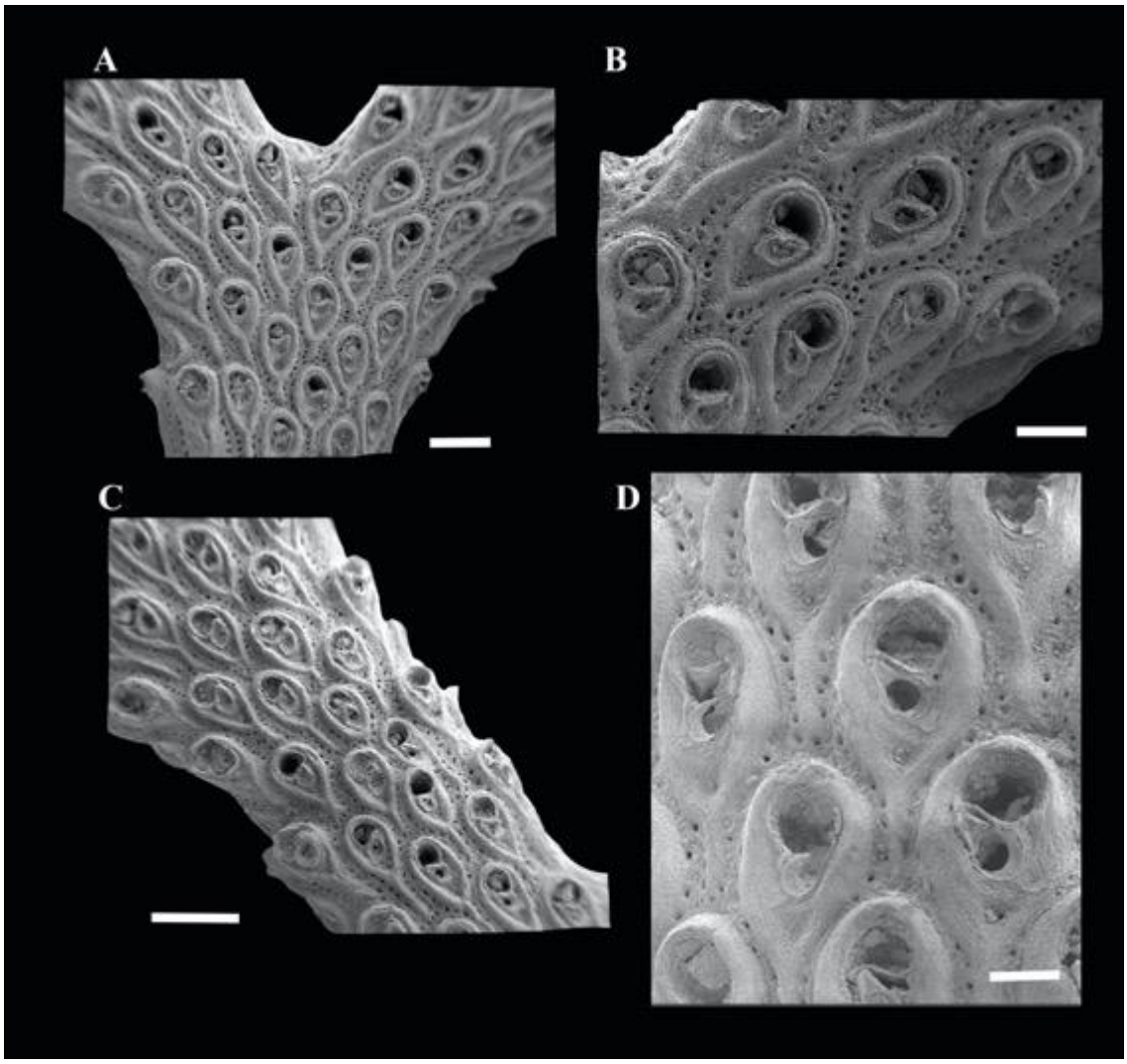

Figure 5. *Adeonellopsis* af *subsulcata* (Smitt, 1873): A. General view of the branched colony, scale bar: 100µm B. Detail of zooid arrangement, scale bar: 100µm C. Detail of colony, scale bar: 100µm D. Close up detail of drop-shaped zooids, avicularia and marginal pores, scale bar: 50µm.

Material examined. INVBRY 2083, INVBRY 2094, INVBRY 2097.

Family Porinidae d'Orbigny, 1852

Genus *Semihaswellia* Canu & Bassler, 1917

***Semihaswellia sinuosa*. Canu & Bassler, 1928a**

(Fig. 6)

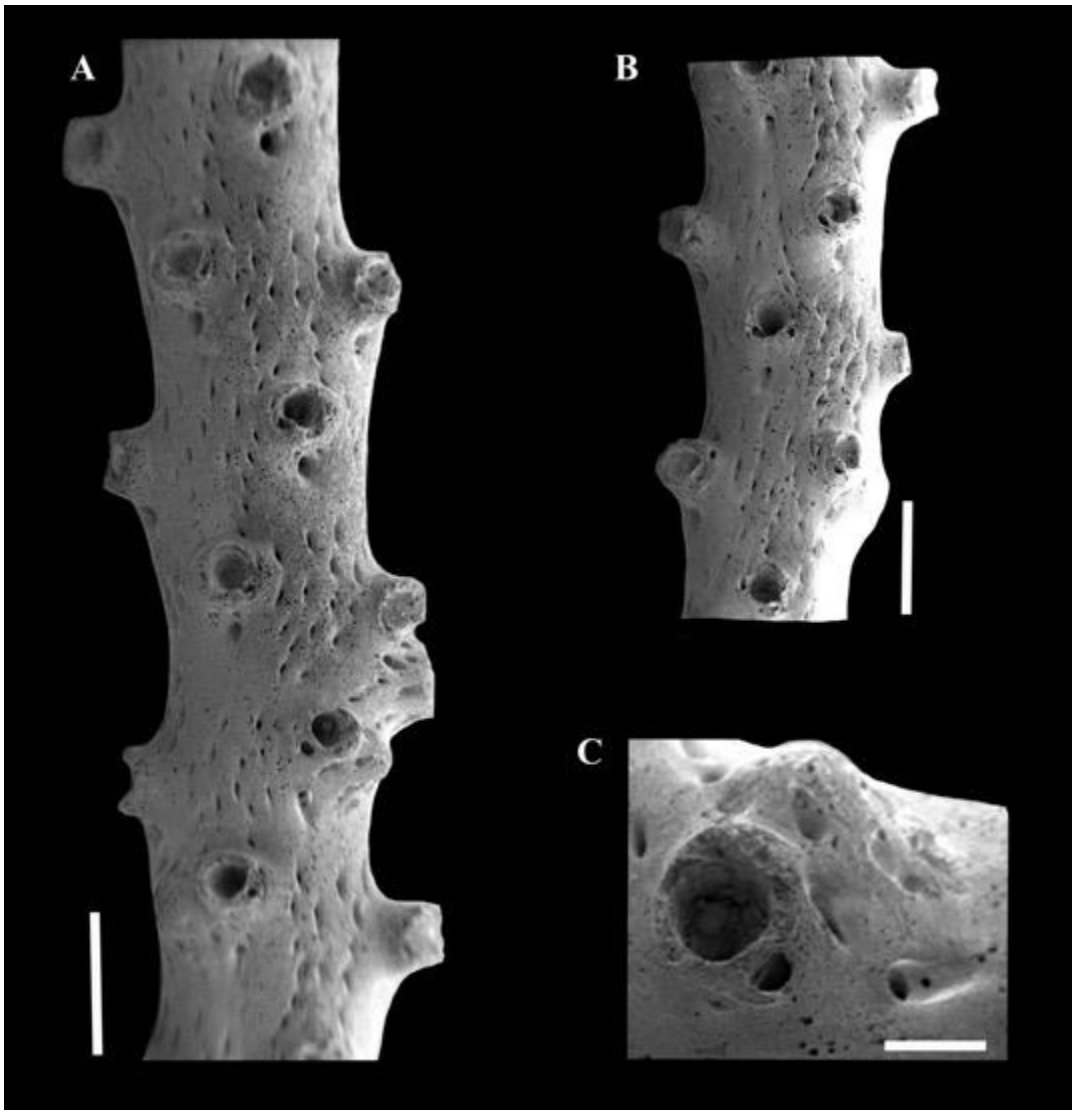

Figure 6. *Semihaswellia sinuosa*. A. General view of the colony, scale bar: 100µm B. Detail of zooid organization in the colony, scale bar: 100µm C. Detail of ovicell and avicularia, scale bar: 50µm.

Material examined. INVBRY 2123, INVBRY 2129.

Superfamily Celleporoidea Johnston, 1838

Family Colatooeciidae Winston, 2005

Genus *Trematooecia* Osburn, 1940

***Trematooecia gemmea* (Winston & Woollacott, 2009)**

(Fig. 7)

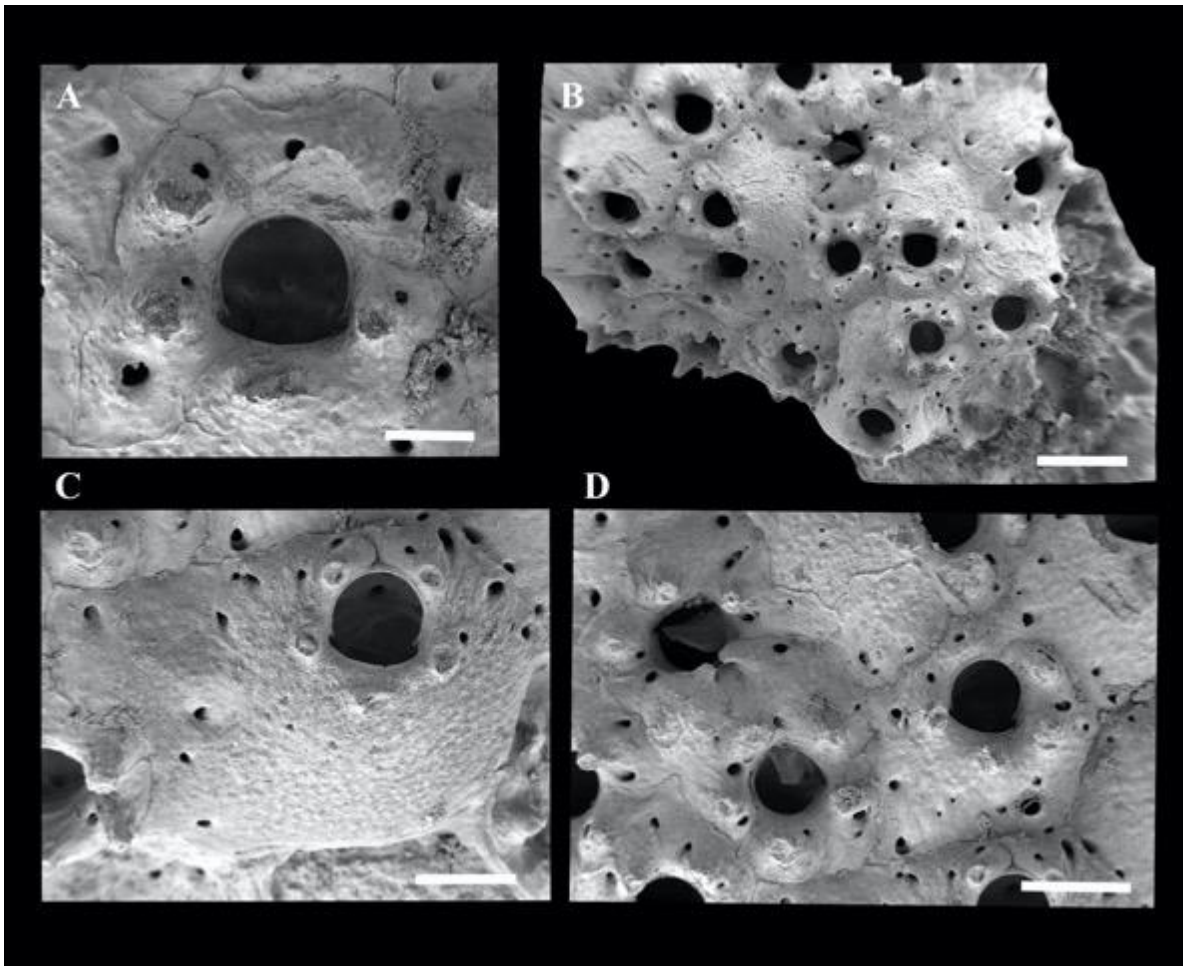

Figure 7. *Trematooecia gemmea* (Winston & Woollacott, 2009). A. Detail of opesia with condyles and ornamentation, scale bar: 50µm B. General view of the encrusting colony, scale bar: 100µm C. Detail of zooids with pores in the interzooidal zone, scale bar: 100µm, D. Zooid arrangement showing different growing directions, scale bar: 100µm.

Material examined. INVBRY 2134.

Family Phidoloporidae Gabb & Horn, 1862

Genus *Plesiocleidochasma* Soule, Soule & Chaney, 1991

***Plesiocleidochasma* sp.**
(Fig. 8)

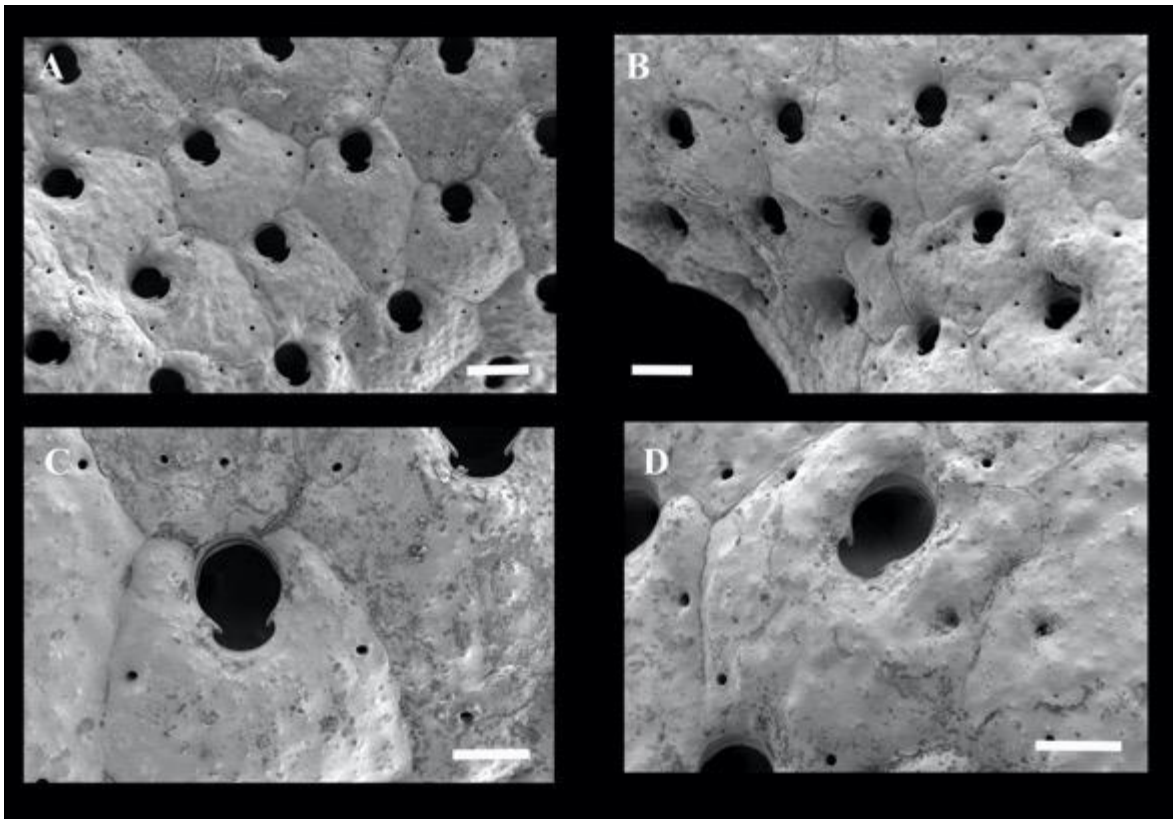

Figure 8. *Plesiocleidochasma* sp. A. General view of the colony, scale bar: 100µm B. Detail of zooid arrangements in the curved colony, scale bar: 100µm C. Detail of opesia and zooid shape, scale bar: 100µm, D. Detail of zooid and opesia with condyles, scale bar: 50µm

Material examined. INVBRY 2136, INVBRY 2138.

Family Celleporidae Johnston, 1838

Genus Turbicellepora Ryland, 1963

***Turbicellepora pourtalesi* Winston, 2005**

(Fig. 9)

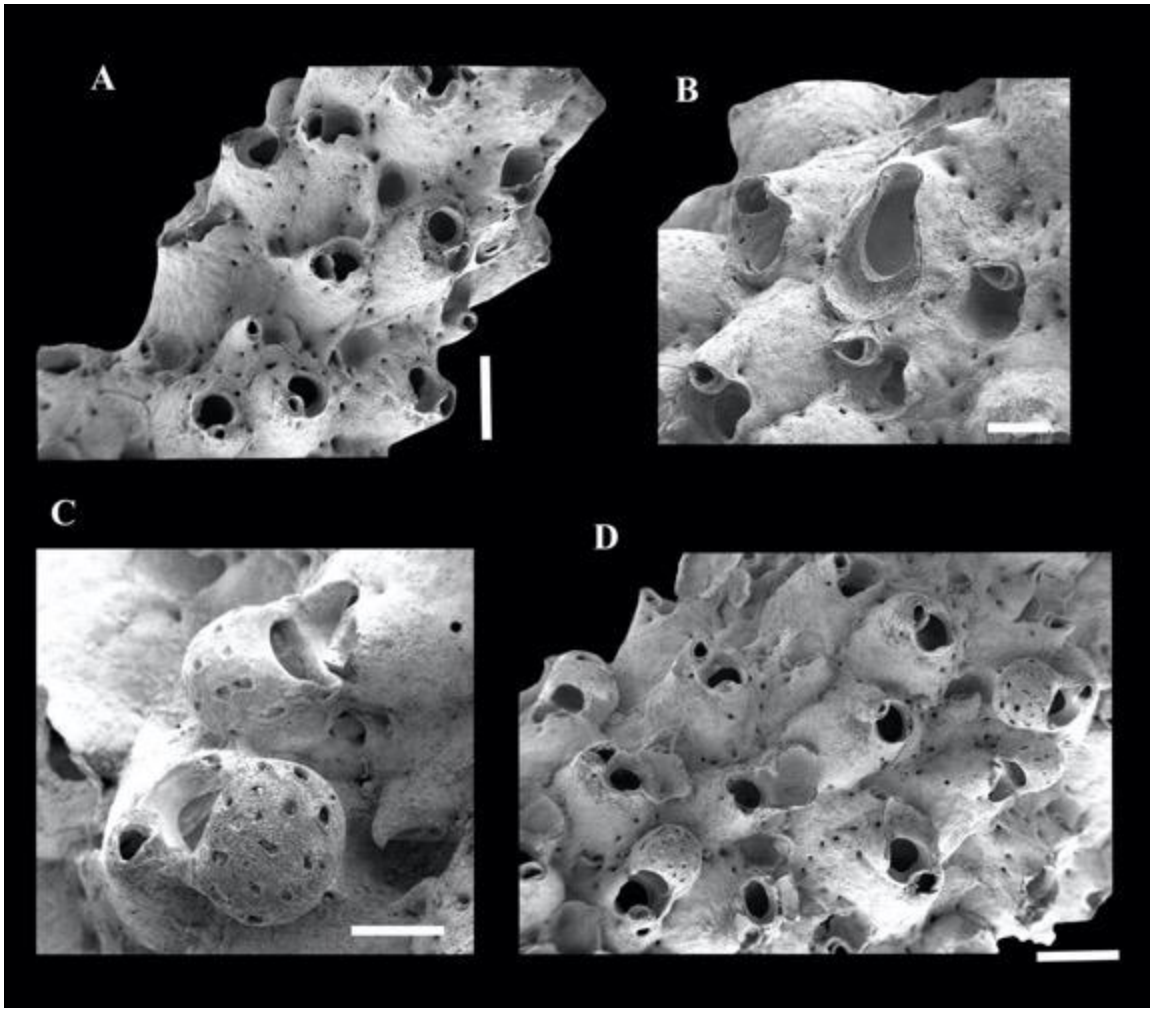

Figure 9. *Turbicellepora pourtalesi* Winston, 2005. A. General view of the colony, scale bar: 100µm B. Detail of suboral avicularia and large spatulated interzooidal avicularium, scale bar: 100µm C. Detail of perforated ovicells, scale bar: 100µm, D. Detail of the colony with zooid arrangement, scale bar: 100µm.

Material examined. INVBRY 2145.

Class Stenolaemata Borg, 1926

Order Cyclostomatida Busk, 1852

Suborder Tubuliporina

Family Oncousoeciidae Canu 1918

Genus *Oncousoecia* Canu, 1919

***Oncousoecia dilatans* (Johnson, 1847)**
(Fig. 10)

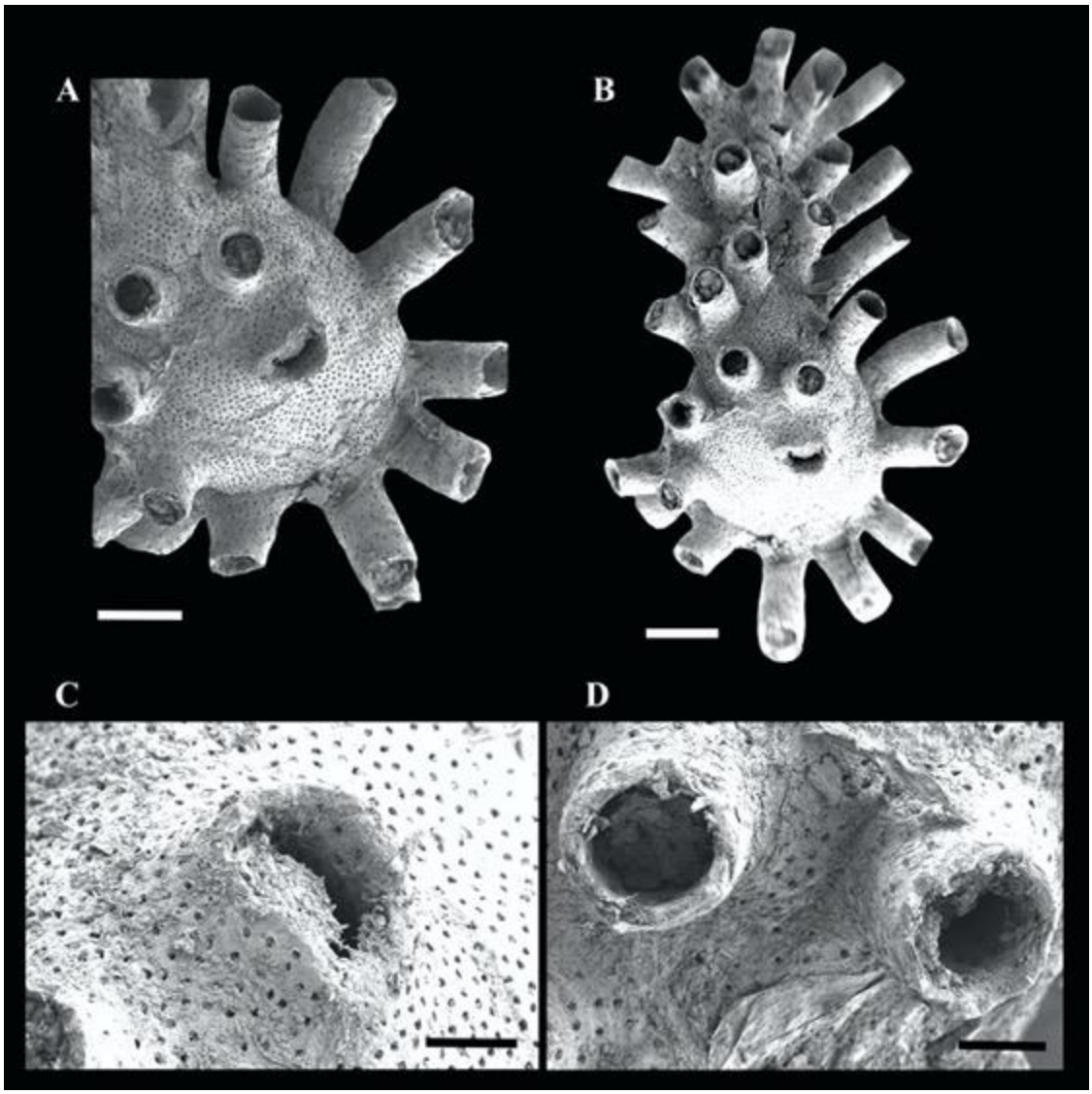

Figure 10. *Oncousoecia dilatans* (Johnson, 1847). A. Detail of colony with gonozooid, Scale bar: 100µm B. General view of the colony, scale bar: 100µm C. Detail of gonozooid opening, scale bar: 50µm D. Detail of zooid peristome, scale bar: 50µm.

Material examined. INVBRY 2147.
Family Stomatoporidae Pergens & Meunier, 1886

Genus *Stomatopora* Bronn, 1825

***Stomatopora* sp.**
(Fig. 11)

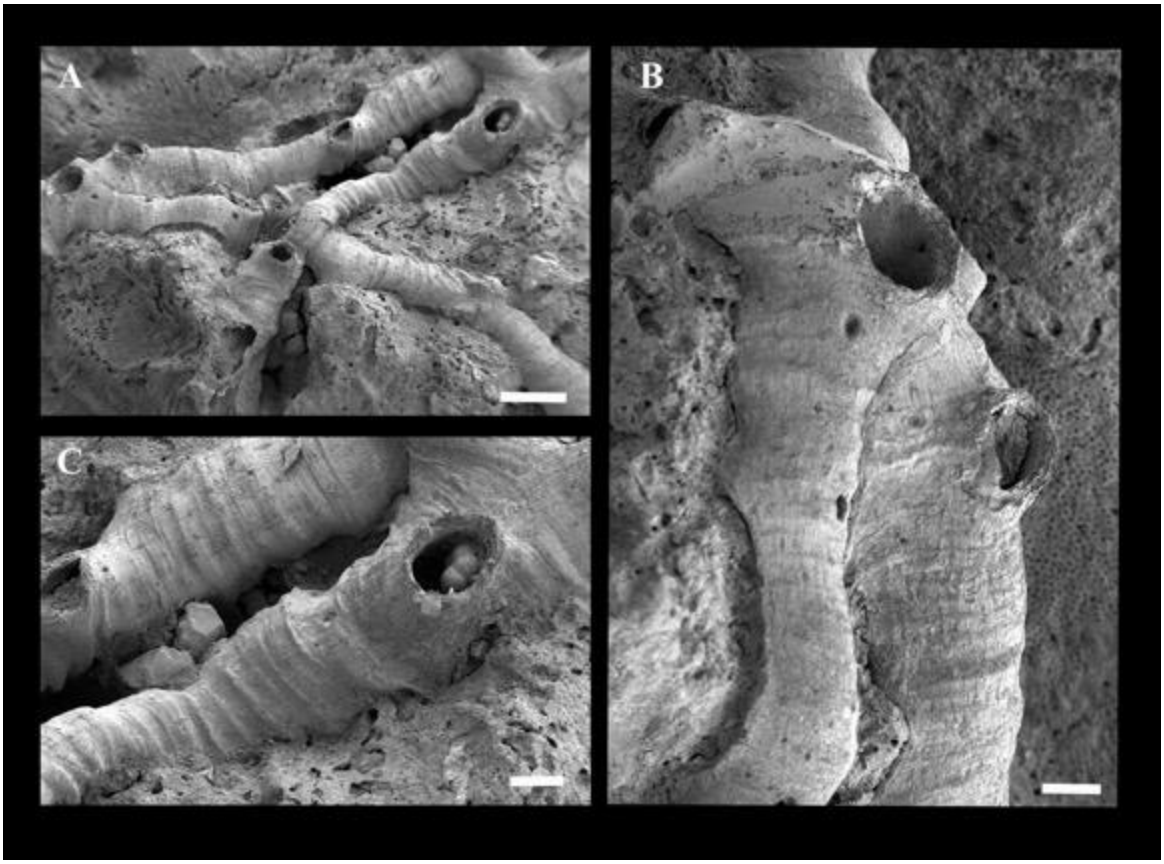

Figure 11. *Stomatopora* sp. A. General view of the encrusting colony, scale bar. 100µm B. Detail of the zooids, scale bar 250µm C. Detail of a zooid, scale bar: 100µm.

Material examined. INVBRY 2143.

Chapter 3: Bryozoan distribution and its relationship with environmental conditions in the Colombian Caribbean

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ABSTRACT

The environmental conditions that determine the extent of occurrence of bryozoans are poorly known in the Caribbean. We made an extensive ecological review of bryozoans collected over a 15-year period in the Colombian Caribbean to determine the relationship between their distributions and environmental variables. Bryozoans were divided based on their growth forms (encrusting, erect and free living) and then grouped by type of sediment, location and depth. We found that erect flexible forms are dominant in the 1-10m depth range associated to hard substrates, while the 50-200m depth range was dominated by erect rigid forms associated with biological substrates and, the 1000-3888m depth range was dominated by delicate flexible-articulated forms attached to soil grains. To better understand these bathymetric trends, four species were selected based on their potential of bioconstruction and growth form, *Adeonellopsis subsulcata*, *Steginoporella magnilabris*, *Halophila antillea* and *Margaretta cereoides* and their presence records were compared with environmental data. Our results showed a relationship between the relative abundance, mineral composition and growth with depth and temperature. The range 50-450m depth, presented the greatest assemblages of calcite-composed species with larger colonies than in shallower areas. The range 1500-3888m depth has more calcite-Mg composed species. This is the first study reporting environmental relationships with bryozoans from 1 to 3888m in the Colombian Caribbean.

Keywords: Environmental variables; Offshore research; growth type; Bioconstruction

INTRODUCTION

The relationship between environmental conditions and marine benthic fauna plays an important role in determining community structure and ecological functioning and distribution (Donnarumma et al., 2019; Arribas et al., 2014; Lloret & Marín, 2009). The occurrence, species richness, spatial distribution and relative abundance of calcified species are determined by the interaction of both biotic and abiotic variables (Vertino et al., 2010; Roberts et al., 2009). For example, temperature, salinity, primary productivity and flow are some of the major drivers in the distribution of corals, sponges, bryozoans and ascidian species worldwide (Eidens et al., 2014; Orejas et al., 2009).

A great number of marine bioconstructing species like bryozoans have, to some extent, a bathymetrical gradient distribution (Brown & Thatje, 2014; Pradillon & Gaill, 2007) which in some species is associated with morphological variations or polymorphisms (Figuerola et al., 2017; Fusco & Minelli, 2010). A reduction of some phenotypic features with increasing depth (Stepien et al., 2017; Hageman & Todd, 2014) is a consequence of limited food availability due to the reduced water flow (Crook et al., 2013). Bryozoan species found at abyssal zones are characterized as presenting delicate and less calcified colonies as an adaptative strategy to the low motion environments (Grischenko et al., 2019; Kaandorp, 1999) and due to carbonate corrosion because of the interaction with low pH caused by the oxidization of carbon dioxide (Palmer, 2009; Anderson et al., 2008).

Bryozoans are conspicuous and diverse in most marine environments with a broad tolerance of salinity and temperature (Amini & Rao, 2004; Taylor & Allison 1998). They are great carbonate producers in high primary productivity environments like upwelling zones, where their growth rate is faster, and the mineral composition of their skeletons is more complex (O'Dea et al., 2007). Typically, in those cases, bryozoans have bigger colony sizes and create habitat complexity for other organisms (Bastos et al., 2018; Wood et al., 2012; Yepes-Narváez. 2013; O'Dea et al., 2007).

The ecological information of bryozoans in the Colombian Caribbean is scarce as most studies have focused mainly on the biodiversity composition (Gracia et al., 2018; Cedeño-Posso et al., 2017; Delgadillo & Fórez, 2015; Flórez et al., 2007) and

updating species checklists (Montoya-Cadavid et al., 2007). However, the information associated with those findings has been key to understand the structure of the assemblages in the Colombian Caribbean (Montoya-Cadavid et al., 2010). To date, 202 species of bryozoans have been described for the continental shelf and upper slope of Colombia belonging mainly to Cheilostomatida (60%), which are particularly conspicuous in the northern part of the Caribbean, in La Guajira where a natural continuous upwelling phenomenon occurs (Corpoguajira & Invemar, 2012; Invemar, 2010). In this area, bryozoan colonies are bigger and more abundant than in the rest of the country (Yepes-Narváez, 2013).

This study aimed to Identify the bryozoans with high potential of bioconstruction and possible habitat in the Colombian Caribbean by re-analysing museum reference collections. We then compared the spatial and bathymetric distribution of the species found with environmental variables taken *in situ* and identified patterns of species distribution.

METHODS

Study area

The continental margin of the Colombian Caribbean is defined by a distinct topography with depths down to 3500m (Lopez, 2005), and an abyssal zone with the deepest isobath recorded at 4220 m (Invemar, 2018).

Due to its geographical location, the area presents both dry and wet seasons and a strong wind system that regulates most of the environmental dynamic of the area (Invemar, 2010). The northernmost part of its coastline experiences a constant oligotrophic upwelling characterized by decreasing surface temperature and increasing primary productivity (Manjarrés *et al*. 2005). During the wet season, environmental variables are modified by the continental runoff, mainly from the Magdalena River, that shapes the geography of the seabed and discharges tons of suspended particulate organic matter (POM), causing lower salinity levels in the Colombian Caribbean sea compared to the North Atlantic (Invemar 2006, Posada & Henao, 2006). Those fluctuations, together with the flow systems, contribute to the composition and spatial distribution of the Colombian marine biodiversity

(Invemar, 2010). Over 1000 samples were collected along the Colombian Caribbean Sea during both climatic seasons at 130 stations with contrasting topography (Figure 1), environmental features and depths (Suppl. I).

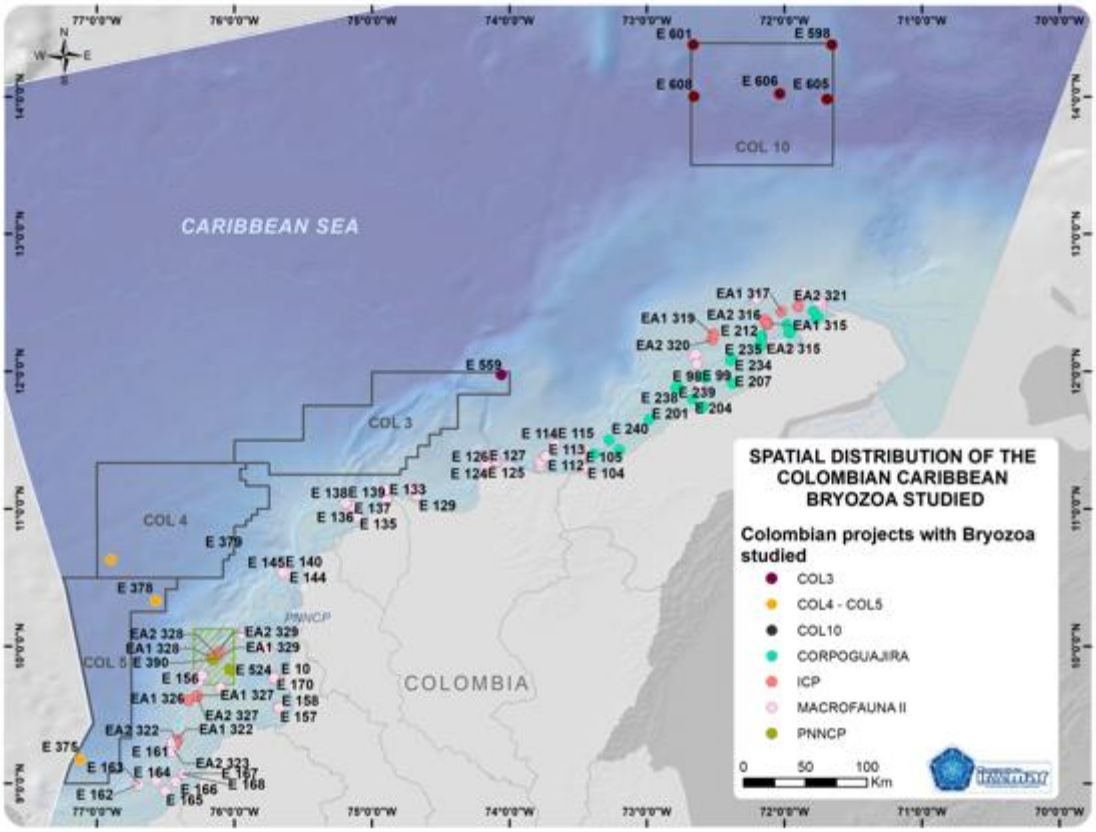

Figure 1. Map of the Colombian Caribbean with the sampling stations with bryozoan samples.

Material examined

This study included both, museum and fresh bryozoan material. The historical samples were collected from 2002 to 2017 during several research expeditions carried out by the Marine and Coastal Research Institute - INVEMAR in partnership with several national organisations and using different methods, such as trawling, dredges, benthic grabs, piston corers and remote operated vehicles - ROVs. Those samples were preserved in 70% ethanol and are part of the reference collection of the Marine Natural History Museum of Colombia – MHNMC under catalogue numbers INVBRY-001 – INVBRY-1985.

Additional biological material was collected by the author between 2017 and 2018 at 32 coastal ecosystem stations (submerged mangrove roots, seagrass meadows and rocky shores) along the Colombian Caribbean from 1 m to 5 m depth, using free diving and scuba diving. Half of the samples were preserved in 70% ethanol spirit for taxonomic identification and the other half in molecular grade ethanol for further genetic studies. A copy of this material was deposited as part of the coastal bryozoan reference collection of the MHNMC.

Taxonomic identification

Fresh and historical samples were separated by morphotypes. Some specimens were cleaned with a solution of Sodium Hypochlorite (10%) and rinsed with Hydrogen Peroxide (4%) and distilled water to remove tissue residues and facilitate subsequent microscopic review, identification and photographic recording. Taxonomic identification was performed based on Hayward and Ryland (1985) for Stenolaemata; Hayward (1985), Winston (1982) and Winston and Woollacoot (2009) for Ctenostomata; and for Cheilostomata Canu and Bassler (1928), Osburn (1940), Winston (1982, 1984, 1986, 2005), Soule *et al.* (1995), Hayward and Ryland (1998, 1999); Flórez and Montoya-Cadavid (2004); Flórez *et al.* (2007); Montoya-Cadavid *et al.* (2007); Montoya-Cadavid and Flórez (2010) and Vieira *et al*. (2008, 2010, 2012).

As a support for taxonomic description, three colonies of every species were selected from which 10 zooids of each were measured, using Scanning Electron Microscopic (SEM) photographs and ImageJ software 1.45s, Java 1.6 .0-20 (64 bit). The identified material was entered into the Museum of Marine Natural History of Colombia – MHNMC and the Marine Biodiversity Information System database - SIBM.

Analysis of the general species richness and relative abundance per depth, sediment and geographic range were carried out, and four representatives of bio-constructing and habitat-forming bryozoan species were selected and are described in this study based on their growth form, size and relative abundance as well as observed biocoenosis, *Adeoneollopsis subsulcata*, *Steginoporella magnilabris*, *Halophila antilleae* and *Margaretta cereoides.*

Carbonate mineralogy of bryozoan species

We performed mineralogical analyses to determine the main mineral composition of the four bryozoan species selected at the Imaging and Analysis Centre (MMU) using semi-quantitative X-ray diffractometry (EDAX). Three sections of the colonies were selected, at the bottom, middle and tips and three replicates measurements were made of each bryozoan section. In addition, we gathered secondary information from the literature (Smith et al., 2006; Cairn & Macintyre, 1992) (Figure 2; Table 1

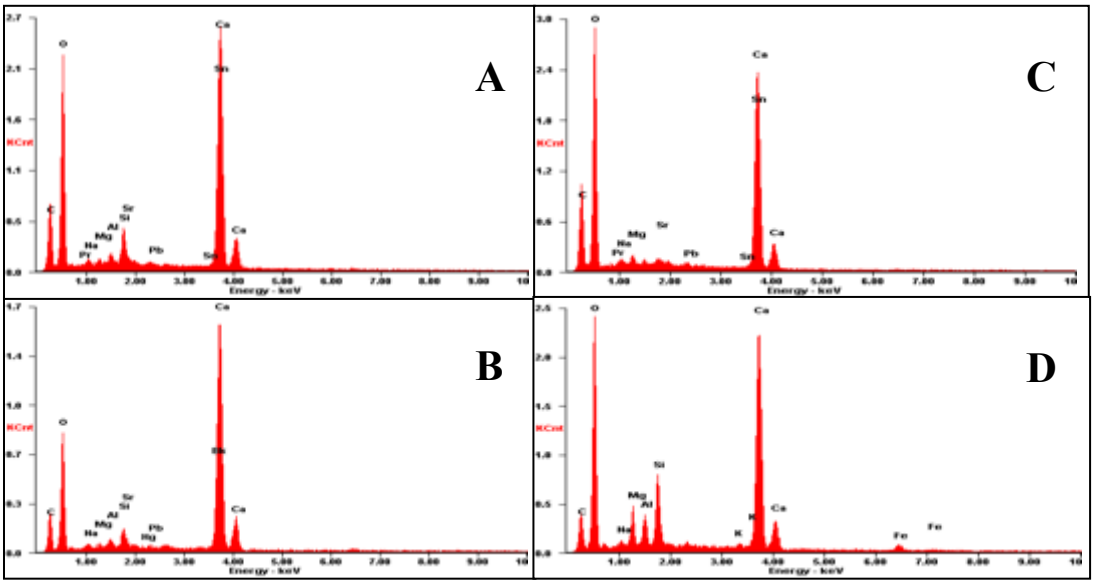

Figure 2. Skeletal diffractometry (EDAX) found in three replicates of A) *A. subsulcata*, B) *S. magnilabris*, C) *M. cereoides* and D) *H. antillaea*

Table 1. Mineral information of the four bryozoans selected. Information obtained both from A) literature and B) measured in this study.

A		***Wt% Calcite***				***Wt%MgCO3***				***Reference***
Species	**Mineralogy**	**Mean**	**Min**	**Max**	**Range**	**Mean**	**Min**	**Max**	**Range**	
Margaretta cereoides	Aragonite	15.3	12.0	22.0	10.0	8.3	7.8	8.5	0.7	Cairns & Macintyre, 1992
	High Mg/Calcite									Smith et al., 2006
Adeonellopsis	Aragonite	0.0	0.0	1.0	1.0	1.0				Smith et al., 2006
	Low Mg/Calcite									Smith et al., 2006

B	*Adeonellopsis subsulcata*		*Steginoporella magnilabris*		*Margaretta cereoides*		*Halophila antillaea*	
Element	***Wt%***	***At%***	***Wt%***	***At%***	***Wt%***	***At%***	***Wt%***	***At%***
CaK	28.83	14.01	25	11.6	22.57	10.17	16.63	8.04
SiK	2.25	1.56			1.68	1.08	10.08	6.96
SrL	1.07	0.24	1.02	0.22				
AlK	0.88	0.63			0.89	0.59	5.06	3.63
SnL	0.86	0.14	0.68	0.11				
PbM	0.75	0.07	0.96	0.09	0.26	0.02		
NaK	0.55	0.47	0.85	0.68	1.36	1.07		
MgK	0.32	0.25	1.02	0.78	3.39	3.29	5.1	5.47
ClK					0.32	0.16		
FeK							2.92	1.01
KK							0.66	0.33
HgM							0	0

wt% means weight percent; At% means atomic percent

Environmental variables

Environmental data measured at the time of collection were Temperature (C), Salinity (PPT), Dissolved oxygen (ml l^{-1}), sediment type and Depth (m) to determine the relationship between those variables and the distribution of bryozoans. The acquisition of historical environmental variables differed among expeditions, because the methods implemented varied. For 10 years CTDO equipment was deployed to obtain temperature, salinity and conductivity parameters and from 2012 CTDO was surrounded by a rosette of Niskin bottles for nutrient measurement, chlorophyll, nitrates, nitrites and phosphates. Recent expeditions implemented additional measurement of deep current systems ADSP and pCO2 probes for the measurement of partial pressure of carbon dioxide in the water. But, as this last information was only possible to obtain from the deep-sea stations COL3, COL4-5 and COL10 we could not compare this information with the other shallower stations (Suppl. II).

Map development

The distributions maps were made using the bryozoan database at the Information's System lab - Labsis at Invemar using ArcGIS. The distribution of bryozoans was evaluated by determining the species extent of occurrence in the sampled stations. For these analyses, sectorization by sub-regions (North, Middle and South Caribbean), bathymetry (1 to 3888 m) and the type of sediment (Sands and Muddy sands, Mud, Rocky bottoms) were followed in order to determine if there was any distribution pattern with respect to these factors (Figure 1).

Statistical analyses

To analyse if temperature, salinity, sediment type and location have an effect on the relative abundance of the 4 species, we performed a generalized linear model (glm), graphics are presented here; significant values were considered as below 0.001. Analyses were run in RStudio.

RESULTS

Bryozoan richness and growth forms

Between 1 and 3888m depth, 87 families, 78 genera and 203 bryozoan species were reviewed and re-identified. Our findings showed that Gymnolaemata (72 families) is the most conspicuous class in the area with representatives of the orders Ctenostomatida (10spp) and Cheilostomatida (162spp), this last one with higher number of families found (61) and 78% of the species. Stenolaemata on the other hand is the least representative class of bryozoans with four families belonging to the order Cyclostomatida (31spp). Some deep-sea species remain under study due to the lack of taxonomic reference for the area at those depths (Suppl. VII). The species presented three main growth forms, erect, encrusting and free-living (Table 2)

Table 2. List of species with their growth forms found in the Colombian Caribbean.

Erect			Encrusting			Free living
Rigid	**Articulated**	**Flexible**	**Massive**	**Membraniporiforme**	**Stolon**	**Lunulitiform**
Biflustra arborescens	*Bugula neritina*	*Amathia distans*	*Celleporaria* albirostris	*Antropora leucocypha*	*Aetea anguina*	*Cupuladria panamenesis*
Adeonellopsis subsulcata	*Canda simplex*	*Amathia vidovici*	*Disporella* cf. *pristis*	*Beania americana*	*Aetea ligulata*	*Cupuladria surinamensis*
Cosciniopsis violacea	*Crisia denticulata*	*Bugula stolonifera*	*Plagioecia* cf. *patina*	*Biflustra arborescens*	*Aetea truncata*	*Discoporella* cf. *marcusorum*
Labioporella dumonti	*Filicrisia* sp.	*Catenicella contei*	*Rhynchozoon spicatum*	*Biflustra denticulata*	*Beania australis*	*Mamillopora cupula*
Mecynoecia delicatula	*Gemelliporina glabra*	*Savinyella lafonti*	*Cigclisula turrita*	*Bryopesanser pesanseris*	*Beania klugei*	
Metrarabdotos sp.	*Licornia* joloisii	*Halophila antillaea*		*Celleporella carolinensis*	*Beania maxilladentata*	
Nevianipora floridiana	*Margaretta buski*			*Drepanophora tuberculata*	*Beania mirabilissima*	
Reteporellina evelinae	*Margaretta cereoides*			*Escharina porosa*	*Hippothoa flagellum*	
Steginoporella magnilabris	*Nellia oculata*			*Exechonella antillea*	*Nolella stipata*	
Stylopoma projecta	*Pasythea tulipifera*			*Floridina antiqua*		
Reteporellina marsupiata	*Sinnotum aegyptiacum*			*Gemelliporida aculeata*		
	Tetraplaria dichotoma			*Hippaliosina rostrigera*		
				Hippomenella fissurata		
				Hippoporidra eddax		
				Jellyella tuberculata		
				Klugerella aragoi		
				Membranipora cf. *tenella*		
				Membranipora tenuis		
				Micropora coriacea		
				Microporella protea		
				Parellisina curvisostris		
				Petraliella bisinuata		
				Petraliella marginata		
				Pleurocodonellia signata		
				Poricella mucronata		
				Pourtalesella rugosa		
				Puellina radiata		
				Reginella floridiana		

To analyse the growth forms per bathymetric gradient in the Colombian Caribbean, we divided the area into five ranges (Figure 3), 1-50m depth was predominantly dominated by erect-flexible-articulated colonies, likely to be influenced by the strong current flows that characterize this zone, followed by the encrusting-membraniporid forms.

The range 50-200m depth was dominated by erect rigid-forms either unilaminar or multilaminar, especially in La Guajira (North) where the abundance of species was the highest for the country. The range 200-600m depth was dominated by encrusting-massive forms and erect-rigid like *C. albirostris*.

The range 600-1000m was less diverse and mainly colonised by slightly calcified species showing an erect-flexible or articulated form such as Bugulidae bryozoans. The range 1000-4000m, due to the lack of sampling effort, was the least diverse and the few species registered for the zone were predominantly Bugulidae with small erect-articulated or flexible forms (Figure 3).

The species with bigger colony size and with the highest bioconstruction complexity were, *A.subsulcata, S. magnilabris, S. projecta, S. smitti, C. violacea, B. arborescens, C. albirostris, M. cereoides, M. buski, G. glabra, R. marsupiata, R. evelinae, P. bisinuata, P. mucronata, C. simplex, L. jolloisii, B. neritina, A. vidovici, A. distans, H. antillaea, P. tulipifera, I. atlantica, E. bellula, N. ocullata, S. dumonti, C. uberrima, C. contei, C. denticulata* and *T. dichotoma* (Figure 4; 5)

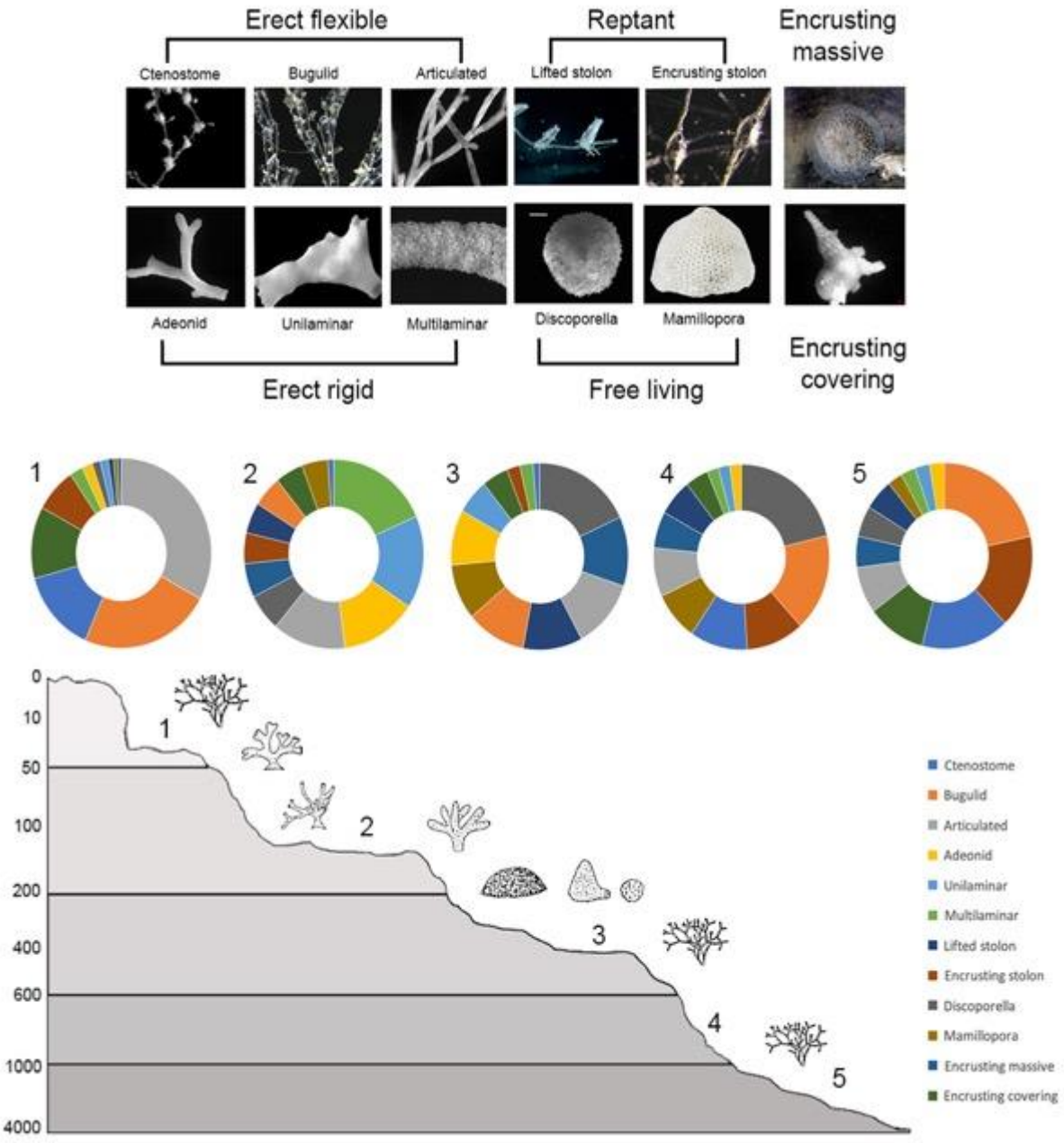

Figure 3. Illustration of the relative abundance of growth forms based on the bathymetric ranges set in this study for the Colombian Caribbean.

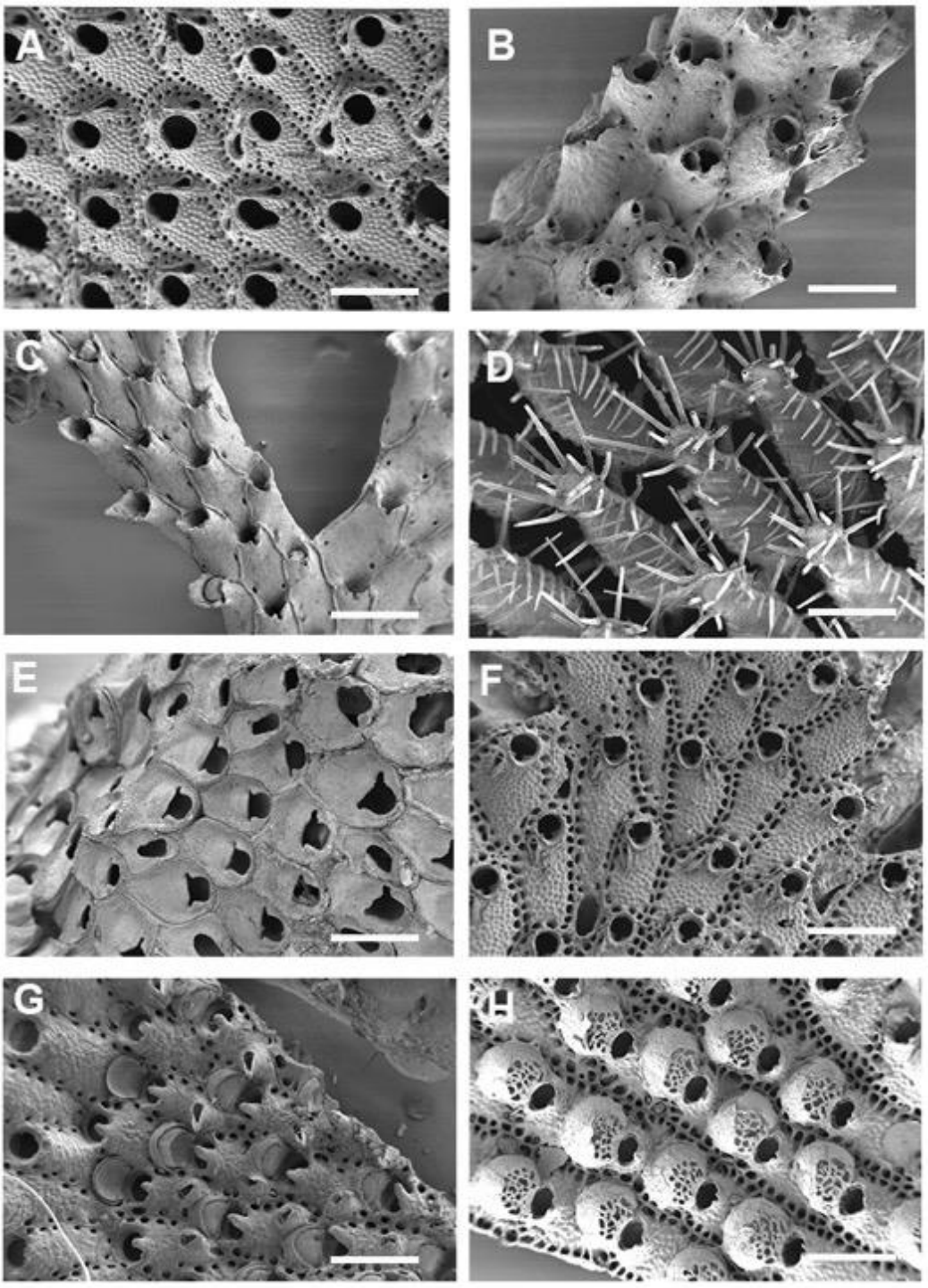

Figure 4. SEM photography of the most common bryozoans with different growth forms found along the Colombian Caribbean. A. *H. rostrigera*, B. *T. pourtalesi*. C. *R. marsupiata,* D. *B. Americana*, E. *F. antiqua*, F. *P. signata*, G. *R. spicatum*, H. *S. pacifica*. Scale bar: 100um.

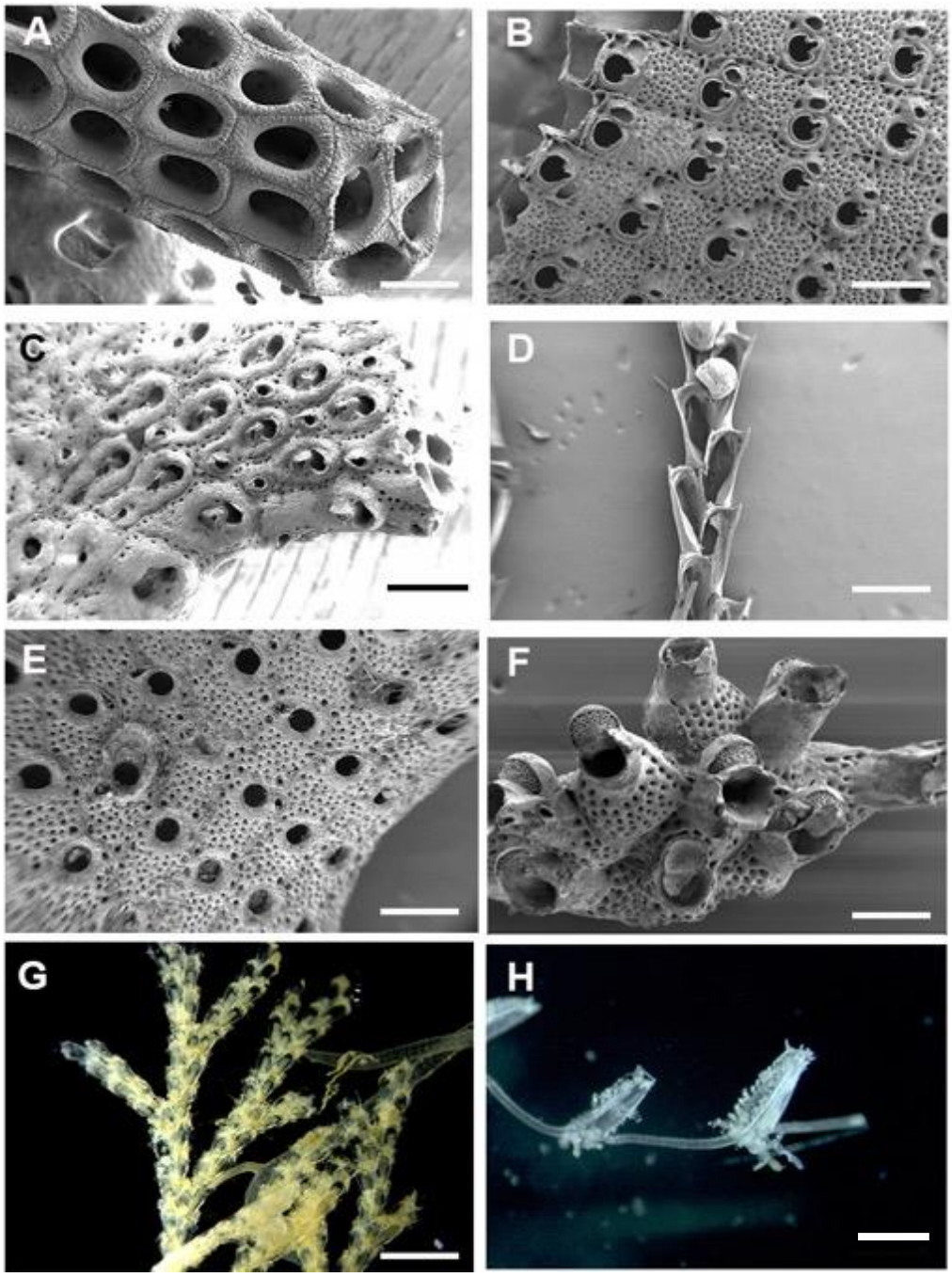

Figure 5. *continuation*. SEM photography of the most common bryozoans with different growth forms found along the Colombian Caribbean. A. *B.arborescens*, B. S*. projecta*, C. *A. subsulcata*, D. *B. neritina*, E. *C. violacea*, F. *C. calciformis*. G. *L. jolloisii*. H. *B. australis*. Scale bar: 100um.

Comparison to environmental conditions

From the samples reviewed in this study we selected four species (*A. subsulcata, S. magnilabris, M. cereoides* and *H. antillaea*) characterized by having a wide distribution in the Colombian Caribbean both geographically and across depth, and by their contrasting growth forms. Here, we compared their relative abundance in the three sections we divided our sampling area (North, Middle and South Caribbean).

Adeonellopsis subsulcata (Smitt, 1873) (Fig. 6)

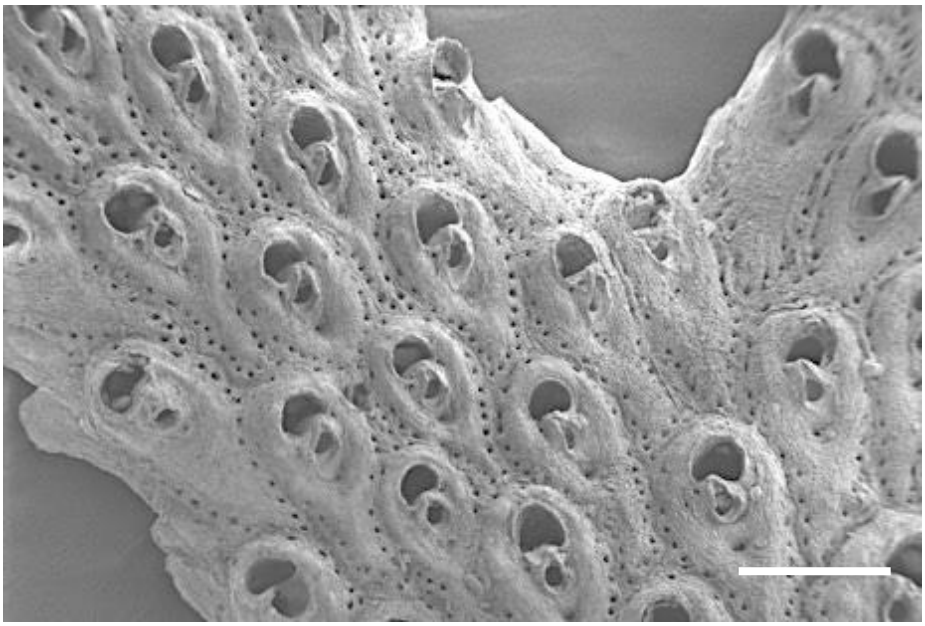

Figure 6. SEM photography of *A. subsulcata* found in the Colombian Caribbean. Scale bar: 100um.

Our results showed that the location did not have an effect on the relative abundance of *A. subsulcata* in the three areas of the Caribbean (p=0.002; Figure 7). In addition, the model showed that temperature (Suppl. III) and salinity (Figure 8) had a significant effect on the relative abundance of this species (p<0.001); higher at a salinity of 36.6 ppt and at 24.5C. The interaction of both factors did not have a significant effect on abundance (p=0.008).

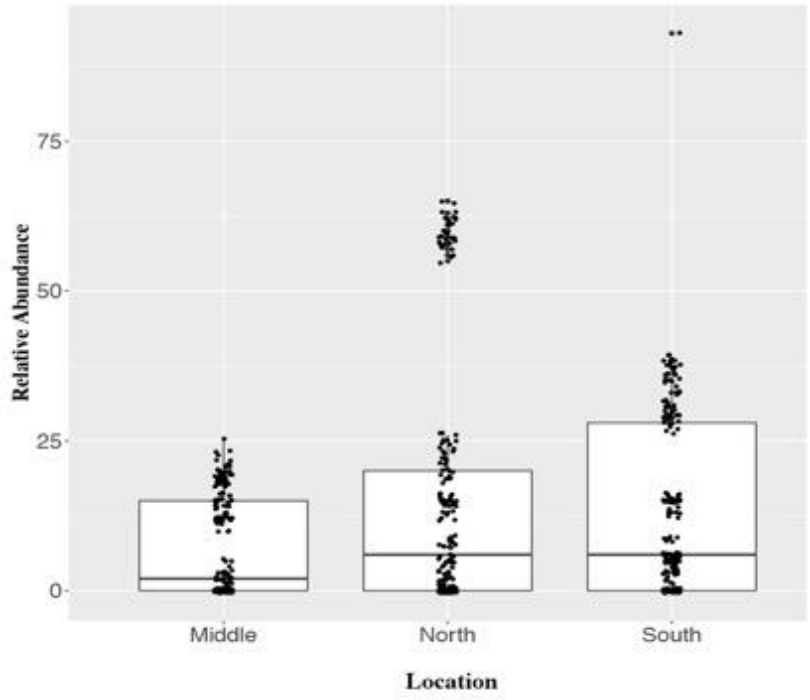

Figure 7. Relative abundance of *A. subsulcata* with respect the three locations in the Colombian Caribbean, showing a higher variance in the North but similar mean abundance with the South zone.

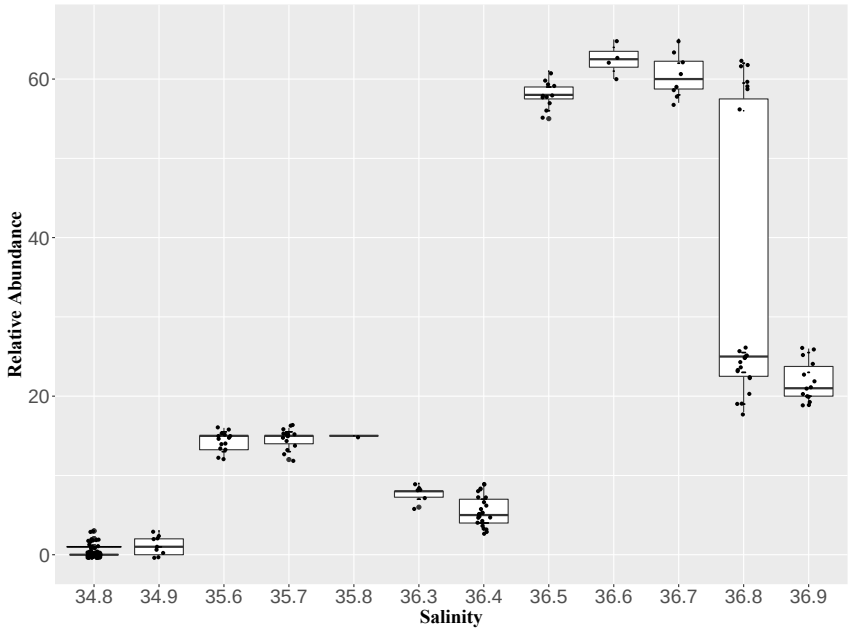

Figure 8. Relative abundance of *A. subsulcata* with respect the salinity in the Colombian Caribbean, showing a higher abundance after 36.5 ppt.

The abundance of *A. subsulcata* was affected by the sediment type ($p<0.001$) (Figure 9). Most colonies were found in mixed Mud-Rock and Mud-Sand stations. Also, the abundance was the highest at 80m depth ($p<0.001$) (Figure 10), and the interaction depth and temperature ($p<0.001$) but no interaction between depth and salinity ($p=0.042$).

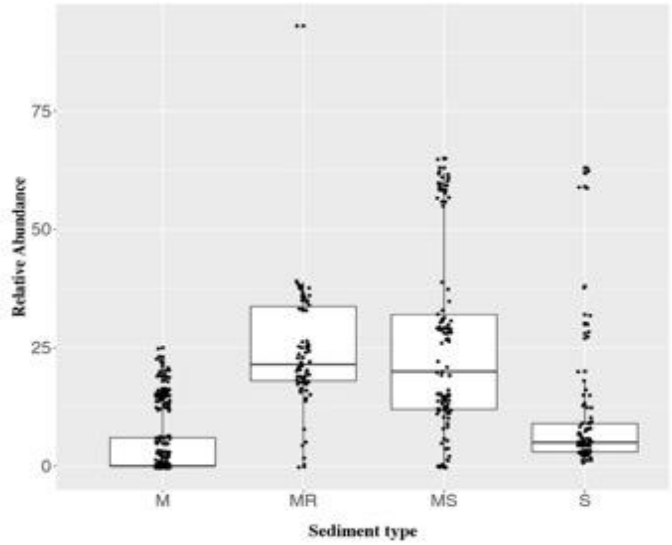

Figure 9. Relative abundance of *A. subsulcata* with respect the sediment type in the Colombian Caribbean, showing a higher variance in the Mud-sand type but similar mean abundance with the Mud-rock sediment.

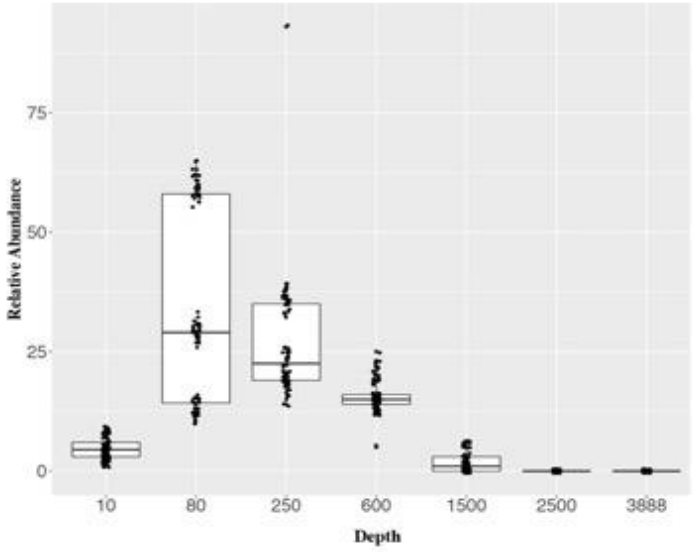

Figure 10. Relative abundance of *A. subsulcata* with respect to depth.

Steginoporella magnilabris (Busk, 1854) (Fig. 11)

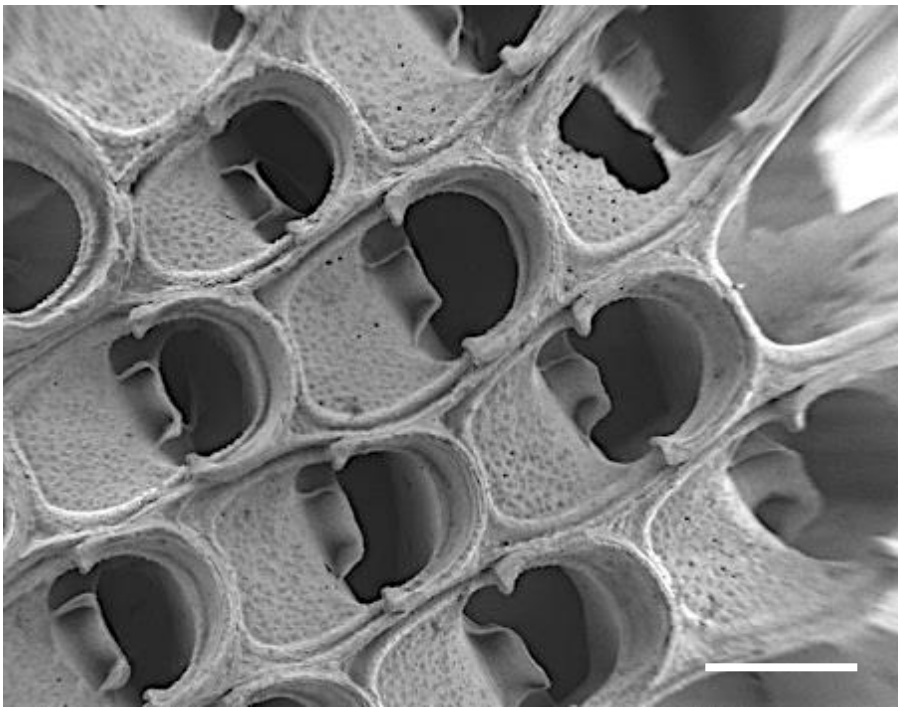

Figure 11. SEM photography of *S. magnilabris* found in the Colombian Caribbean. Scale bar: 100um.

Our results showed that the location had an effect on the relative abundance of *S. magnilabris* ($p<0.001$). It was high in the Middle zone and lower in the North (Figure 12). Temperature (Suppl. IV) had a significant effect on the relative abundance of this species ($p<0.001$), and the abundance was higher at 22.7C. Salinity (Figure 13) did not show significant effects on the relative abundance ($p=0.003$), but, the interaction of both factors with the location had a significant effect on the relative abundance ($p<0.001$).

The abundance of *S. magnilabris* was affected by the sediment type (Figure 14). Most colonies were found in mixed Mud-Rock and Mud-Sand stations ($p<0.001$). In addition, the abundance was higher at 250m depth showing an effect of the depth on the abundance ($p<0.001$) (Figure 15) and the interaction depth and temperature ($p<0.001$) but no interaction between depth and salinity ($p=0.004$).

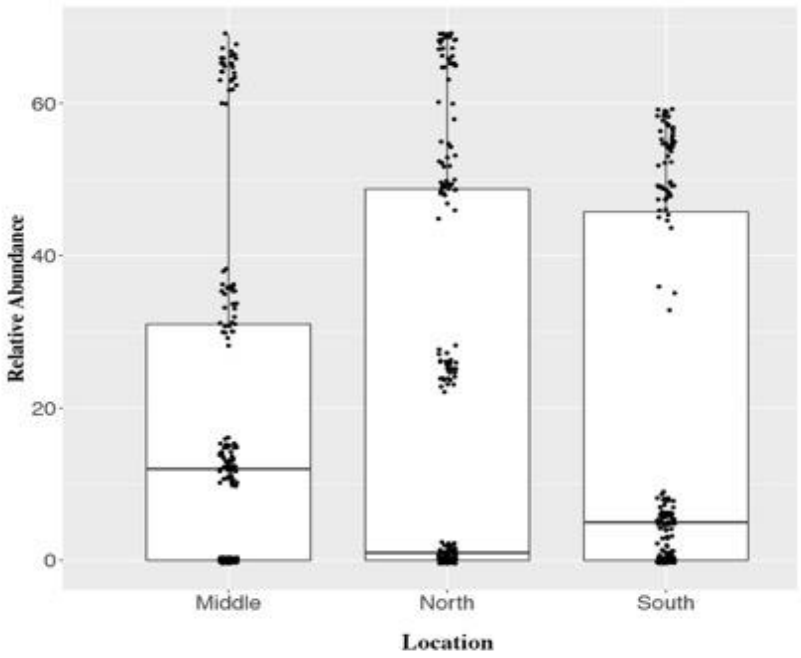

Figure 12. Relative abundance of *S. magnilabris* in the three locations in the Colombian Caribbean, showing a low abundance in the North relative to the Middle zone.

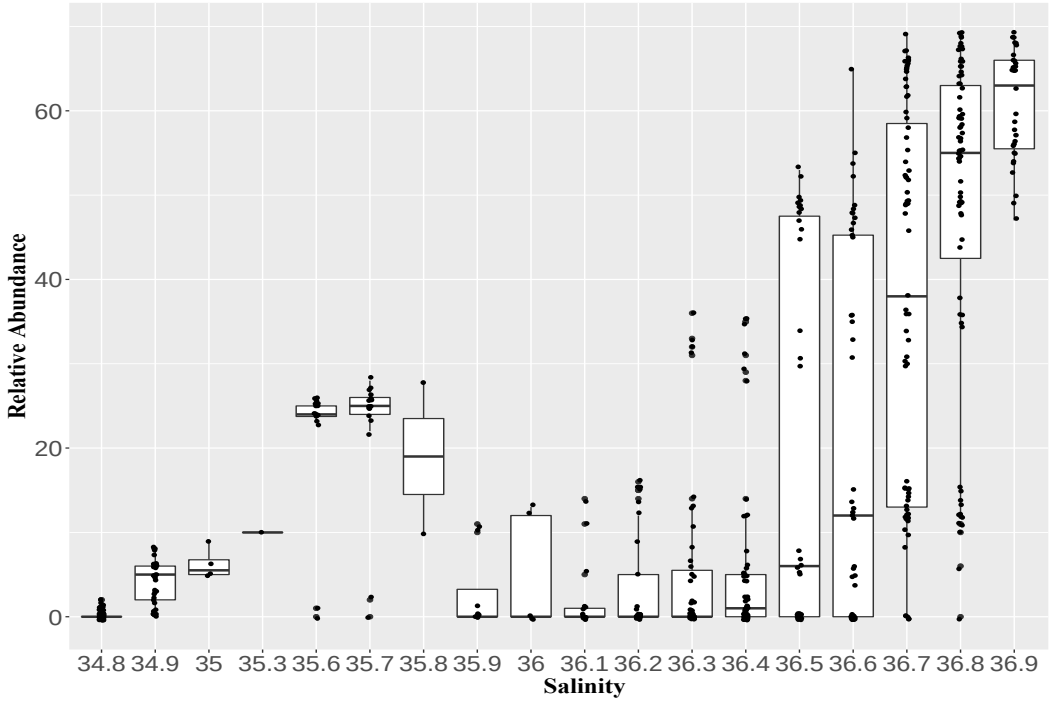

Figure 13. Relative abundance of *S. magnilabris* with respect the salinity in the Colombian Caribbean, showing a higher abundance after 36.9 ppt.

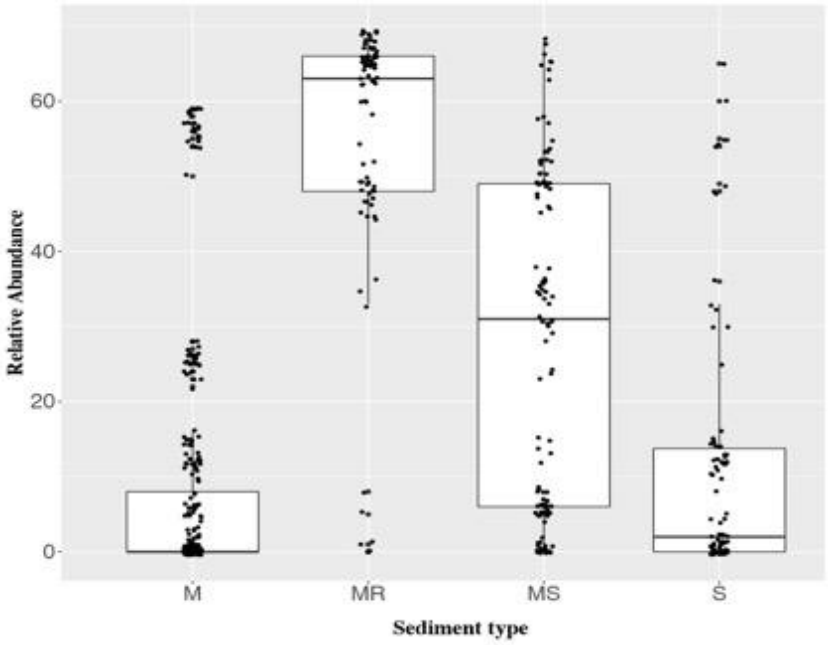

Figure 14. Relative abundance of *S. magnilabris* with respect the sediment type in the Colombian Caribbean, showing a high abundance in the Mud-Rock type.

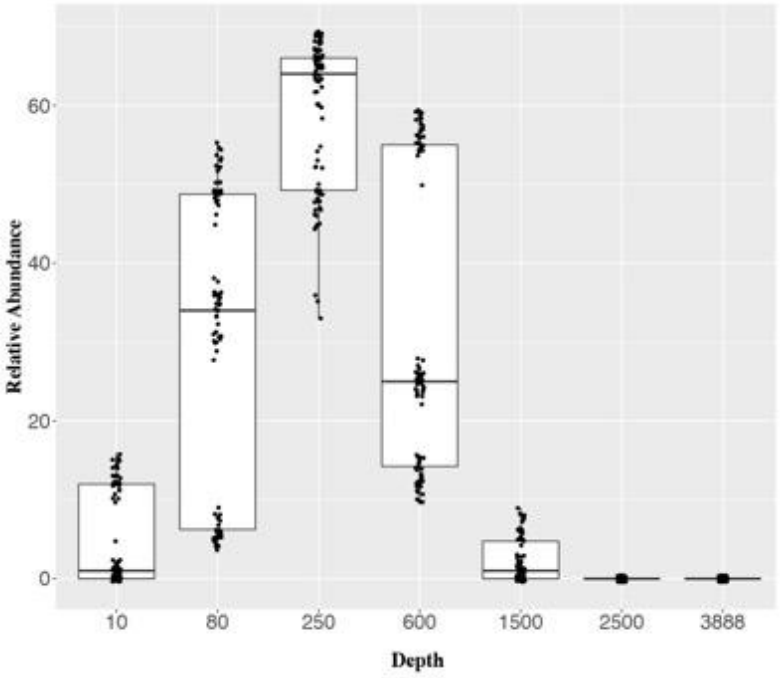

Figure 15. Relative abundance of *S. magnilabris* with respect the depth, showing higher abundance at 250m depth.

Margaretta cereoides (Ellis & Solander, 1786) (Fig. 16)

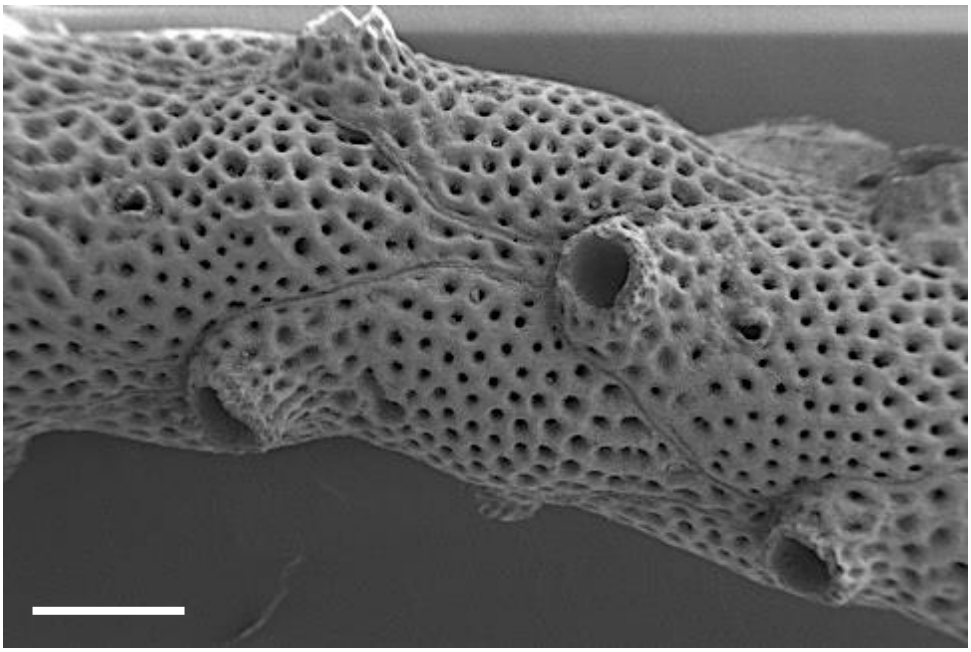

Figure 16. SEM photography of *M. cereoides* found in the Colombian Caribbean. Scale bar: 100um.

Our results showed that the location did not have a significant effect on the relative abundance of *M. cereoides* in the three areas of the Caribbean ($p=0.03$). The variation was high in the Middle zone, but the abundance was higher in the North (Figure 17). In addition, temperature (Suppl. V) had a significant effect on the relative abundance of this species ($p<0.001$), it was higher at 24C, but salinity (Figure 18) did not have a significant effect ($p=0.02$), although it was higher at a salinity of 36.9 ppt. The interaction of temperature and location had a significant effect on the relative abundance ($p<0.001$), but the interaction of salinity and location did not show significant effect ($p=0.07$).

Also, the abundance of *M. cereoides* was affected by the sediment type (Figure 19). Most colonies were found in mixed Mud-Rock and Mud-Sand stations ($p<0.001$). In addition, the abundance was higher at 80m depth showing an effect of the depth on the abundance ($p<0.001$) (Figure 20) and the interaction depth and temperature ($p<0.001$) but no interaction between depth and salinity ($p=0.006$).

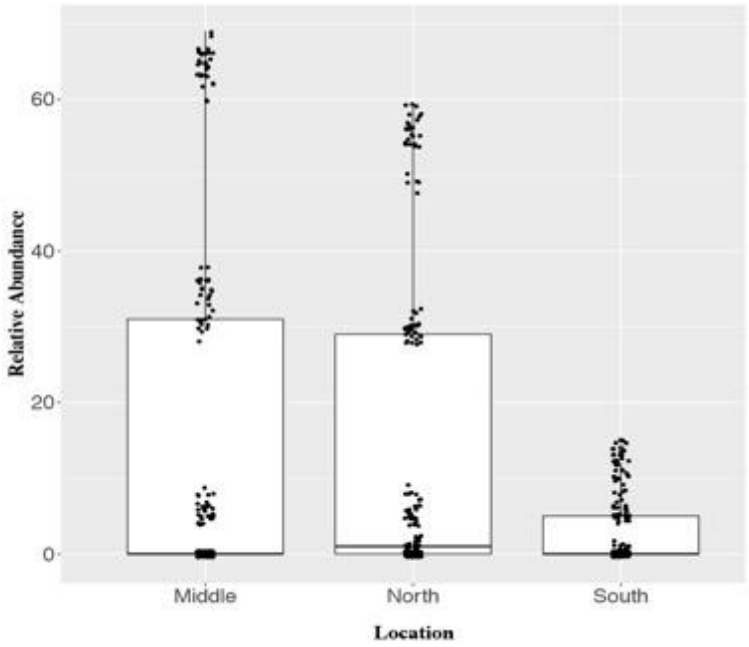

Figure 17. Relative abundance of *M. cereoides* with respect the three locations in the Colombian Caribbean, showing low abundance in all the zones.

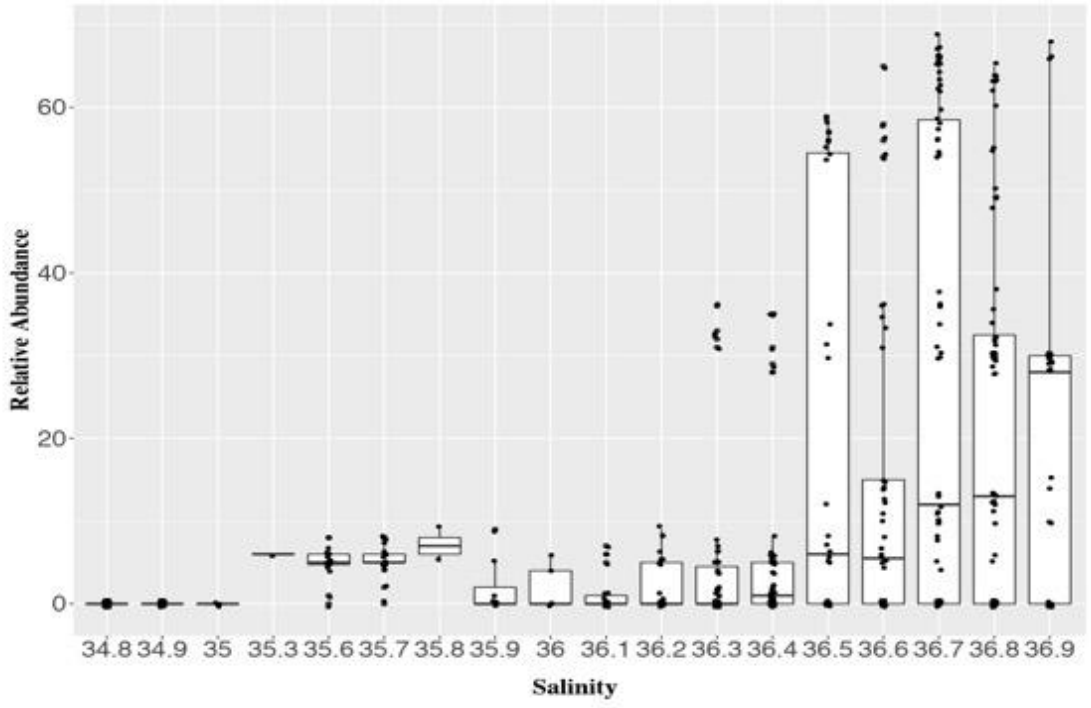

Figure 18. Relative abundance of *M. cereoides* with respect the salinity in the Colombian Caribbean, showing a higher abundance after 36.9 ppt.

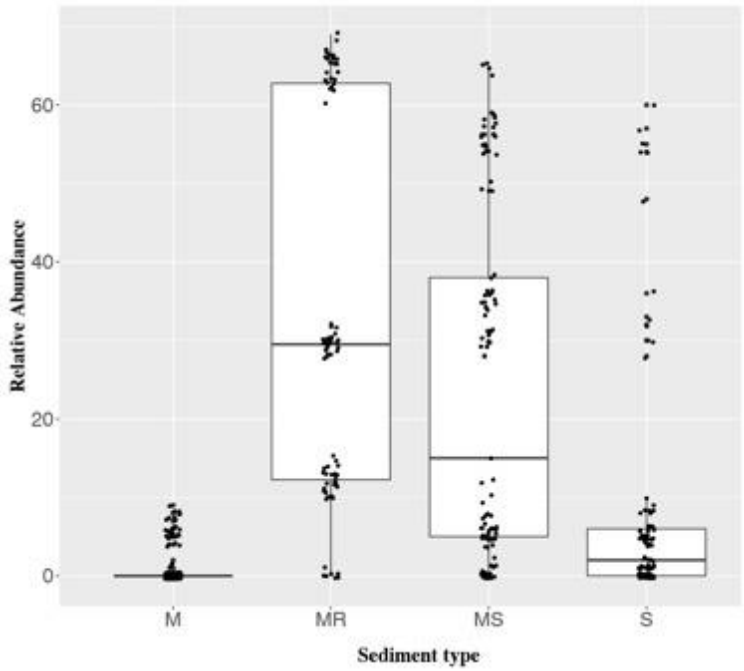

Figure 19. Relative abundance of *M. cereoides* with respect the sediment type in the Colombian Caribbean, showing a high abundance in the Mud-Rock type.

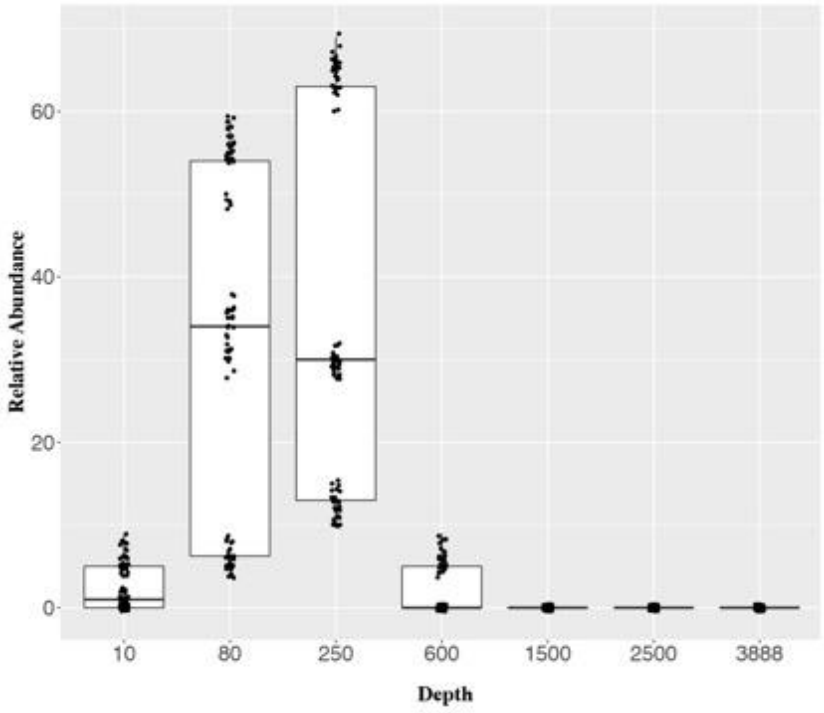

Figure 20. Relative abundance of *M. cereoides* with respect the depth, showing higher abundance at 80m depth.

Halophila antillaea Winston, 2005 (Fig. 21)

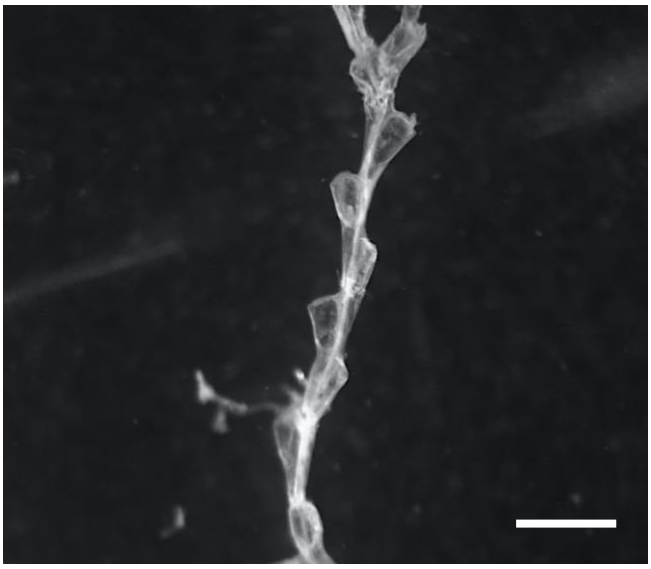

Figure 21. SEM photography of *H. antillaea* found in the Colombian Caribbean. Scale bar: 100um.

Our results showed that the location has a significant effect on the relative abundance of *H. antillaea* in the three areas of the Caribbean ($p<0.001$). The variation was high in the North zone, as well as the abundance (Figure 22). In addition, temperature (Suppl. VI) had a significant effect on the relative abundance of this species ($p<0.001$), it was higher at 27.2C, and the salinity (Figure 22) also has a significant effect ($p<0.001$), being higher at a salinity of 35.6ppt. The interaction of temperature and location had a significant effect on the relative abundance ($p<0.001$), but the interaction of salinity and location did not show significant effect ($p=0.05$).

Also, the abundance of *H. antillaea* was affected by the sediment type (Figure 24). Most colonies were found in mixed Mud-Sand stations ($p<0.001$). In addition, the abundance was higher at 80m depth showing an effect of the depth on the abundance ($p<0.001$) (Figure 25) and the interaction depth and temperature ($p<0.001$) but no interaction between depth and salinity ($p=0.002$).

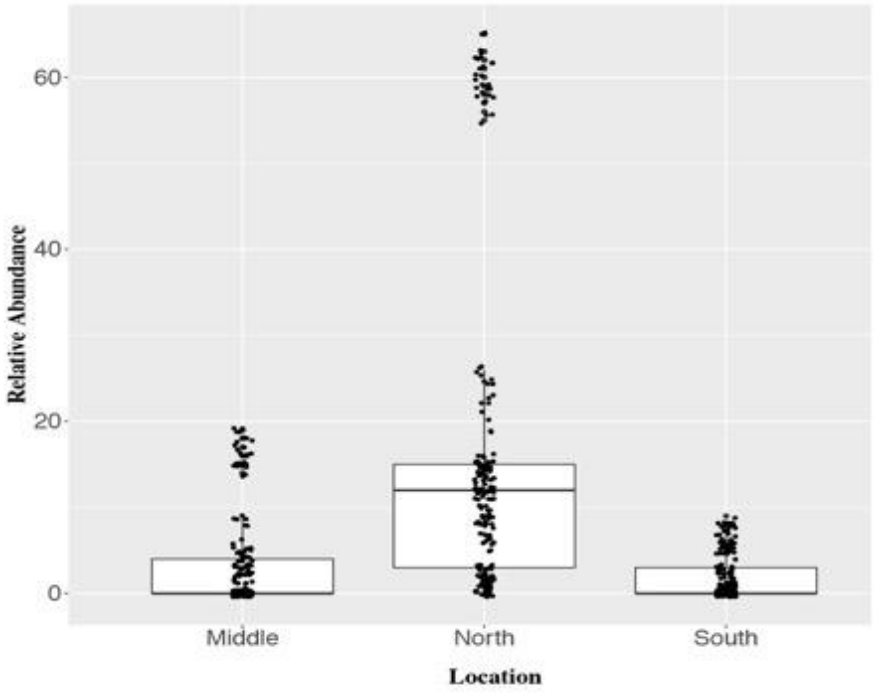

Figure 22. Relative abundance of *H. antillaea* with respect the three locations in the Colombian Caribbean, showing high abundance in the North zone.

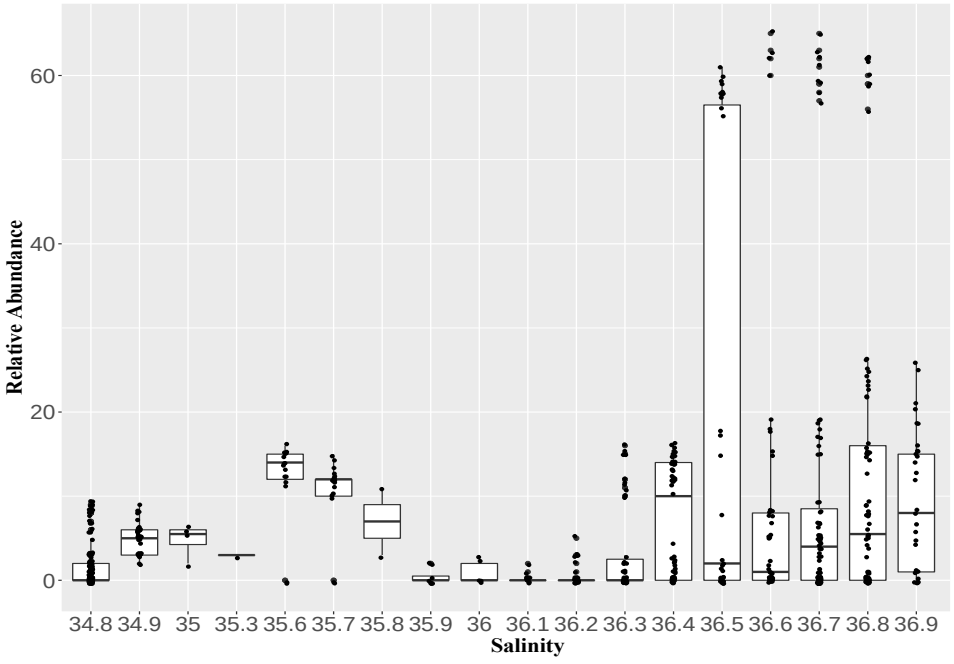

Figure 23. Relative abundance of *H. antillaea* with respect the salinity in the Colombian Caribbean, showing a higher abundance after 35.6 ppt.

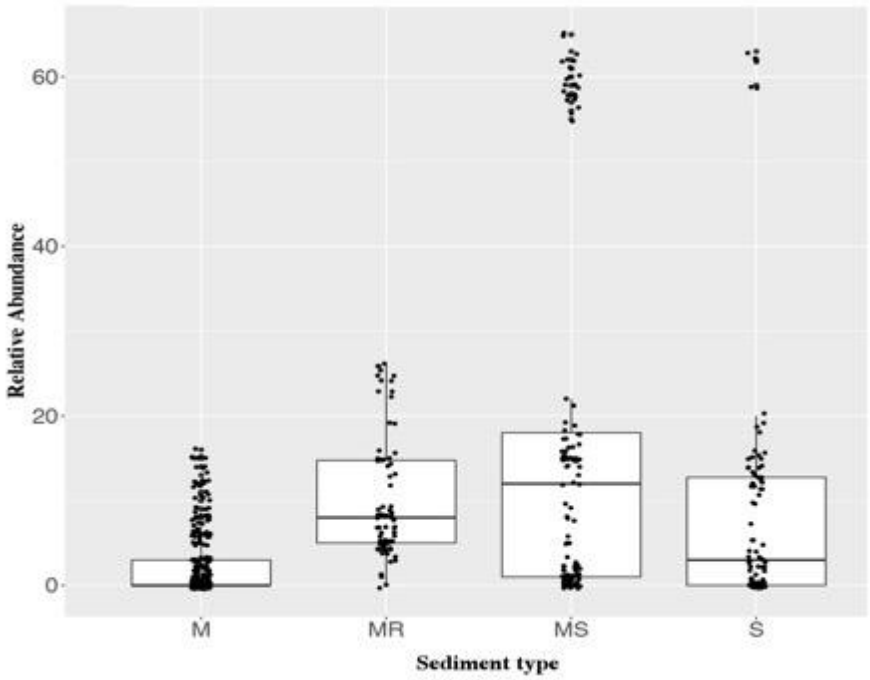

Figure 24. Relative abundance of *H. antillaea* with respect the sediment type in the Colombian Caribbean, showing a high abundance in the Mud-Sand type.

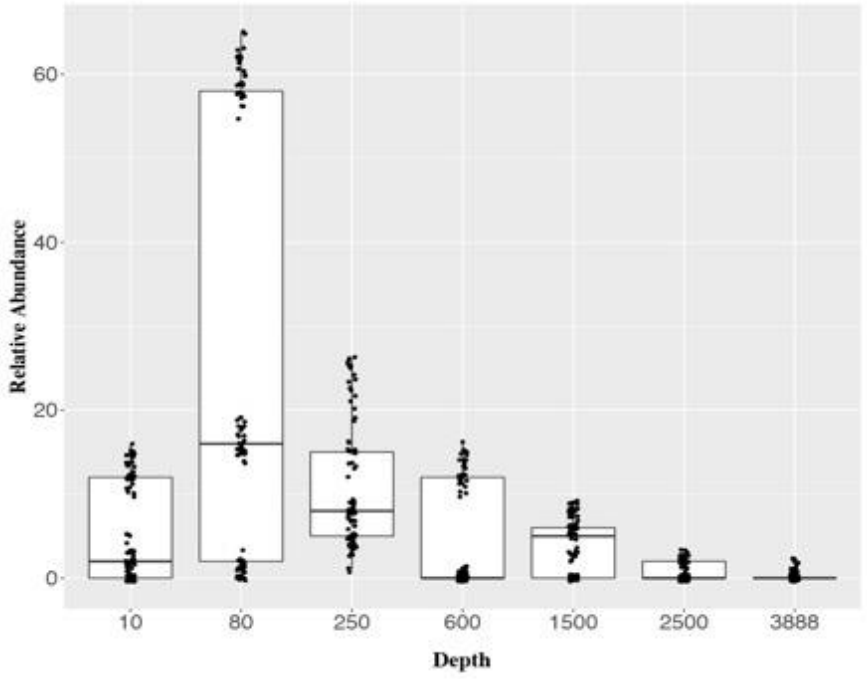

Figure 25. Relative abundance of *H. antillaea* with respect to the depth, showing a higher abundance at 80m depth.

DISCUSSION

Bathymetric and geographical distribution of bryozoans

Bryozoans inhabit the Colombian Caribbean Sea from 1 to 3880m depth. This study showed that their distribution along bathymetric ranges depends on physical and environmental conditions and each species has different environmental requirements for its relative abundance and growth. The Colombian continental shelf and the upper slope have diverse geological formations that are influenced by the constant continental interactions such as Magdalena river, in the Mid-Caribbean, whose delta modifies the geology of the deep-sea (Magdalena fan) (Idárraga-Garcia et al., 2019; Invemar, 2010) and contributes to salinity fluctuations in the area. (Figure 26). About 10% of the bryozoan assemblages were identified in this area from 1-80m depth and are characterized for having higher tolerances to salinity and temperature fluctuations and present mainly flexible growth forms that allow them to dominate this high energetic area. Example species are *Bugula neritina*, *Conopeum seuratum* and *Amathia vidovici*.

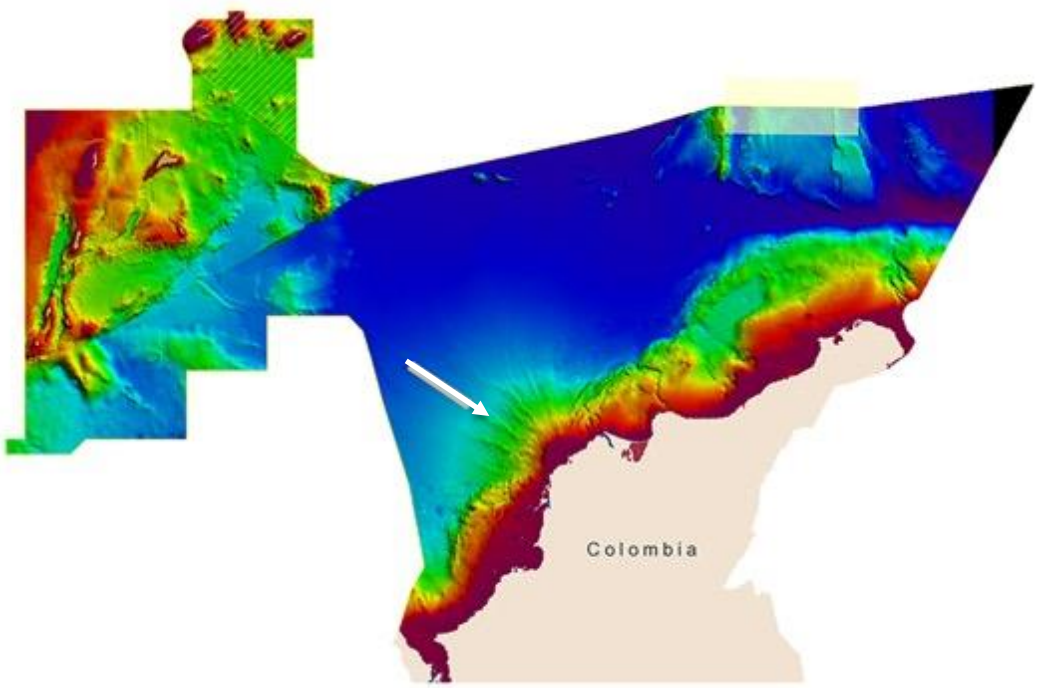

Figure 26. Bathymetry and geo-forms in the Colombian Caribbean. Shelf in red, deep-sea in blue. The Magdalena fan (arrow). Image credits Dimar, 2020.

However, the vast majority of the species in this area are found from 80 to 1000m depth at which several growth forms thrive such as the rigid-erect (e.g *Steginoporella* spp., *A. subsulcata* and *Celleporaria* spp.), the massive encrusting (*F. antiqua, R. spicatum* and *C. turrita*) and the free-living forms (*Cupuladria* spp and *Discoporella* spp.) this trend is possibly influenced by the high deep-sea productivity in the area (Santodomingo et al., 2013; Ercilla et al., 2002), and triggered by the Magdalena river which injects tons of continental nutrients, positively impacting growth and reproduction as evidenced in the high abundance and richness found (Invemar, 2010). A similar situation occurs at the south Colombian Caribbean where bryozoan diversity from 1 to 80m is low, most species present flexible growth forms and high tolerance to salinity and temperature fluctuations. The sediment at that depth is mainly mud, originated from the interaction of runoff from mainland, the diapiric activity of mud volcanos, and the methane seeps from the Sinú-San Jacinto fold belt that enriches the sediments with minerals and generates lower pH in the sediment (Gracia et al., 2011; Invemar, 2010).

The middle and south zones have the highest species richness from 80 to 250m depth, possibly due to the enrichment from shallower areas and the more stable environmental conditions such as slower water flow and nutrient availability. Here, erect-articulated and unilaminar rigid-erect forms are dominant.

In contrast, the northern zone of the Colombian Caribbean holds the greatest abundance and species richness form 1 to 80m depth (Yepes-Narvaez, 2013), mainly influenced by the seasonal upwelling phenomena that have modified the chemistry and physical processes such as water currents and sediment removal in the area (Eidens et al., 2014; Corpoguajira & Invemar, 2012) that in addition to high turbidity, supports a relatively high primary productivity that will benefit skeleton growth (Berning, 2007; Smith, 2007; Taylor, 2005, Winston, 1983), However, diversity decreases from 250m depth, and the driver of this trend could be that the deep-sea sediments removal creates an unstable environment for larval settlement at those depths and in addition to stronger water flow. These phenomena cause a reduction of food availability in the deep which is crucial for the growth and reproduction of bryozoans (O'Dea & Okamura, 1999; Winston, 1988).

With regards to geographical distribution, our results partially contradict Navas et al. (2010) findings about an exponential reduction in faunal assemblages from north to south Colombian Caribbean in the continental shelf and the upper slope because we found that what is actually triggering the distribution patterns, at least for bryozoans are the depth, flow and food availability. As explained earlier, La Guajira (north) holds the richest bryozoan biodiversity due to higher food availability in the range 1-80m depth and exponentially decreases after 250m, however, in the middle and south at the same bathymetric range, species assemblages are reduced, but at deeper ranges 80-250m and 250-600m depth, the diversity is higher than that found at the same depth in the northern part of the Caribbean.

Bryozoans in the North zone are bigger in size and are the main structuring organisms, especially in La Guajira, in the absence of stony corals due to the high turbidity systems typical from this location. This is the most explored area in the Colombian Caribbean Sea, and it holds the greatest species record for the country (Figure 27). *Adeonellopsis subsulcata* is one of the most dominant species here and has an erect-rigid growth form that allows it to create habitat complexity (Yepes-Narvaez, 2013).

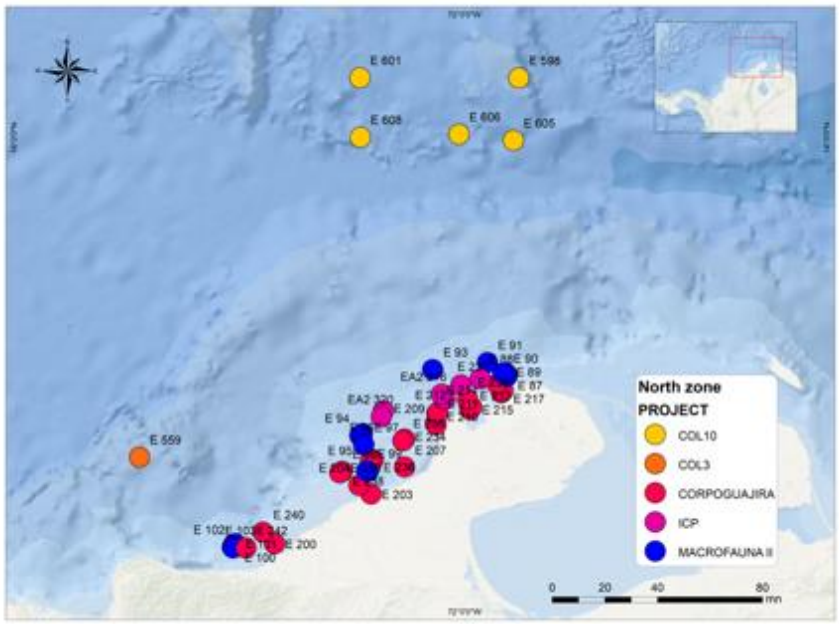

Figure 27. Bryozoan distribution in the north zone of the Colombian Caribbean based on the collections of five expeditions in the area.

The least explored area in the northern Caribbean is the bathymetric range from 1000m due to several limiting factors including harsh climatic conditions that limit longer exploration campaigns and the sampling costs attributed to this type of research. However, this area holds species that could be new to science and give paleo-environmental information about those depths (covered in chapter 2).

The Middle zone is the least explored in the Colombian Caribbean, hence the low number of bryozoan species recorded (Flórez et al., 2007). However, the bryozoan species list from 9.9 to 20m depth here is the highest at that depth compared to any other zone of the Caribbean and reveals a possible connection with deeper bryozoans in the south Caribbean (Montoya-Cadavid et al., 2007) (Figure 28). This area could be considered an intermediate between the dynamic north Caribbean and the calmer shallow water of the South, for instance finding species from both other zones here is not rare. The dominant growth form here are the erect-rigid *S.magnilabris* and the erect-articulated *M. cereoides* as well as the encrusting laminar forms associated artificial substrates in the line coast (Gracia et al., 2018).

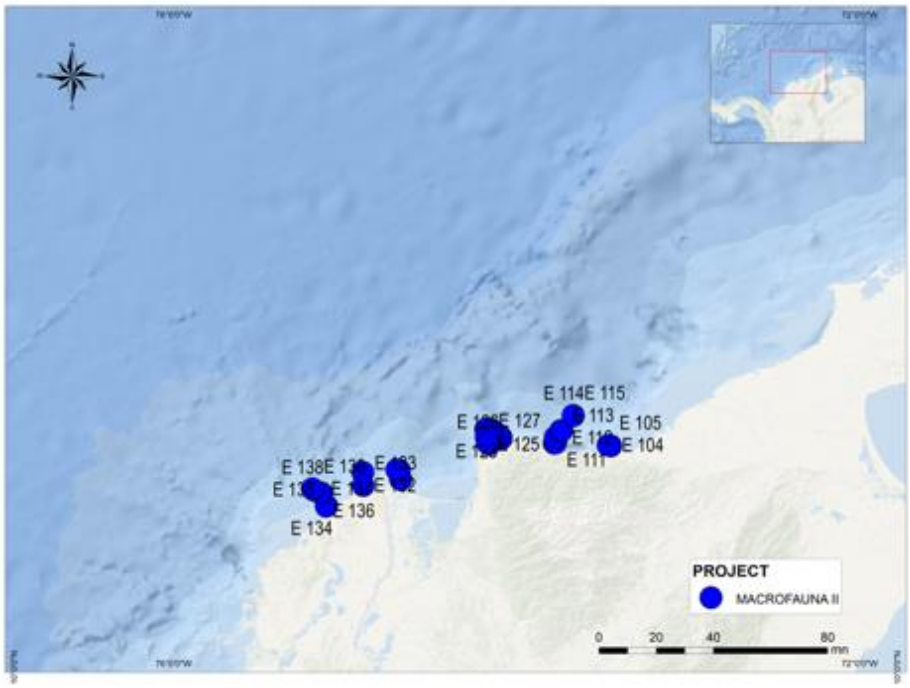

Figure 28. Bryozoan distribution in the middle zone of the Colombian Caribbean.

Finally, the South zone, is the second most explored area in the Colombian Caribbean since the recent declaration of the Deep-sea Corals Natural National Park – PNNCP (MADS, 2013). Most of the sampling efforts have focused on baseline explorations at the limiting margins of the park and very few efforts have been put in place to study the shallower fauna, hence the reduced species records (Montoya-Cadavid et al., 2010). Bryozoans in this zone are less abundant and diverse from 1-80m and most have been identified as associating with artificial substrates (Delgadillo &. Flórez, 2015) or in natural substrates associated with mangrove roots (Chapter 4). The dominant growth form at the shallower range is the erect-flexible *Amathia* spp. or erect-articulated form typical of *Paralicornia* spp and *Bugula neritina.* From 80-600m depth a vast diversity and abundance of bryozoans is found (Figure 29); the dominant species at these depths are *S. magnilabris* and *A. subsulcata.* in addition, a high deep-sea diversity is found surrounding the *Madracis myriaster* bioherms at the PNNCP (Chapter 2). The knowledge is still incipient and further efforts should be implemented to explore different areas of the Colombian Caribbean

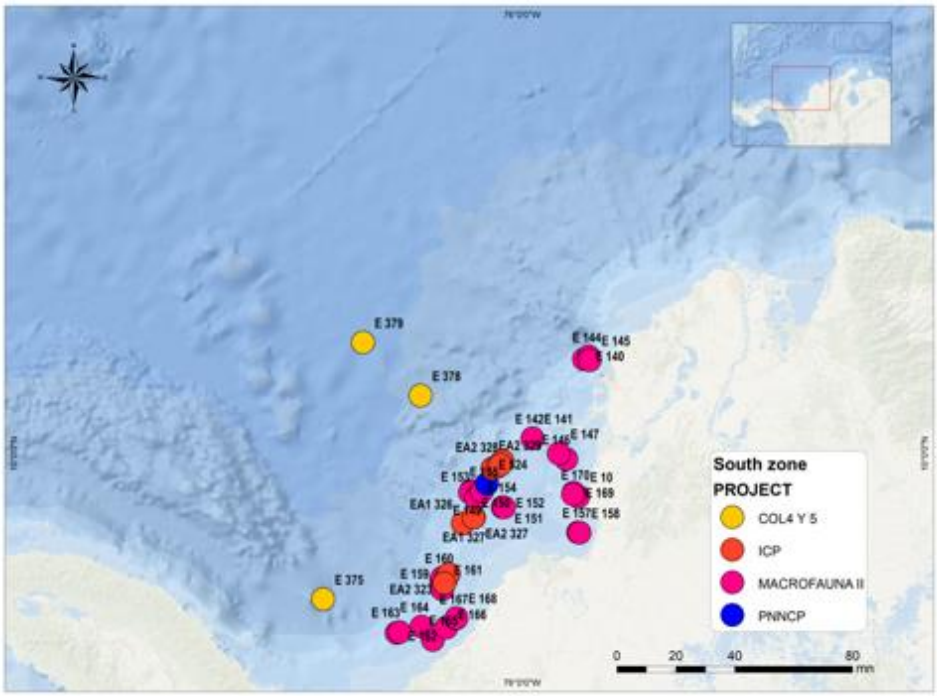

Figure 29. Bryozoan distribution in the south zone of the Colombian Caribbean based on the collections of four expeditions in the area.

Significance of environmental variables

We found that bryozoans have different environmental needs for their growth and distribution. The environmental variables measured in this study compared to the bryozoan relative abundance, showed that salinity is not a good indicator for *A. subsulcata, S. magnilabris* or *M. cereoides,* as specimens of these were found across salinity ranges with similar variances and no trend was observed at higher or lower salinity levels. However, this is different for *H. antillaea* whose highest relative abundances were delimited by the salinity range between 21.7 and 27.2ppt, lower or higher salinities represented a reduction in relative abundance for this particular species. On the other hand, temperature is good indicator of the abundance of these species along a bathymetric range because it limits the distribution of species and their relative abundance, deeper stations presented fewer species records and smaller sized colonies, these stations were the coldest, while shallower stations 1-10m depth had the hottest temperatures and the lowest abundances of individual species. The optimum temperature range for the four species analysed was between 22.7 and 24 C.

Bryozoan mineralogy significance and relationship distribution

The literature revision of the mineralogical compositions of the four species analysed, could not be compared between all the species because, to our knowledge, there are not enough preliminary studies in the calcification patterns of Caribbean species. The EDAX readings on the chemical composition, revealed that *M. cereoides* and *A. subsulcata* present the highest proportions of Mg and Calcite while *H. antillae* present the lowest calcite readings compared to the previous species but their Mg composition is high compared to *S. magnilabris*, which also present high calcite concentrations and high Mg. The distribution of these species could explain their mineralogical composition. *H. antillaea* and *M. cereoides* inhabit mostly clear and energetic environments which reduces the carbonate intake, and their colonies are flexible as an adaptation to the water movement and to ensure bigger food catch. The rest, invest in bigger colonies as a consequence of the productive environments they live in.

CONCLUSIONS

There is a relationship between the environmental conditions and the bathymetric and geographical distribution of bryozoans in the Colombian Caribbean. The northern zone has the greatest diversity from 1-80m depth and in the middle and south the greatest from 80-600m depth. Growth forms are structural modifications of the colony shape in response to different physical and environmental stressors. Flexible colonies are typical of shallow water bryozoans in high energy habitats and in deep-sea environments where there are limiting factors that do not allow bigger colony growth. Rigid colonies on the other hand can be unilaminar or multilaminar and their level of complexity depends on the energy and food availability. Temperature and depth are good indicators of the distribution and relative abundance of bryozoans. The mineralogical composition of bryozoans is also influenced by the environmental conditions and food availability.

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Supplementary material I: List of collection sites and associated information

EXPEDITION	STATION	LATITUDE	LONGITUDE	ECOREGION	DEPTH	SEDIMENT TYPE	ENV. VAR. M.	COL. METHOD
ICP	EA2 329	9° 56' 14.1" N	76° 7' 26.8" W	Coral Archipelagos	72	Mud-Sand	CTDO	Trawl
ICP	EA1 329	9° 57' 38.7" N	76° 6' 43.3" W	Coral Archipelagos	72	Mud-Sand	CTDO	Trawl
ICP	EA2 328	9° 55' 36.4" N	76° 8' 23.8" W	Caribbean Offshore	73	Mud-Sand	CTDO	Trawl
ICP	EA1 328	9° 55' 2.5" N	76° 10' 6.6" W	Caribbean Offshore	78	Mud-Sand	CTDO	Trawl
ICP	EA2 327	9° 38' 15" N	76° 16' 26.8" W	Caribbean Offshore	82	Mud-Sand	CTDO	Trawl
ICP	EA1 327	9° 38' 17.8" N	76° 16' 20.8" W	Coral Archipelagos	95	Mud-Sand	CTDO	Trawl
ICP	EA1 326	9° 36' 18" N	76° 20' 10.5" W	Coral Archipelagos	98	Mud-Sand	CTDO	Trawl
ICP	EA2 323	9° 16' 3.5" N	76° 26' 26.8" W	Caribbean Offshore	124	Mud-Sand	CTDO	Trawl
ICP	EA2 322	9° 19' 7.2" N	76° 24' 59.1" W	Caribbean Offshore	125	Mud-Sand	CTDO	Trawl
ICP	EA1 322	9° 17' 36.7" N	76° 25' 46.9" W	Caribbean Offshore	126	Mud-Sand	CTDO	Trawl
ICP	EA2 321	12° 28' 19.4" N	71° 54' 2.6" W	Guajira	128	Rock	CTDO	Box core
ICP	EA2 320	12° 13' 55.2" N	72° 31' 37.7" W	Guajira	190	Rock	CTDO	Box core
ICP	EA1 319	12° 16' 12.4" N	72° 30' 50.2" W	Guajira	220	Rock	CTDO	Box core
ICP	EA1 317	12° 26' 1.4" N	72° 1' 21.4" W	Guajira	270	Rock	CTDO	Box core
ICP	EA2 316	12° 22' 32.7" N	72° 8' 44.6" W	Guajira	240	Rock	CTDO	Box core
ICP	EA2 315	12° 20' 44.8" N	72° 7' 21" W	Guajira	230	Rock	CTDO	Box core
ICP	EA1 315	12° 20' 45.9" N	72° 7' 52.7" W	Guajira	250	Rock	CTDO	Box core
COL10	E 608	14° 0' 9.8" N	72° 39' 35.1" W	Caribbean offshore	3700	Mud	CTDO-Nutrients	Box core
COL10	E 606	14° 1' 19.4" N	72° 2' 5.9" W	Caribbean offshore	3888	Mud	CTDO-Nutrient	Box core
COL10	E 605	13° 58' 58.6" N	71° 41' 24.5" W	Caribbean offshore	3600	Mud	CTDO-Nutrient	Box core
COL10	E 601	14° 22' 46.9" N	72° 39' 46" W	Caribbean offshore	3290	Mud	CTDO-Nutrient	Box core
COL10	E 598	14° 22' 42.7" N	71° 39' 19.1" W	Caribbean offshore	2887	Mud	CTDO-Nutrient	Box core
COL4 Y 5	E 379	10° 37' 47.4" N	76° 54' 4.1" W	Caribbean offshore	1796	Mud	CTDO-Nutrient	Box core
COL4 Y 5	E 378	10° 19' 46.9" N	76° 34' 32" W	Caribbean offshore	2910	Mud	CTDO-Nutrient	Box core
COL4 Y 5	E 375	9° 10' 37.5" N	77° 7' 34.2" W	Caribbean offshore	3134	Mud	CTDO-Nutrient	Box core
COL3	E 559	11° 58' 31.6" N	74° 3' 33" W	Caribbean offshore	1673	Mud	CTDO-Nutrient	Box core
PNNCP	E 524	09°49'47.5" N	76°12'01.6" W	Coral Archipelagos	180	Coral rubble	CTDO	Dredge
PNNCP	E 390	9°54'09,1" N	76°09'19,0" W	Coral Archipelagos	110	Mud-Sand	CTDO	ROV
CORPOGUAJIRA	E 200	11° 25' 43.2" N	73° 12' 8.6" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 201	11° 39' .9" N	72° 59' 3.8" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 203	11° 44' 34.1" N	72° 35' 31.5" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 204	11° 47' 55.2" N	72° 40' 10.4" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 207	11° 54' 51" N	72° 22' 48.6" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 209	12° 11' 25.5" N	72° 10' 58.6" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 210	12° 10' 52.1" N	72° 10' 47.2" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 211	12° 15' 16.9" N	72° 10' 28.4" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 212	12° 15' 34.7" N	72° 10' 10.8" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 213	12° 16' 42.7" N	71° 58' 1" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 214	12° 17' 31.2" N	71° 57' 17.2" W	Guajira	10	Sand	CTDO	Trawl
CORPOGUAJIRA	E 215	12° 23' 6.6" N	71° 46' 1" W	Guajira	10	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 217	12° 23' 49.7" N	71° 45' 22.8" W	Guajira	10	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 231	12° 26' 22.8" N	71° 47' 37.3" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 232	12° 20' 32.3" N	71° 58' 53" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 234	12° 5' 25.5" N	72° 22' 51.7" W	Guajira	50	mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 235	12° 4' 49.2" N	72° 23' 26.9" W	Guajira	50	Sand	CTDO	Trawl
CORPOGUAJIRA	E 236	11° 57' 37.1" N	72° 34' 45.3" W	Guajira	50	Sand	CTDO	Trawl
CORPOGUAJIRA	E 237	11° 57' 19.9" N	72° 35' 41.1" W	Guajira	50	Sand	CTDO	Trawl
CORPOGUAJIRA	E 238	11° 53' 10.3" N	72° 46' 28.4" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 239	11° 52' 40.1" N	72° 47' 25.2" W	Guajira	50	Sand	CTDO	Trawl
CORPOGUAJIRA	E 240	11° 30' 6.9" N	73° 16' 40.2" W	Guajira	50	Mud-Sand	CTDO	Trawl
CORPOGUAJIRA	E 242	11° 24' 4.7" N	73° 23' 7.4" W	Guajira	50	Sand	CTDO	Trawl
MACROFAUNA II	E 10	9° 45' 21.9" N	75° 40' 59" W	Guajira	9.9	Sand	CTDO	Trawl
MACROFAUNA II	E 87	12° 29' 44.9" N	71° 43' 40" W	Gulf Morrosquillo	72	Sand	CTDO	Trawl
MACROFAUNA II	E 88	12° 29' 17.9" N	71° 43' 51.9" W	Guajira	73	Sand	CTDO	Trawl
MACROFAUNA II	E 89	12° 30' 34.9" N	71° 44' 18.9" W	Darién	152	Sand	CTDO	Trawl
MACROFAUNA II	E 90	12° 30' 33" N	71° 45' 20.9" W	Gulf Morrosquillo	150	Sand	CTDO	Trawl
MACROFAUNA II	E 91	12° 34' 35" N	71° 51' 16.9" W	Gulf Morrosquillo	305	Sand	CTDO	Trawl
MACROFAUNA II	E 92	12° 32' .9" N	72° 11' 30" W	Darién	493	Sand	CTDO	Trawl
MACROFAUNA II	E 93	12° 31' 50.9" N	72° 12' 6" W	Darién	496	Sand	CTDO	Trawl
MACROFAUNA II	E 94	12° 6' 45" N	72° 39' 48.9" W	Darién	151	Sand	CTDO	Trawl
MACROFAUNA II	E 95	12° 7' 35" N	72° 38' 48.9" W	Darién	154	Sand	CTDO	Trawl
MACROFAUNA II	E 96	12° 3' 24" N	72° 38' 17" W	Darién	70	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 97	12° 3' 16.9" N	72° 38' 17.9" W	Darién	70.1	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 98	11° 53' 4.9" N	72° 36' 38.9" W	Darién	21.4	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 99	11° 53' 21.9" N	72° 37' 12" W	Darién	22	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 100	11° 25' 33.9" N	73° 27' 39.9" W	Darién	150	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 101	11° 25' 45.9" N	73° 27' 9" W	Gulf Morrosquillo	153	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 102	11° 24' 23" N	73° 28' 18" W	Gulf Morrosquillo	70	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 103	11° 24' 3.9" N	73° 28' 1.9" W	Coral Archipelagos	71.6	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 104	11° 17' 31.9" N	73° 27' 6" W	Coral Archipelagos	20	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 105	11° 17' 40.9" N	73° 27' 57.9" W	Caribbean offshore	21	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 108	11° 18' 28" N	73° 46' 50" W	Caribbean offshore	70	Mud-Sand	CTDO	Trawl

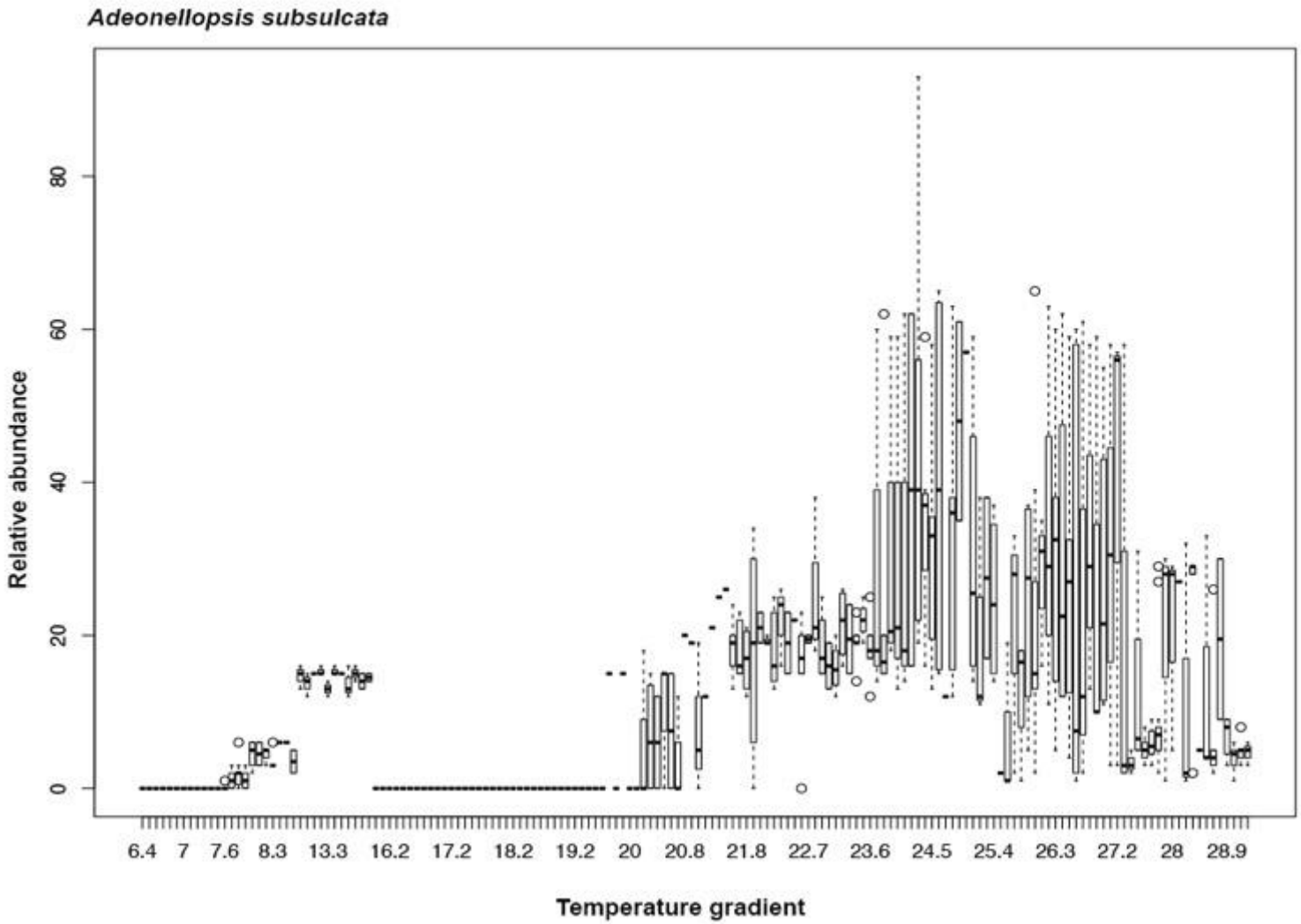
MACROFAUNA II	E 109	11° 18' 30.9" N	73° 46' 28.9" W	Coral Archipelagos	71	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 110	11° 20' 30.9" N	73° 46' 0" W	Coral Archipelagos	150	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 111	11° 20' 30" N	73° 46' 27.9" W	Caribbean offshore	152	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 112	11° 22' 51.9" N	73° 44' 35" W	Caribbean offshore	300	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 113	11° 22' 57" N	73° 44' 8" W	Coral Archipelagos	300	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 114	11° 28' 4" N	73° 40' 14" W	Coral Archipelagos	498	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 115	11° 28' 13" N	73° 40' 14.9" W	Coral Archipelagos	504	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 116	11° 20' 4.9" N	74° 5' 25" W	Coral Archipelagos	35	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 117	11° 20' 26" N	74° 5' 27.9" W	Coral Archipelagos	20.4	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 118	11° 21' 29" N	74° 6' 16.9" W	Caribbean offshore	76	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 119	11° 21' 24" N	74° 6' 21.9" W	Caribbean offshore	74	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 120	11° 23' 7" N	74° 8' 40.9" W	Caribbean offshore	151	Mud	CTDO	Trawl
MACROFAUNA II	E 121	11° 23' 12.9" N	74° 8' 56" W	Caribbean offshore	150	Mud	CTDO	Trawl
MACROFAUNA II	E 122	11° 23' 13.9" N	74° 10' 50" W	Gulf Salamanca	150	Mud	CTDO	Trawl
MACROFAUNA II	E 123	11° 23' 30" N	74° 10' 17" W	Gulf Salamanca	154	Mud	CTDO	Trawl
MACROFAUNA II	E 124	11° 20' .9" N	74° 10' 0" W	Gulf Salamanca	72.3	Mud	CTDO	Trawl
MACROFAUNA II	E 125	11° 20' 31.9" N	74° 10' 37.9" W	Gulf Salamanca	72	Mud	CTDO	Trawl
MACROFAUNA II	E 126	11° 18' 2.9" N	74° 9' 36" W	Gulf Salamanca	26.6	Mud	CTDO	Trawl
MACROFAUNA II	E 127	11° 18' 29" N	74° 10' 9.9" W	Gulf Salamanca	39.5	Mud	CTDO	Trawl
MACROFAUNA II	E 128	11° 5' 57.9" N	74° 40' 36.9" W	Magdalena	20	Mud	CTDO	Trawl
MACROFAUNA II	E 129	11° 5' 45.9" N	74° 40' 35" W	Magdalena	20	Mud	CTDO	Trawl
MACROFAUNA II	E 130	11° 9' 5" N	74° 41' 9.9" W	Gulf Salamanca	70.4	Mud	CTDO	Trawl
MACROFAUNA II	E 131	11° 9' 10" N	74° 41' 57" W	Gulf Salamanca	70	Mud	CTDO	Trawl
MACROFAUNA II	E 132	11° 8' 17.9" N	74° 53' 48.9" W	Gulf Salamanca	153	Mud	CTDO	Trawl
MACROFAUNA II	E 133	11° 3' 38.9" N	74° 53' 39" W	Gulf Salamanca	148	Mud	CTDO	Trawl
MACROFAUNA II	E 134	10° 56' 35.9" N	75° 6' 29" W	Tayrona	20.9	Mud	CTDO	Trawl
MACROFAUNA II	E 135	10° 56' 25" N	75° 6' 36" W	Tayrona	20	Mud	CTDO	Trawl
MACROFAUNA II	E 136	11° 1' 0" N	75° 8' 17.9" W	Tayrona	72	Mud	CTDO	Trawl
MACROFAUNA II	E 137	11° 1' 12" N	75° 8' 56" W	Tayrona	70	Mud	CTDO	Trawl
MACROFAUNA II	E 138	11° 2' 8.9" N	75° 11' 6" W	Tayrona	150	Mud	CTDO	Trawl
MACROFAUNA II	E 139	11° 2' 29" N	75° 11' 27.9" W	Tayrona	145	Mud	CTDO	Trawl
MACROFAUNA II	E 144	10° 32' 56" N	75° 37' 19.9" W	Tayrona	309	Mud	CTDO	Trawl
MACROFAUNA II	E 145	10° 31' 45.9" N	75° 37' 6.9" W	Tayrona	309	Mud	CTDO	Trawl
MACROFAUNA II	E 140	10° 32' 6" N	75° 39' 5" W	Tayrona	487	Mud	CTDO	Trawl
MACROFAUNA II	E 141	10° 5' 16" N	75° 56' 33" W	Tayrona	151	Mud	CTDO	Trawl
MACROFAUNA II	E 142	10° 5' 15" N	75° 56' 34" W	Tayrona	150	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 146	9° 58' 12" N	75° 45' 2.9" W	Tayrona	67	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 147	10° 0' 5" N	75° 47' 35" W	Caribbean offshore	89	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 149	9° 47' 29" N	76° 17' 21.9" W	Caribbean offshore	507	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 150	9° 46' 50" N	76° 17' 44.9" W	Caribbean offshore	500	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 151	9° 41' 52" N	76° 6' 38" W	Caribbean offshore	70.9	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 152	9° 41' 47" N	76° 6' 11" W	Palomino	70.5	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 153	9° 45' 37" N	76° 15' 19" W	Palomino	270	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 154	9° 44' 48.9" N	76° 15' 38" W	Palomino	280	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 155	9° 47' 12" N	76° 13' 45" W	Palomino	160	Mud-Sand	CTDO	Trawl
MACROFAUNA II	E 156	9° 47' .9" N	76° 14' 12" W	Palomino	155	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 157	9° 33' 15.9" N	75° 41' 4.9" W	Palomino	22	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 158	9° 33' 9" N	75° 40' 33.9" W	Palomino	22	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 159	9° 17' 3" N	76° 27' 29" W	Palomino	158	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 160	9° 17' 52" N	76° 27' 14" W	Palomino	160	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 161	9° 13' 59.9" N	76° 27' 11.9" W	Palomino	70.6	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 162	8° 59' 8.9" N	76° 42' 38.9" W	Guajira	151	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 163	8° 59' 25" N	76° 41' 44" W	Guajira	150	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 164	9° 1' 18" N	76° 34' 10.9" W	Guajira	70	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 165	8° 56' 43" N	76° 30' 20.9" W	Guajira	20.6	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 166	9° 0' 55" N	76° 25' 48" W	Guajira	20.8	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 167	9° 4' 1.9" N	76° 22' 26" W	Guajira	20.6	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 168	9° 4' 13" N	76° 22' 17" W	Caribbean offshore	19.6	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 169	9° 46' 46.9" N	75° 42' 34.9" W	Caribbean offshore	20	Sand-Rock	CTDO	Trawl
MACROFAUNA II	E 170	9° 46' 10.9" N	75° 42' 47" W	Caribbean offshore	20.1	Sand-Rock	CTDO	Trawl

Supplementary material II: Environmental data collected per station

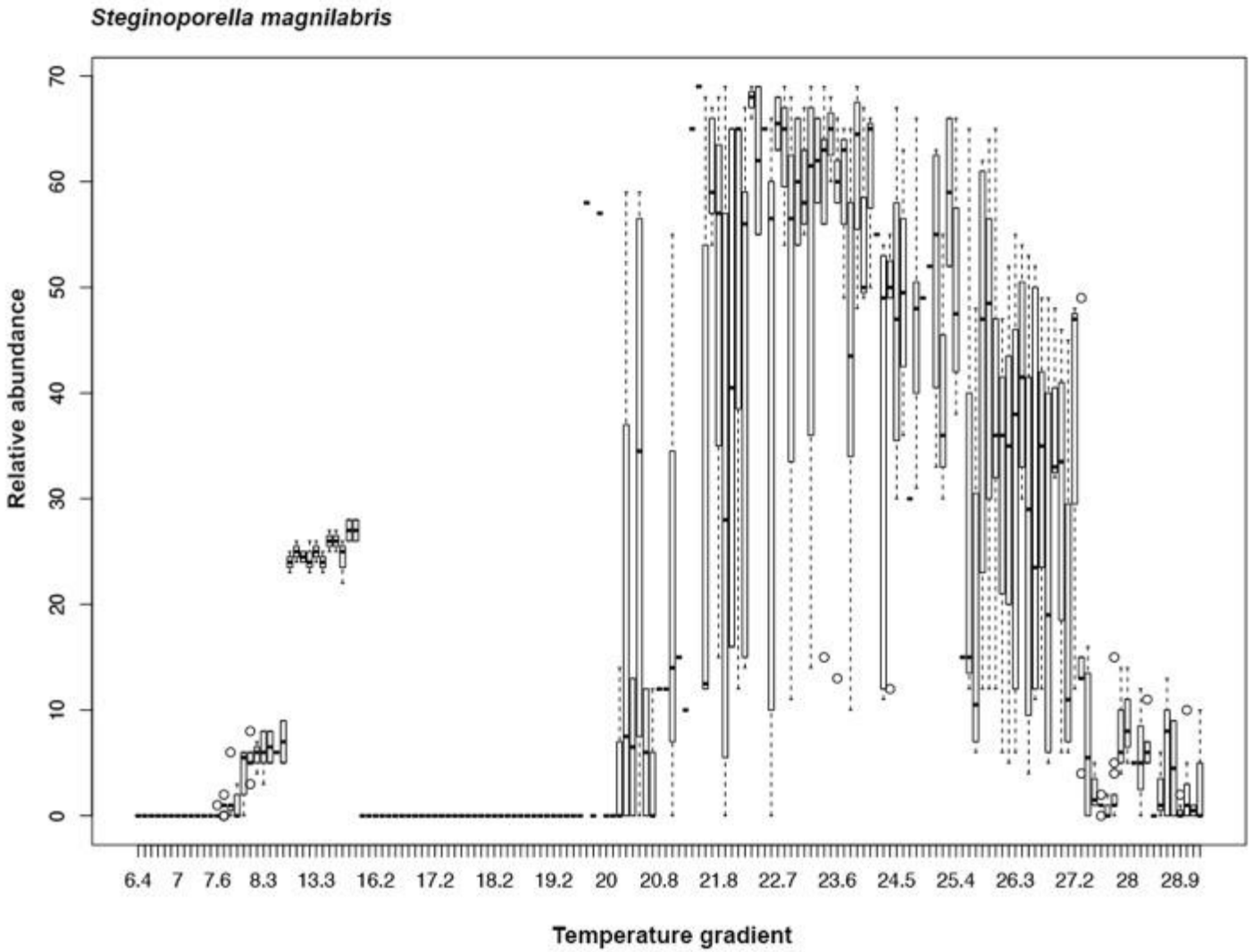
Station	Depth	Sediment	TC	Sal	OD
E 10	9.9	Sand	27.8	36.3	6
E 200	10	Sand	27.8	36.3	6
E 201	10	Sand	27.8	36.3	6
E 203	10	Sand	27.8	36.3	6
E 204	10	Sand	27.8	36.3	6
E 207	10	Sand	27.8	36.3	6
E 209	10	Sand	27.8	36.4	6
E 210	10	Sand	27.8	36.4	6
E 213	10	Sand	27.8	36.4	6
E 214	10	Sand	27.8	36.4	6
E 215	10	Mud-Sand	27.8	36.4	6
E 217	10	Mud-Sand	27.8	36.4	6
E 168	19.6	Sand-Rock	27.8	36.4	6
E 104	20	Mud-Sand	26.9	36.5	5.9
E 128	20	Mud	26.8	36.5	5.9
E 129	20	Mud	26.7	36.5	5.9
E 135	20	Mud	26.6	36.5	5.9
E 169	20	Sand-Rock	26.6	36.5	5.9
E 170	20.1	Sand-Rock	26.5	36.5	5.8
E 117	20.4	Mud-Sand	26.4	36.6	5.8
E 165	20.6	Sand-Rock	26.3	36.6	5.8
E 167	20.6	Sand-Rock	26.2	36.6	5.8
E 166	20.8	Sand-Rock	26	36.6	5.8
E 134	20.9	Mud	25.1	36.7	5.4
E 105	21	Mud-Sand	25	36.7	5.3
E 98	21.4	Mud-Sand	24.9	36.7	5.3
E 99	22	Mud-Sand	24.8	36.7	5.4
E 157	22	Sand-Rock	24.6	36.7	5.4
E 158	22	Sand-Rock	24.6	36.7	5.3
E 126	26.6	Mud	24.5	36.7	5.2
E 116	35	Mud-Sand	24.4	36.7	5.1
E 127	39.5	Mud	24.3	36.8	5.1
E 211	50	Mud-Sand	24.2	36.8	5.1
E 212	50	Mud-Sand	24.1	36.8	5.1
E 231	50	Mud-Sand	24	36.8	5.1
E 232	50	Mud-Sand	23.9	36.8	5.1
E 234	50	mud-Sand	23.8	36.8	5.1
E 235	50	Sand	23.7	36.8	5.1
E 236	50	Sand	22.9	36.8	5.2
E 237	50	Sand	22.8	36.8	5.2
E 238	50	Mud-Sand	22.8	36.9	5.1
E 239	50	Sand	22.7	36.9	5.2
E 240	50	Mud-Sand	22.6	36.9	5.1
E 242	50	Sand	22.6	36.9	5.1
E 146	67	Mud-Sand	22.5	36.9	5.1
E 96	70	Mud-Sand	22.4	36.9	5.1
E 102	70	Mud-Sand	22.3	36.9	5
E 108	70	Mud-Sand	22.3	36.9	5
E 131	70	Mud	22.2	36.9	5
E 137	70	Mud	22.1	36.9	5
E 164	70	Sand-Rock	22.1	36.9	4.9
E 97	70.1	Mud-Sand	22	36.9	4.9
E 130	70.4	Mud	21.9	36.9	4.9
E 152	70.5	Mud-Sand	21.8	36.9	4.9
E 161	70.6	Sand-Rock	21.7	36.8	4.9
E 151	70.9	Mud-Sand	21.7	36.8	4.9
E 109	71	Mud-Sand	21.6	36.8	4.9
E 103	71.6	Mud-Sand	21.5	36.8	4.8
EA2 329	72	Mud-Sand	22.9	36.8	5.2
EA1 329	72	Mud-Sand	22.8	36.8	5.2
E 87	72	Sand	22.8	36.9	5.1
E 125	72	Mud	22.7	36.9	5.2
E 136	72	Mud	22.6	36.9	5.1
E 124	72.3	Mud	22.6	36.9	5.1
EA2 328	73	Mud-Sand	22.5	36.9	5.1
E 88	73	Sand	22.4	36.9	5.1
E 119	74	Mud-Sand	22.3	36.9	5
E 118	76	Mud-Sand	22.8	36.9	5.1
EA1 328	78	Mud-Sand	22.7	36.9	5.2

EA2 327	82	Mud-Sand	22.6	36.9	5.1
E 147	89	Mud-Sand	21.6	36.7	5.7
EA1 327	95	Mud-Sand	28.7	35.9	6.8
EA1 326	98	Mud-Sand	29.2	35.3	6.7
E 390	110	Mud-Sand	13.9	35.7	4.2
EA2 323	124	Mud-Sand	13.8	35.7	4.2
EA2 322	125	Mud-Sand	13.8	35.7	4.2
EA1 322	126	Mud-Sand	13.7	35.7	4.2
EA2 321	128	Rock	13.7	35.7	4.2
E 139	145	Mud	13.7	35.7	4.2
E 133	148	Mud	13.6	35.7	4.2
E 90	150	Sand	13.6	35.7	4.2
E 100	150	Mud-Sand	13.6	35.7	4.2
E 110	150	Mud-Sand	13.5	35.7	4.2
E 121	150	Mud	13.5	35.7	4.2
E 122	150	Mud	13.5	35.7	4.1
E 138	150	Mud	13.4	35.7	4.1
E 142	150	Mud-Sand	13.4	35.7	4.1
E 163	150	Sand-Rock	13.4	35.7	4.1
E 94	151	Sand	13.3	35.6	4.1
E 120	151	Mud	13.3	35.6	4.1
E 141	151	Mud	13.3	35.6	4.1
E 162	151	Sand-Rock	13.2	35.6	4.1
E 89	152	Sand	13.2	35.6	4.1
E 111	152	Mud-Sand	13.2	35.6	4.1
E 101	153	Mud-Sand	13.1	35.6	4.1
E 132	153	Mud	13.1	35.6	4.1
E 95	154	Sand	12.9	35.6	4.1
E 123	154	Mud	12.9	35.6	4.1
E 156	155	Sand-Rock	12.9	35.6	4.1
E 159	158	Sand-Rock	8	34.9	3.8
E 155	160	Mud-Sand	8	34.9	3.8
E 160	160	Sand-Rock	7.9	34.9	3.8
E 524	180	Coral rubble	7.9	34.9	3.8
EA2 320	190	Rock	7.9	34.9	3.8
EA1 319	220	Rock	7.9	34.9	3.8
EA2 315	230	Rock	7.9	34.9	3.8
EA2 316	240	Rock	7.9	34.9	3.8
EA1 315	250	Rock	7.9	34.9	3.8
EA1 317	270	Rock	7.8	34.8	3.8
E 153	270	Mud-Sand	7.8	34.8	3.8
E 154	280	Mud-Sand	7.8	34.8	3.8
E 112	300	Mud-Sand	7.8	34.8	3.8
E 113	300	Mud-Sand	7.8	34.8	3.8
E 91	305	Sand	7.8	34.8	3.8
E 144	309	Mud	7.8	34.8	3.8
E 145	309	Mud	7.8	34.8	3.8
E 140	487	Mud	7.8	34.8	3.8
E 92	493	Sand	7.8	34.8	3.8
E 93	496	Sand	7.8	34.8	3.8
E 114	498	Mud-Sand	7.8	34.8	3.8
E 150	500	Mud-Sand	7.8	34.8	3.8
E 115	504	Mud-Sand	7.8	34.8	3.8
E 149	507	Mud-Sand	7.8	34.8	3.8
E 559	1673	Mud	6.7	34.8	4.1
E 379	1796	Mud	6.8	34.8	4.2
E 598	2887	Mud	6.6	34.8	4.3
E 378	2910	Mud	6.6	34.8	4.1
E 375	3134	Mud	6.6	34.8	4.1
E 601	3290	Mud	6.6	34.8	4.3
E 605	3600	Mud	6.6	34.8	4.1
E 608	3700	Mud	6.6	34.8	4.5
E 606	3888	Mud	6.8	34.8	4.2

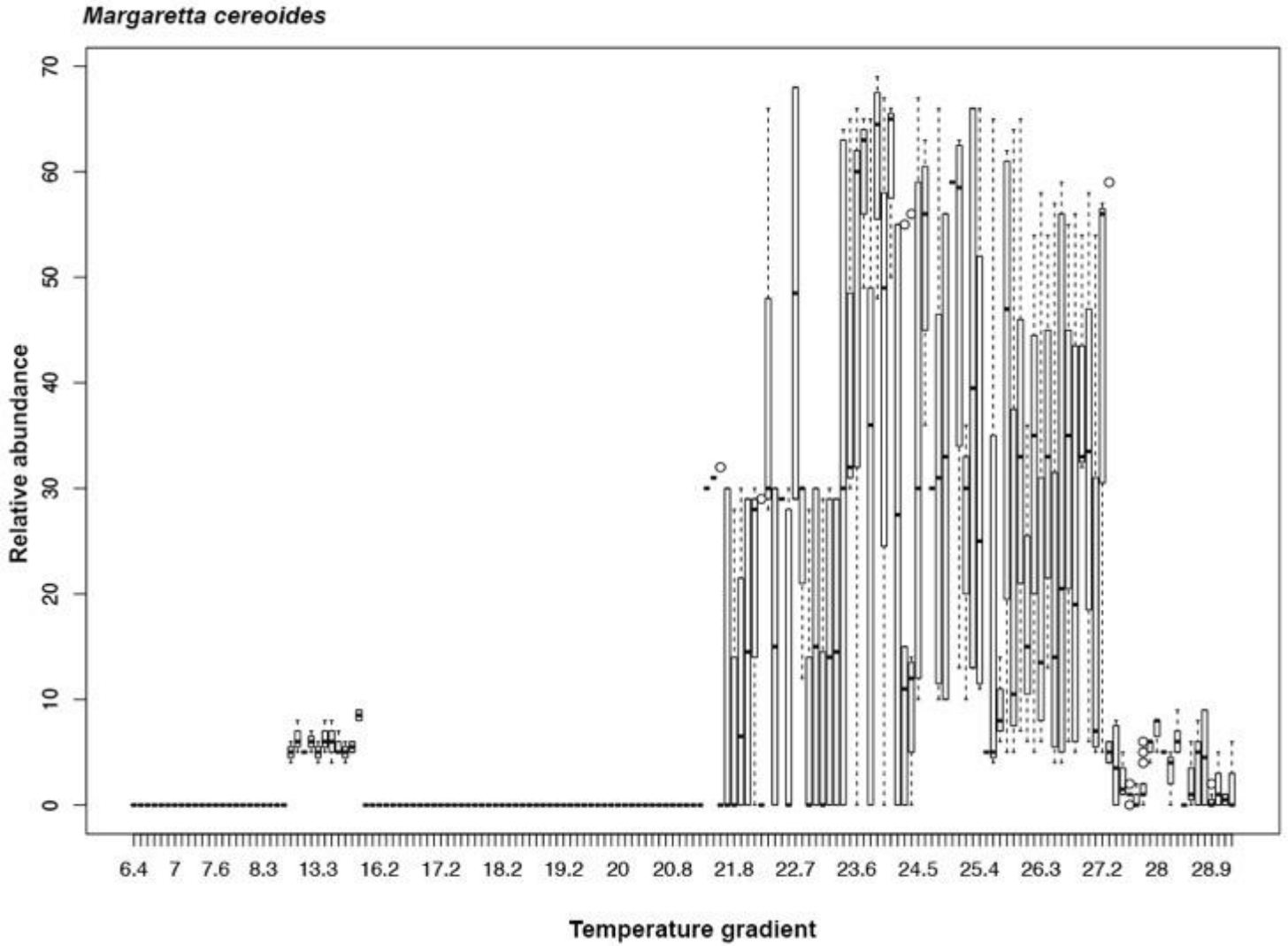
Supplementary material III: Relative abundance of *Adeonellopsis subsulcata* in a temperature gradient in the Colombian Caribbean.



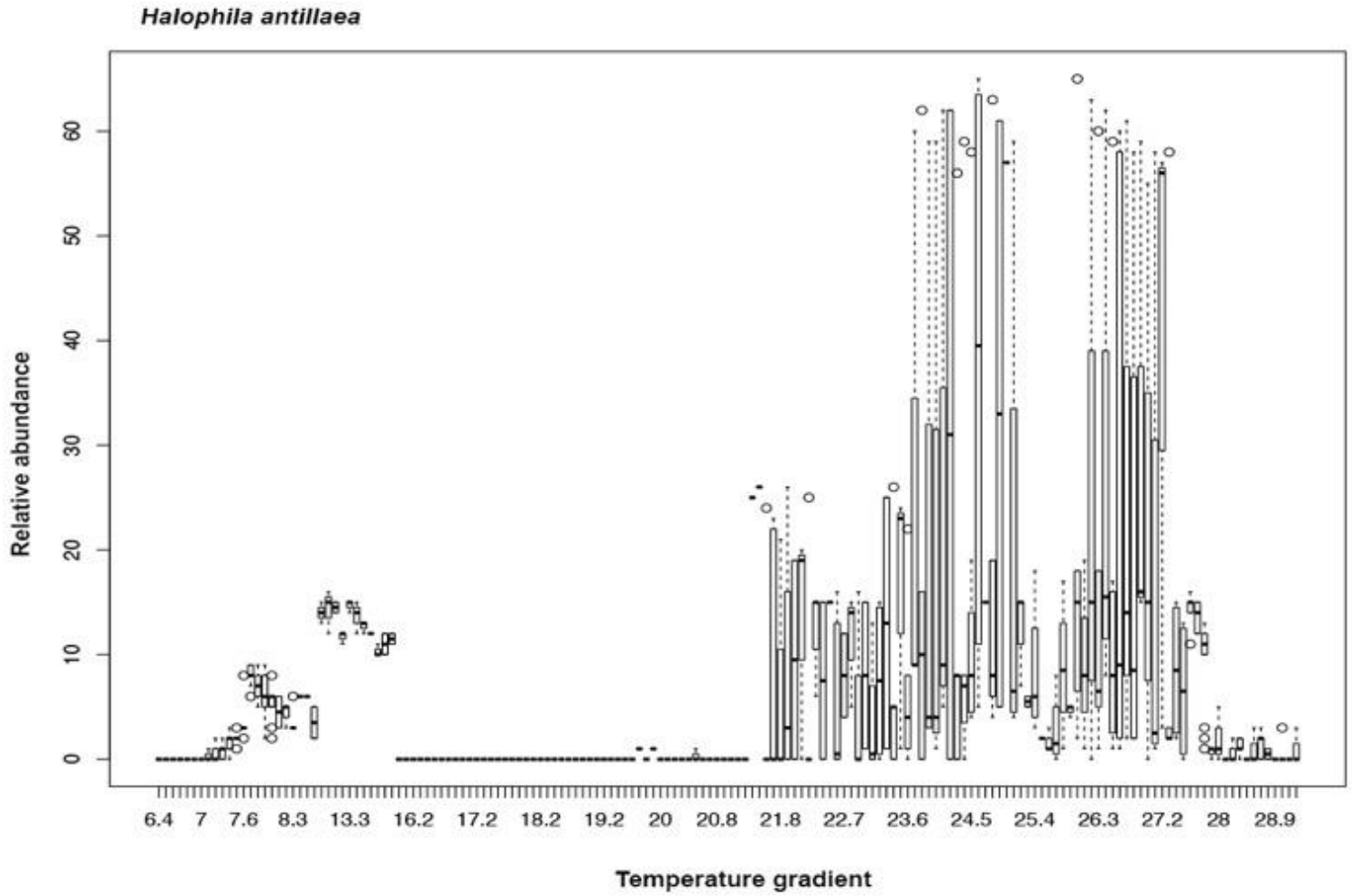
Supplementary material IV: Relative abundance of *Steginoporella magnilabris* in a temperature gradient in the Colombian Caribbean.



Supplementary material V: Relative abundance of *Margaretta cereoides* in a temperature gradient in the Colombian Caribbean.



Supplementary material VI: Relative abundance of *Halophila antillaea* in a temperature gradient in the Colombian Caribbean.



Supplementary material VII. Deep-Sea bryozoan species found in COL10 that require further taxonomic identification.

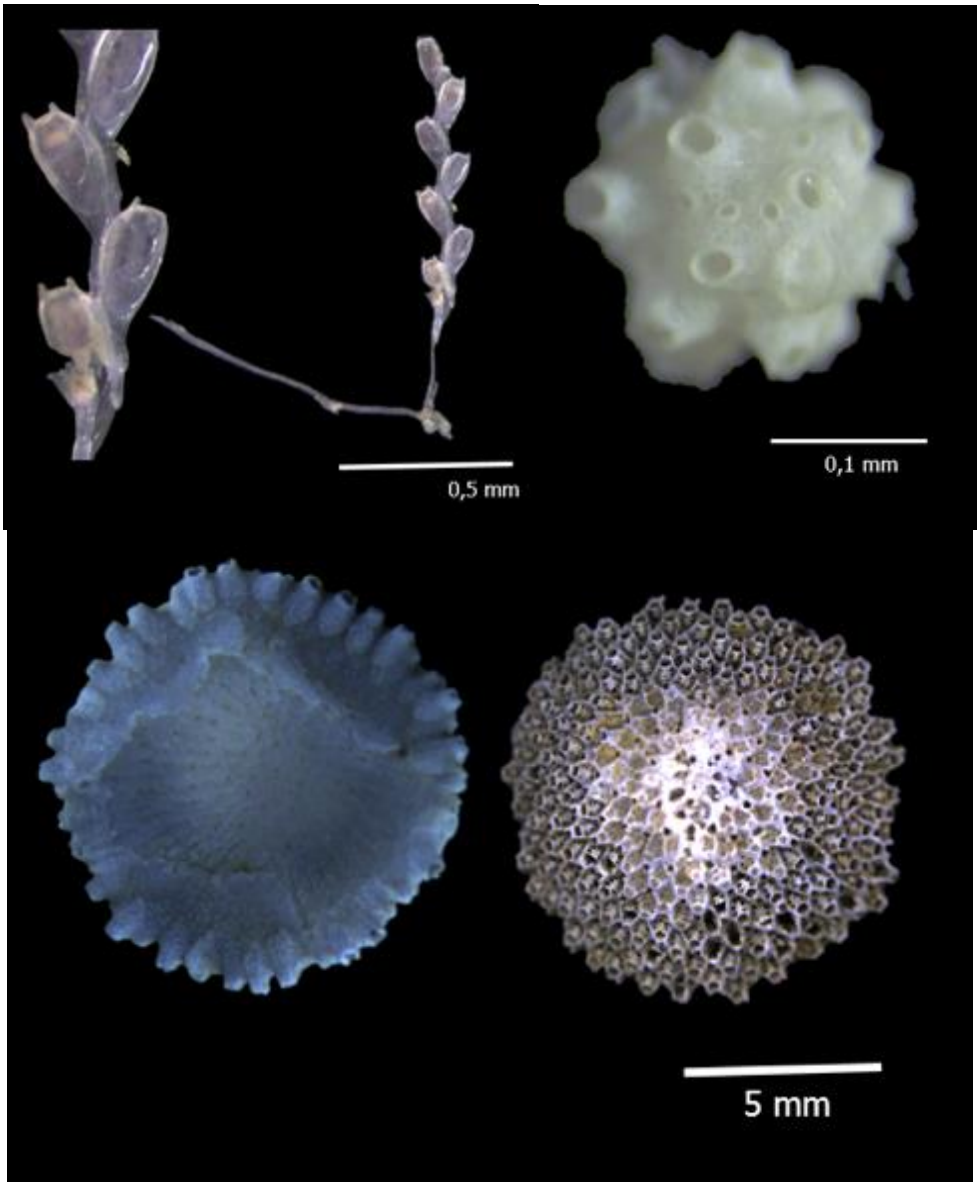


Figure 1. *Top left*: Bugulidae; *Top right:* incertae sedis *Bottom: Discoporella* sp.

Chapter 4: Relationship between environmental factors and the reproductive strategy of *Bugula neritina* (Phylum: Bryozoa)

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ABSTRACT

Bryozoans have the evolutionary ability to reproduce sexually and asexually and to switch between these strategies in response to changes in the environment. The degree of adaptability of an individual species is reflected in its ability to produce sexual structures such as ovicells in response to natural fluctuations in its environment. To examine this in a widely distributed bryozoan, several colonies of *Bugula neritina* were collected in contrasting geographical areas of the Atlantic and Pacific Oceans from 1 to 5m depth. This sampling approach allowed us to evaluate the reproductive strategies developed under different climatic seasons, substrates, and environmental factors and determine if there is a relationship between these factors and reproduction. Zooid size and colony size were measured as part of the evaluation of their reproductive strategy. Our results showed that colonies inhabiting similar ecosystems with contrasting environmental factors differed in their reproductive strategies and morphometry. In addition, we suggest a possible trade-off between zooid features and ovicell production, when colonies invested in sexual polymorphisms, a reduction in zooid and colony size was observed. The environmental factors that mostly affect the reproductive strategy of *B. neritina* were salinity, temperature and primary productivity. Specimens in the Colombian Caribbean reproduce sexually during the dry season and invest in colony growth by budding during the rainy season.

Keywords: Bryozoa, Colombia, SFF Malpelo, Caribbean, environmental conditions, ovicell density.

INTRODUCTION

In marine organisms, reproductive strategies refer to the amount of energy allocated to the production of gametes and protection of the offspring (Corriero et al., 1996; Vance, 1973), sexual maturation could be considered a reproductive strategy (Clutton-Brock, 1991). Several invertebrates like bryozoans, have developed different reproductive strategies in the marine environment to ensure their ecological success within the ecosystems they inhabit (Ryland, 1976). These strategies are a consequence of internal and external stressors that might affect their populations such as environmental disturbances and reproductive physiology (Ostrovsky, 2013; Blackburn, 1999; Cancino et al., 1991; Pearse et al., 1989). Bryozoans can invest in highly energetically expensive sexual (mictic) reproduction (Thomsen & Håkansson, 1995) and fast asexual (amictic) growth (O'Dea et al., 2008) and animals have the ability to switch between them as a response to their environment (Ostrovsky, 2013; Hoshi et al., 2003; Barnes & Clarke, 1994; Thorson, 1950).

Sexual reproduction promotes gene flow between geographically distant populations, increases genetic diversity and allows the colonization of new habitats (McEdward, 1995; Thompson et al., 1987). Bryozoans are hermaphrodites with gonads separated basal-distally (Woollacott, 1999). In these animals, fertilization can take place in different parts of the zooecium, either inside the zooid cavity, the ovicell or during the release of eggs (Jenkins et al., 2017; Ostrovsky, 2009; Silén, 1966); self-fertilization has also been observed in dioecious species with sexual dimorphism (Hughes & Wright, 2014; Hughes et al., 2002; Hunter & Hughes, 1993). Either way, sexual reproduction is a mandatory strategy in the bryozoan life cycle not only for dispersal but for ecological success (Ostrovsky, 2013).

After fertilization, bryozoans develop one or more planktotrophic or lecithotrophic swimming larvae (Jenkins et al., 2017; Ryland, 1974), which depending on their feeding needs, could settle in short time after release (Wendt, 1996; Winston, 1978;1988). Once attached to a substrate, larvae undergo metamorphosis to the primary zooid or ancestrula (Pechenik et al., 1998; Reed, 1991) (Figure 1). The energetic costs of sexual reproduction can be expensive for the populations, as it includes larvae breeding per mature zooid, the maintenance of embryos, and the

performance of larvae after being released in the plankton (Johnson, 2010; Ostrovsky & Shunatova, 2002). In the case of non-feeding larva as in *Bugula neritina*, survival, metamorphic competence, growth time and fitness (Wendt, 1998; 2000; Pechenik, 1990) will depend on the larvae's motility and ability to cope with the environment and the rapid selection of suitable habitats for establishment (Ostrovsky, 2013; O'Dea & Jackson, 2009; Shunatova & Ostrovsky, 2002).

Because of the clonal nature of cheilostome bryozoans, they have the ability to regenerate (O'Dea, 2006; Winston, 1983; Levinsen, 1907), and perform zooid budding immediately after the development of the ancestrula, which has many adaptative benefits for the colony success, including faster colonial growth and faster rate of food capture (McKinney & Jackson 1989) (Figure 1). Also, cheilostomes that invest higher energy in asexual growth have the ability to undertake division of a parent colony for the dispersal of subpopulations with equal genetic information as in the case of branching tree-like bryozoans and cupuladriids (Ostrovsky, 2013; Ostrovsky et al., 2009; O'Dea et al., 2008; Winston, 1983), in which a whole new colony grows from a single piece (Thomsen & Häkansson, 1995; O'Dea et al., 2004). This strategy guarantees a larger spatial distribution than through sex but has been recognised as less cost effective because it reduces the potential of adaptation of a species and their populations, leading to local extinctions (O'Dea, 2006; Keough & Chernoff, 1987).

Studies mentioning dispersal through fragmentation in *Bugula neritina* are scarce, but a few have indicated that this strategy represents an important ecological feature that allows the establishment of clones in habitats that could not be colonised by larvae otherwise (McKinney & Jackson, 1989; Connell & Keough, 1985). However, their growth and success after transplantation will depend on the environmental factors in the newly colonised areas (Schuster et al., 2019).

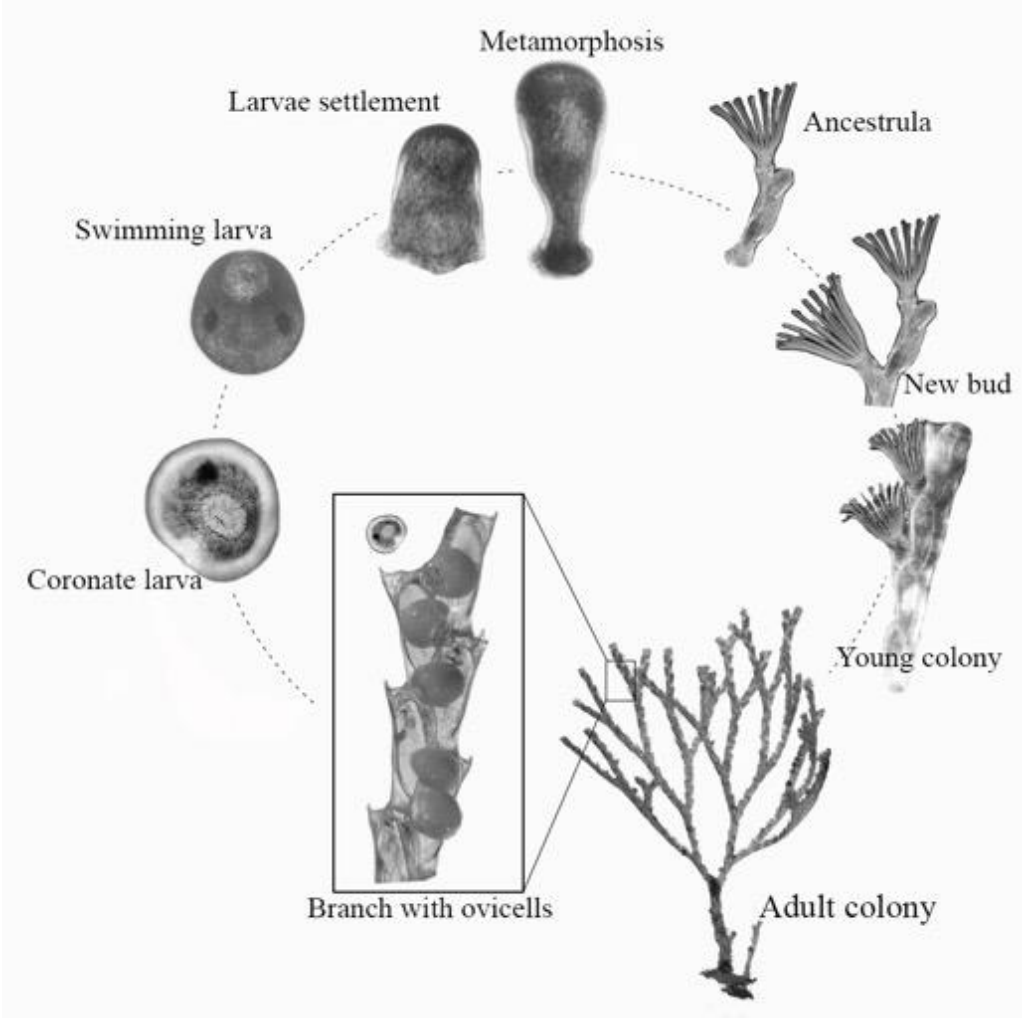

Figure 1. Reproductive life cycle of *Bugula neritina. (*Diagram based on Yang et al., 2018; Wong et al., 2010 and VYN biological sample from Colombian Caribbean).

Bryozoan species with mostly asexual strategies, produce fewer fertile zooids than those that engage in sexual reproduction, because of the expensive costs of larval production and brooding, this may be the reason why fragmentation may overtake a brooding strategy in some cases (Thomsen & Håkansson, 1995). The investment in sexual reproduction will leave fewer resources for asexual reproduction and vice versa (Hughes et al., 2002). The best reproductive strategy will also differ across environments (Ostrovsky, 2009), seasonality (McEdward, 1995) and substrates (host) (Clarke, 1988; Cancino, et al., 1991). Species with a widespread distribution are likely to perform reproduction in different climatic seasons (Lidgard, 1990; Ryland, 1976) in comparison to the tropical ones which are expected to reproduce continuously (Jackson & Wertheimer, 1985).

Also, sexuality is favoured under high primary productivity (O'Dea et al., 2004) as large energy intake is required to support gamete production, while clonal growth requires less energy to regenerate fragments (Håkansson & Thomsen, 2001; O'Dea, 2006). An adaptive feature of sexual reproduction is the ability to suspend propagation under less favourable conditions and to store energy resources (Hunter et al., 1998; Harvell & Grosberg, 1988; Hall & Hughes, 1996). Reproduction will resume when conditions improve and thrive back at the end of natural disturbances, this represents an advantage over the asexual strategy despite requiring additional energy for gametogenesis and brooding (Wendt, 1998; 2000).

Ectotrophic larvae like *B. neritina* are more resilient to low primary productivity than planktotrophic larvae in other species, but the establishment of the colony will depend on the available energy source to feed the ancestrula that then will promote budding (Jaeckle, 1994; Keough, 1989;1986). For instance, food availability is the main environmental factor determining the establishment and the extent of occurrence in bryozoans (Ostrovsky, 2013). Other factors such as temperature, salinity, pH, turbidity, sedimentation rate, depth, and current patterns also have important contributions to the establishment of larvae and their success (Schuster et al., 2019; Pecquet et al., 2017). Some bugulids have high tolerances to variations in salinity which allow them to inhabit estuarine areas and settle for longer periods than stenohaline species (Winston &Wollacott, 2008; Abbott, 1973).

Bugula neritina is a model system for the understanding of the relationship between biotic and abiotic factors, the sexual reproductive strategy and clonal growth. This species belongs to the "*Bugula neritina* species complex" in which to date three genotypes have been identified (Types S, D and N) who's main distinguishable feature is their geographical and bathymetric preferences (Fehlauer-Ale, et al., 2013; 2015). Due to its worldwide distribution, it has been classified as an invasive and introduced fouling organism in several temperate and tropical parts of the world, and the Caribbean (Ryland et al., 2011), but its native origin has not been identified to date.

By understanding the environmental factors involved in its reproductive strategy, biocontrol can be implemented on its non-native populations. The use of species distribution models can be useful to predict potential colonisation areas by analysing

habitats with suitable environmental conditions for the establishment of larvae that encourages sexual reproduction and the fouling nature of *B. neritina*.

This research focused on understanding the reproductive strategies observed in *B. neritina* in different parts of the Atlantic and Pacific oceans in regards the production of sexual polymorphisms such as ovicells, the presence of mature zooids, their density per colony and the relationship between those features and the environmental factors of the inhabited areas. Here we suggest that engagement in sexual reproduction is related to seasonality, and environmental changes by measuring and counting the number of ovicells in two different climatic seasons in the Caribbean and South West coast of England. Differences were observed under different temperature and salinities at the same location. Some areas were characterized for presenting the lowest ovicell production during the sampled times indicating a possible link with the environmental conditions and the switch to asexual strategies.

METHODS

Species used

Order: Cheilostomata Busk, 1852
Suborder: Flustrina Smitt, 1868 (part)
Superfamily: Buguloidea Gray, 1848
Family: Bugulidae Gray, 1848
Genus: *Bugula* Oken, 1815

***Bugula neritina* (Linnaeus, 1758)**
(Figs. 1,3,4)
Bugula neritina Osburn, 1914, p. 186; 1927, p. 126; 1940, p. 389; Marcus, 1937: 67; Winston, 1982: 129.

Description. Red pigmented Erect, branched unilaminar colonies. Present large elongated zooids tapered proximally (averaging 307µm in width and 914µm in length) with pointed outer distal corners. Zooids are biserial along the unjointed

branches. The frontal wall is covered by the frontal membranous flap. This species has large, hyperstomial and globular ovicells distally attached to the corners of zooids by a short stalk, opposing the pointed corners.

Distribution: *B. neritina* is reported almost worldwide and considered to be an invasive species in some parts of the world (Porter, 2012). It ranges from warm-temperate to subtropical coastal waters. Molecular studies demonstrated that it actually corresponds to a complex cryptic species complex with three recognized genotypes (Types S, D and N), the main differences between them are their bathymetric and geographical distribution, however, type S is distributed worldwide from intertidal zones to 2m depth.

Ecological remarks. This species is one of the most well-known bryozoans due to its invasive and fouling nature (Porter, 2012) and also because it has been studied in the pharmaceutical sector for its bioproduct Bryostatin, a natural anti-leukaemia and anti-Alzheimer chemical. *Bugula neritina* reproduces from January to June in the western Atlantic coasts of Florida (Winston, 1982) and from the summer months until before winter season in temperate areas like the south coast of England. There is no evidence of reproductive seasonality in the Caribbean or Tropical Eastern Pacific coast of Colombia.
Within the ovicells, a specialised lining enables the production and transmission of nutrients to the embryos that develop within the brood chamber lumen (Woollacott & Zimmer, 1972; 1975). The increase in ovicell size leads to the subsequent increase in nutrient provisioning for the larva (Woollacott & Zimmer, 1975). The increased parental provisioning promotes higher survival of the non-feeding larva since it has to endure a swimming period and metamorphosis to a feeding ancestrula.

Study areas

Fresh colonies of *B. neritina* were obtained from six locations of the Atlantic (Panama, Colombia, South coast of England) and Pacific (Tropical Eastern Pacific, Colombia) oceans at different depths (Table 1). Samples were associated with submersed mangrove roots, submersed rocks and pontoon areas. A map of the

geographical positions of the sampled areas for *B. neritina* collections is shown in figure 2.

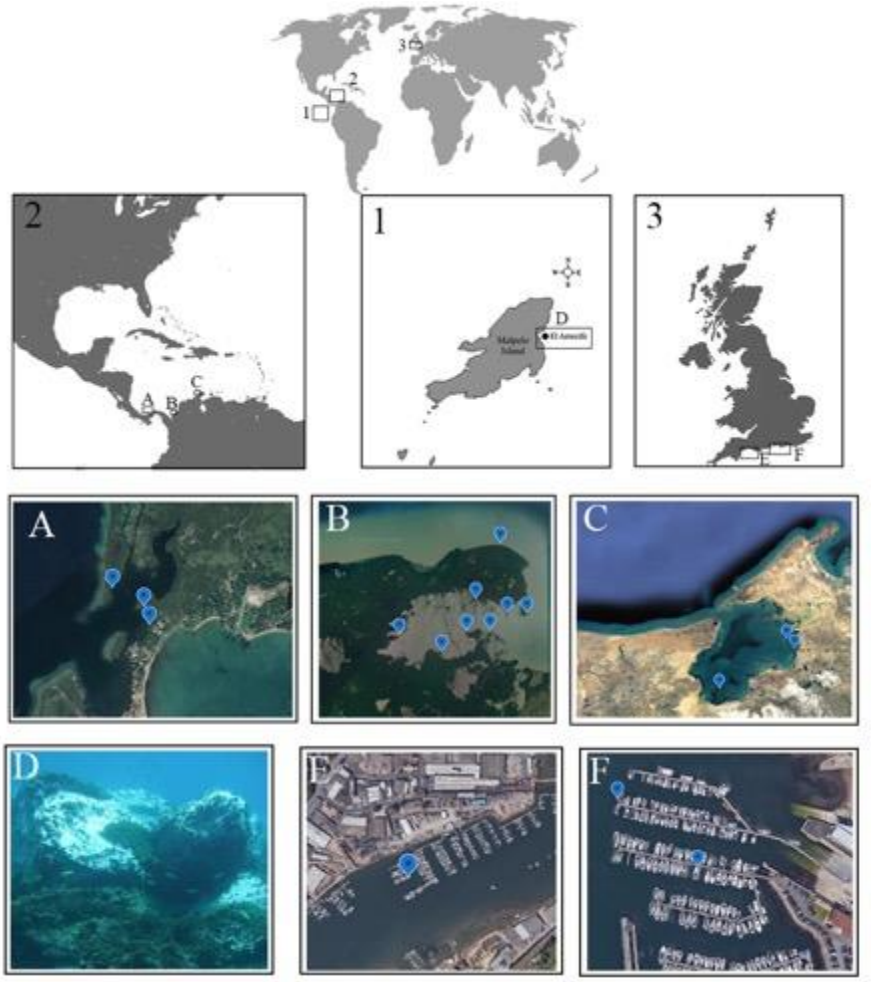

Figure 2. Geographical position of each sampled location used in this study. *Tropical Atlantic Ocean*: A) Bocas del Toro, Panamá. B) Bahia Cispatá, Colombia. C) Bahia Portete, Colombia. *North Atlantic Ocean*: F) Portsmouth, UK. G. Plymouth Sound, UK. *Tropical Eastern Pacific*: D. SFF Malpelo, Colombian Pacific.

Description of sampling locations

Atlantic Ocean

Bocas del Toro, Panamá, Caribbean

It is a province of Panamá composed of several small islands and part of the mainland. In this region, wet season is overcast, and dry season is cloudy; the environmental temperature varies from 22 to 30 C all year long, the warmest months are August and September. The annual upwelling season from January to April,

introduces colder, more saline and nutrient-rich water (Schaefer et al., 1958). During the rainy season, May to December, waters become warmer, diluted and poorer in nutrients (Smayda, 1966; Wooster, 1959), rain can fall up to 20 consecutive days and accumulate up to 56 cm of precipitation decreasing salinity. The average sea surface temperature ranges between 25 to 29°C. Sampling in this area was carried out in June 2017 in three mangrove areas near The Smithsonian Research Institute in Bocas del Toro province (Table 1; Figure 2).

Bahia Portete, La Guajira, Colombian Caribbean.

La Guajira is the northern part of Colombia and South America, in the Caribbean Sea (Corpoguajira & Invemar, 2012). It is characterized by a warm and very dry weather with environmental temperature ranging from 29°C to 39°C. The rainy season occurs between June and November followed by very dry months. The continental shelf has fine muddy sediments which supports a rich biodiversity of coastal fauna. In general, the sampling area is shallow with warm sea surface (Andrade, 2000) with an average current velocity of 57-87 cm/s (Bernal et al., 2006) highly influenced by the introduction of freshwater by the runoff rivers. This area is characterized by high upwelling events and high turbidity which allows the presence of marine suspension feeders like bryozoans. Sampling was made in October 2016 and April 2018 in three mangrove areas at the Bahia Portete National Park in the Uribia municipality (Table 1; Figure 2).

Bahia Cispatá, Cordoba, Colombian Caribbean.

It is located on the Colombian Caribbean coast at the southwest of the Gulf of Morrosquillo in the department of Córdoba. It is characteristically an area with brackish waters highly influenced by several channels from the lower basin of the Sinú river (Invemar-CVS, 2010). This area has a unimodal weather, with a defined dry season between December and March with a maximum environmental temperature of 40°C, followed by a constant wet season from April to November with temperature between 26 and 28° C (IAvH & CVS, 2006). The area consists predominantly of submersed mangrove trees which roots provide an ideal habitat for marine invertebrates and fishes; the most common species providing shelter to marine fauna is *Rhizophora mangle* (Sánchez-Perez et al., 2005). The area is also characterized by severe rain disturbances and big fluctuations of salinity,

temperature, high turbidity and high primary productivity; the mean sea surface temperature is 27°C. Sampling was made in December 2016 and July 2018 in seven mangrove areas (Table 1; Figure 2). It is important to mention that during the dry season the area was affected by the actions of the 2016 Caribbean hurricane season.

Portsmouth Marina, England, United Kingdom.

The Gosport marina in Portsmouth, Hampshire is an important commercial marina. Its geographical position confers a mild climate, the sea surface average temperature in winter ranges between 5 °C to 10 °C; in summer the temperature ranges from 15°C to 19.5°C (Arenas et al., 2006). The water is eutrophic (El-Shenawy et al., 2010) and because of the marine traffic of the zone, it is considered one of the entrance spots for the introduction of non-native species. Sampling was made in May 2017 and October 2018 in a pontoon area (Table 1; Figure 2).

Plymouth Marina, England, United Kingdom.

The Coxside pontoon is a traditional tidal harbour connected to Plymouth Sound, on the south coast of Devon. It is characterized by a low traffic activity and is located right next to an important harbour area (Sims et al., 2004; Cooper, 1985). The sea surface temperature ranges between 4 °C to 10 °C in winter and in summer the temperature ranges from 14°C to 22°C (de Castro et al., 2018; Bremekamp, 2012). Sampling was made in May 2016 and October 2018 in two pontoons (Table 1; Figure 2).

<u>Tropical Eastern Pacific Ocean</u>

Sanctuary of Flora and Fauna Malpelo, Colombia.

It is located offshore in the central region of the Colombian Pacific Ocean and is surrounded by several smaller islands (Kiesser & Hoffman, 1975). The island is part of the underwater "Dorsal Malpelo" ridge. It was declared a marine protected area characterized by climatic conditions influenced by El Niño- Southern Oscillation, as well as strong surface and deep currents. The sea surface temperature ranges from 24 to 25.5° C through the year. It has a high biodiversity and recruitment of marine

invertebrates, including bryozoans (Yepes-Narvaez & Chasqui, 2019). Sampling was made in October 2016 in two sites attached to the natural rock (Table 1; Figure 2).

Table 1: List of collection sites of *Bugula neritina* and associated information. Ambient, m: marine; es: estuarine.

Ocean	***Country***	***Area***	***Ecosystem (ambient)***	***Locality***	***Coordinates***	***Depth (m)***
Atlantic	Colombia	Cispatá				
Atlantic	Colombia		Mangrove (m)	Punta Bonita	9°24'28.5"N 75°46'7,7"W	2
Atlantic	Colombia		Mangrove (m)	Punta Terraplén	9°24'46.2"N 75°47'42.0"W	2
Atlantic	Colombia		Mangrove (m)	Punta La Rula	9°24'28.5"N 75°48'46"W	1
Atlantic	Colombia		Mangrove (es)	Caño Salado	9°25'17.9"N 75°49'0.1"W	1
Atlantic	Colombia		Mangrove (m)	Caño Navío	9°24'17.1"N 75°50'22.3"W	1
Atlantic	Colombia		Mangrove (es)	Mestizos	9°25'43.6"N 75°49'20.3"W	1
Atlantic	Colombia		Mangrove (m)	Punta Róbalo	9°24'46.6"N 75°48'00.5"W	1
Atlantic	Colombia	La Guajira				
Atlantic	Colombia		Mangrove (m)	Bahia Portete	12°14'32" N 71°52'21"W	2
Atlantic	Colombia		Mangrove (m)	Bahia Portete	12°13'52" N 71°51'49"W	2
Atlantic	Colombia		Mangrove (m)	Bahia Portete	12°10'48" N 71°57'30"W	2
Atlantic	Panamá	Bocas del Toro				
Atlantic	Panamá		Mangrove (m)	STRI	9° 21'02"N 82°15'28" W	2
Atlantic	Panamá		Mangrove (m)	STRI	9° 21'05"N 82°15'47" W	3
Atlantic	Panamá		Mangrove (m)	STRI	9° 21'07"N 82°15'33" W	2
Pacific	Colombia	SFF Malpelo				
Pacific	Colombia		Rock (m)	El Arrecife	4°0'14.5"N 81°36'15.30"W	5
	Colombia		Rock (m)	El Arrecife	4°0'16.7"N 81°36'17.50"W	5
Atlantic	UK	Portsmouth				
Atlantic	UK		Pontoon (m/es)	Marina	50°48'35.24"N 1°06'05.73"W	1
Atlantic	UK	Plymouth				
Atlantic	UK		Pontoon (m)	Marina	50°21'40.18"N 4°7'56.00"W	1
Atlantic	UK		Pontoon (m)	Marina	50°21'49.18"N 4°7'28.17"W	2

Environmental factors

The sampling of environmental factors included two climatic seasons in most of the stations. The environmental factors measured *in situ* were, temperature and salinity using a portable conductivity meter (Thermo fisher Orion Star A322) and notes on the water turbidity, water movement and climatic season were made at the time of collection based on local reports and *in situ* observations.

Sampling design

In total 360 samples of *Bugula neritina* were hand collected through scuba diving or snorkelling in the Atlantic and Pacific oceans (Figure 2, Table 1). For mangrove areas*, B. neritina* colonies were collected in a 2m horizontal transect on top of submersed *R. mangle* roots from intertidal to 3m depth. For pontoon areas, samples were hand collected when spotted in a 3m transect under the floating deck in the intertidal zone at 1m depth. The pacific collections at SFF Malpelo corresponded to a systematic monitoring of the Marine Protected Area (MPA), when spotted, *B. neritina* colonies were collected at 5m depth and labelled. All samples collected were preserved in 96% molecular grade ethanol.

Sample preparation and measurements

Preserved samples were measured in the laboratory, the branching type, size of the colony, colour and number of sexual polymorphisms per branch were recorded with the associated environmental information. Subsequently, twenty branches of each sample were detached from the colony and cleaned in 40% sodium hypochlorite solution for one minute, rinsed and soaked in distilled water for another minute, before being soaked in the same solution again for another thirty seconds. This process of alternating bleaching solution and distilled water allowed the sample to be cleaned gently and avoid damage of key taxonomical features. After this, multiple photographs were taken of different portions of the colonies with a Zeiss AxioVision brightfield microscope and measured (Table 2). A portion of those was then gold-palladium coated for SEM imaging using a Zeiss Supra 40VP microscope. Thirty zooids were selected randomly from each subset of branches at all collection sites to make morphometric measurements using ImageJ software, measurements included, ovicell width (Wov), ovicell length (Lov) if ovicells were present; opesia

width (Wop) and opesia length (Lop); zooid width (Wz) and zooid length (Lz). In addition to the morphometric characteristics, ovicell density was measured by counting the number of ovicells per branches in each colony and position of the ovicells per colony for each location. Data was then compared with the environmental conditions at the sampling sites and suggestions on the possible reproductive strategy of *Bugula neritina* were made accordingly.

Species Distribution Modelling

To include additional environmental factors associated with the area of collection, a species distribution model for *B. neritina* was created using ModestR (García-Roselló et al., 2013). This model is a user-friendly platform with a powerful potential for the creation of species distribution maps. It was selected based on its quality to draw distribution maps according to the existing presence data stored in the Global Biodiversity Information Facility database (GBIF) and to discriminate between habitat type, which allows for convenient data cleaning and data verification. In addition, the species extent of occurrence can be improved by the addition of new presence data through the creation of new data folder through a Darwin core using our collection areas (Table 3; Figure 4).
For this purpose, an intensive data cleaning was performed in order to avoid misidentifications and errors in the presence data. This information was then coupled with 12 bioclimatic variables obtained from the Worldclim databases (worldclim.com), these gridded layers are modelled from data collected between 1970-2000 with different spatial resolutions, (30 sec (around 1 km^2) to 10 mins (around 340 km^2) and included the predictions of circulation models (GCMs) made by the IPCC Fifth Assessment Report (IPCC, 2004) and simulations of temperature and rainfall concentration pathways (RCPs) based on the high-resolution gridded data proposed by Hijmans et al. (2005).

Those conditions were also combined with the insitu variables collected during collections for each location and compared with the reproductive strategies observed in our model species using ModestR (García-Roselló et al., 2013; Guisande et al., 2017) to predict which environmental factors were likely determining their distribution and reproduction per zone (Caribbean, Pacific,

southwest UK). To avoid misinterpretation of the data in accordance with the newly identified *B. neritina* genotypes (S, D and N), the model was run individually per each zone (Figure 4; 6; Table 3).

Statistical analysis

All data were tested for normality using Shapiro test, if normal, data was analysed with one-way ANOVA and a Tukey's HSD post-hoc test analysis to determine if the colony size and ovicell density differed significantly between the areas sampled. Analyses were run in RStudio. Significant values were considered as below 0.05. For each test, 20 branches per location were analysed. When only two observations per variable were observed. To analyse if temperature and salinity have an effect on the ovicell density and the colony growth, a generalized linear model (glm) was used. For each area (Caribbean, Pacific and South West England) temperature and salinity ranges were different. For the Caribbean salinity had 3 observations and temperature 3; Pacific had for salinity 2 observations and for temperature 2; the South West England had for temperature 3 observations and for salinity 3.

RESULTS

Colony growth

Colonies associated with areas of high turbidity and primary productivity, (e.g. La Guajira) were mainly bigger than the rest and display around six primary bifurcations followed by about 24 secondary younger branches all covered with ovicells from the middle of the colony to the tips where the majority of orange embryos were spotted (Figure 3, A); these colonies were also pale brown coloured and attached to a natural substrate (*Rhizophora mangle*). The second most common branching pattern we identified was tree-shaped; with around four primary bifurcations and about 12 secondary younger branches. The colonies were bright red pigmented, shorter in height than the first-mentioned type and with yellow-orange embryos in the ovicells in the tip of the colony only, this type of colony growth was commonly found in areas with direct contact with the open sea like Panamá and SFF Malpelo. These locations also have strong water currents and stable salinity. The last growth pattern we observed was identified as delicate branched, we consider this growth

type mostly asexual or immature, characterized by presenting about three primary bifurcations and four secondary young branches approximately. These colonies presented the smallest density of ovicells and mostly located at the middle of the colony (Figure 4, Table 2). This pattern was observed in areas with high environmental disturbances and variations in salinity and temperature such as Cispatá Bay and the South West coast of England.

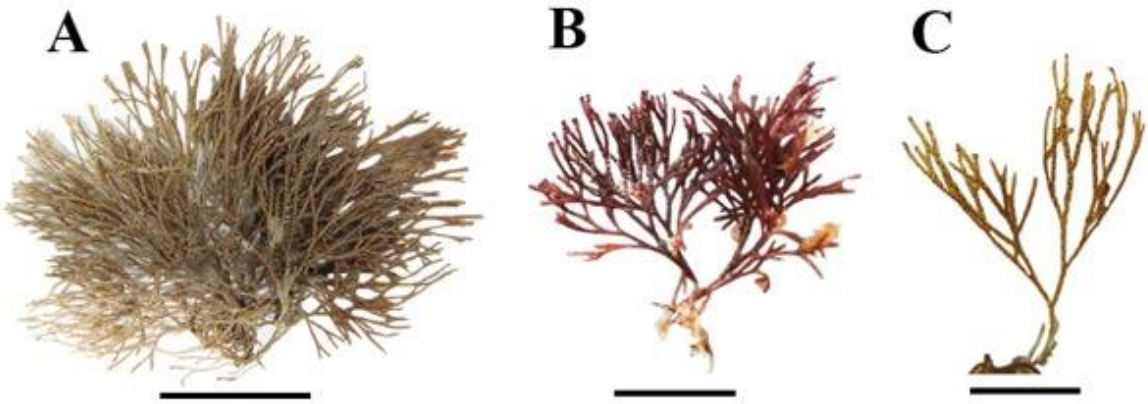

Figure 3. Colony types and complexity observed in *Bugula neritina* samples, A) Bushy, complex and big colony (La Guajira), scale bar: 2 cm. B) Tree, less complex than A, shorter branches (Panamá, Malpelo), scale bar: 1.5 cm. C) Delicate branched, more elongated than wide with few branches (Cispatá, Plymouth, Portsmouth), scale bar: 1 cm.

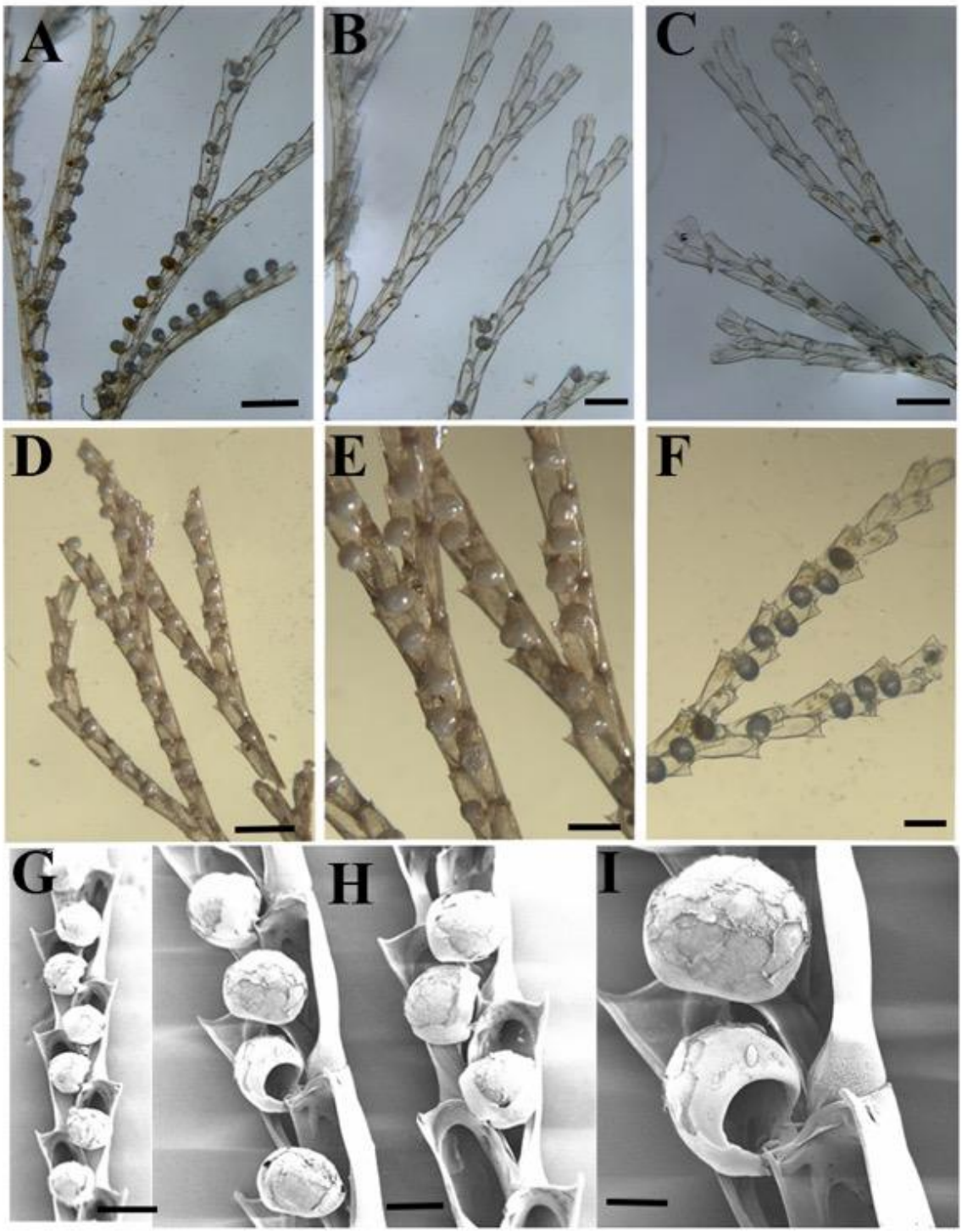

Figure 4: *Bugula neritina*. A-F Brightfield microscope images. A. sample from La Guajira, scale bar: 0.5 cm. B. Frontal view of sample from Cispatá scale bar: 0.5cm C. Sample from Portsmouth, scale bar: 0.5 cm. D. Sample from Panamá, scale bar: 0.8 cm. E. Sample from SFF Malpelo, scale bar: 0.5 cm. F. Sample from Plymouth, scale bar: 0.4 cm. G-I SEM images, Fragments from La Guajira. G. Frontal view of zooid organization, scale bar: 0.5 cm. H. Side view and detail of ovicells and pointed distal margin, scale bar: 0.3 cm. I. Detail of ovicells, scale bar: 0.2 cm.

Table 2: Morphometric features (µm) of *B. neritina* colonies from all the sampled locations. *Caribbean*: Bahia Cispatá (Cis); La Guajira, (Gua); Bocas del Toro, Panamá (Pan) *Pacific*: SFF Malpelo (Mal); *SW England:* Portsmouth Marina, (Port); Plymouth Marina (Ply).

Morphological measurements	***Caribbean Sea***			***Pacific Ocean***	***SW England***	
	Cis	***Gua***	***Pan***	***Mal***	***Port***	***Ply***
Wov(µm)						
n	10	30	30	20	10	10
Mean	579.026	289.927	281.828	301.736	431.153	298.156
Min	562.026	273.279	251.748	258.378	360.734	264.537
Max	595.422	310.271	330.542	332.964	482.317	321.447
SD	16.396	14.5337	29.1546	22.5503	36.1789	18.8681
Lov (µm)						
n	10	30	30	20	10	10
Mean	497.411	258.696	248.798	249.608	381.912	231.896
Min	476.438	227.97	216.562	227.665	341.886	216.714
Max	518.384	318.031	315.945	279.059	421.07	248.57
SD	20.973	27.2909	34.2045	17.9959	20.9007	9.19395
Wop (µm)						
n	30	30	30	30	30	30
Mean	312.175	190.171	116.491	148.81	244.926	169.416
Min	210.526	148.534	88.945	118.45	177.539	142.156
Max	382.26	262.035	163.871	186.857	328.027	216.801
SD	47.6664	31.4999	28.7133	18.6614	42.4413	26.2416
Lop (µm)						
n	30	30	30	30	30	30
Mean	961.181	685.635	740.569	475.25	890.408	562.287
Min	580.037	511.99	718.172	331.882	727.559	523.401
Max	1121.09	839.597	783.372	615.812	1030.19	613.388
SD	151.398	93.9371	25.2197	95.6466	103.691	35.3209
Wz(µm)						
n	30	30	30	30	30	30
Mean	392.575	266.694	226.591	226.034	372.987	247.805
Min	274.377	211.463	208.755	196.964	322.166	227.856
Max	550.423	338.657	260.335	258.794	415.572	274.882
SD	69.0505	38.6809	20.2208	19.5063	25.6176	16.3021
Lz(µm)						
n	30	30	30	30	30	30
Mean	1265.39	815.815	820.582	667.48	1052.75	679.882
Min	970.328	689.072	786.415	540.961	835.464	646.388
Max	1462.88	1014.64	886.004	755.715	1279.88	751.202
SD	135.064	101.762	39.754	84.0132	150.539	31.9095

Wov: Width ovicell; Lov: Length ovicell; Wop: Width opesia; Lop: Length opesia; Wz: Width zooid; Lz: Length zooid

Significant environmental factors

Based on the presence/absence data and modelled oceanographic conditions associated with the occurrences of *B. neritina*, the environmental factors that most strongly contribute to their distribution and reproductive strategy were bathymetry, silicates, temperature and salinity for the Caribbean samples; and nitrates, salinity and temperature for the South west England specimens (Figure 5). For the Pacific Ocean specimens, the model showed that bathymetry, dissolved organic Carbon and particulate organic carbon were contributing the most to *B. neritina* presence (Table 3).

Table 3: The environmental factors with their contribution percentage (min. cont. (%) that correlated with the distribution and reproductive strategy of *B. neritina* at the three zones sampled. In bold the greatest contributions.

Environmental Factor	***Caribbean***	***SW England***	***SFF Malpelo***
	Min. Cont. (%)	***Min. Cont. (%)***	***Min. Cont. (%)***
Bathymetry (Bat)	**95.21**	80.54	**98.01**
Dissolved Organic Carbon (Car)	81.35	81.20	**95.76**
Particulate Organic Carbon (POC)	80.45	84.71	**95.43**
Calcium Concentration (Con)	83.44	80.31	80.14
Chlorophyll a (Chl)	90.05	85.01	80.23
Silicate (Sil)	**95.95**	85.26	81.50
Temperature (T)	**97.23**	**95.42**	88.91
Salinity (Sal)	**97.29**	**97.25**	90.01
Nitrate (Nit)	85.33	**94.29**	91.20

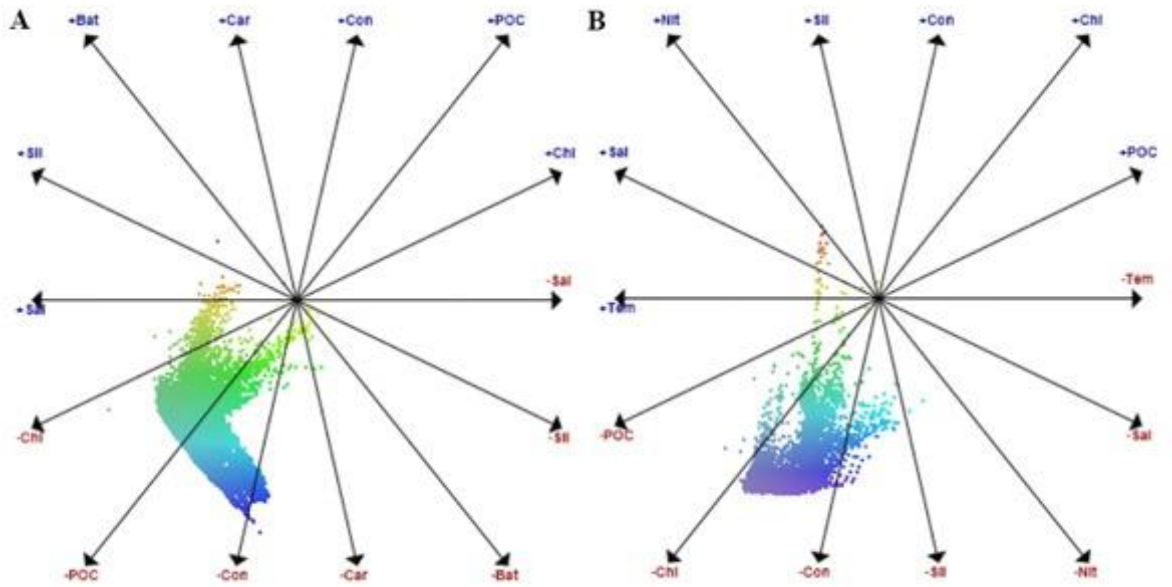

Figure 5: Environmental factors most favourable for *B. neritina* in A) the Caribbean and B) SW England. The factors shown in red (-) represent low concentrations while those in blue (+) represent high concentrations. Bathymetry (Bat), silicates (Sil), salinity (Sal), chlorophyll a (Chl), particulate organic carbon (POC), calcium concentration (Con), and dissolved organic carbon (Car).

Species distribution model

The species distribution model map created from combinations of the significant environmental factors using Alpha Shape and Kernel diversity shows suitable areas for the establishment of *B. neritina* and correlates to the extent of occurrence of our sampled colonies. Our modelled map was validated with the biological collections made in an 80%. In the map, bright red dots represent the maximum likelihood of environmental factors combination that determine the presence of *B. neritina* in that area, while a blue intense colour represent the least likelihood of occurrences. Our results also suggest that in addition of the density of ovicells per branch as means of reproductive strategy, the morphometric information measured of the specimens collected also differed from localities and environmental factors (Table 4).

Table 4: The significant environmental factors that correlated with the distribution of *B. neritina* and main morphometric differences.

	Cis	***Gua***	***Pan***	***Mal***	***Port***	***Ply***
Environmental factor	Low Sal; High Chl; High T	High Sal; High Chl; High Sil	Low Chl; High T; High Sil	High POC; High Sal; High Bat	Low Sal; High Nit; Low T	Low T; High Sal; Low Nit
Ovicell (Ov)	Large Ov size	Small Ov size	Small Ov area	Small Ov area	Small Ov area	Small Ov area
Opesia (Op)	Largest Op area	Small Op area	Small Op area	Small Op area	Smallest area	Small Op area
Zooid (Z)	Largest Z size	Small Z size	Small Z size	Smallest Z size	Large Z size	Large Z size
Ovicells density (mean)	12	97	89	85	10	9
Branches per colony (mean)	4	24	13	12	6	5
Colony length (cm)	6.2	4.5	4.1	4.3	7.1	7.5

Comparative analysis

A subset of thirty branches per locality were cleaned, measured and photographed in order to illustrate the ovicell densities, their distribution in the colony as well as to evidence morphological differences between all the sampled stations. The reduced density of ovicells in Cispatá and Portsmouth samples was evident in comparison to the samples from La Guajira, SFF Malpelo and Panamá (Figure 4; Table 2). Comparisons were made per collection zone (Caribbean, Tropical Eastern Pacific and South West England):

Caribbean

Ovicell density: Overall, the ovicell density was significantly different between the sampled areas (Bocas, Cispatá, Guajira) at all collection times (Anova, $p<0.05$), with the biggest difference between Cispatá and Bocas del Toro (Tukey HSD, $p<0.05$) (Figure 4; Table 5). Within stations, there were significant differences in the ovicell density between La Guajira and Cispatá Bay in both climatic seasons sampled, the rainy season (Anova, $p<0.05$) and the dry season (Anova, $p=0.06$), likely influenced by the environmental changes typical of each season. During the dry season, ovicell density was significantly lower in Cispatá (mean=5) than in La Guajira (mean=15), while during rainy season no ovicells were found in *B. neritina* samples in Cispatá stations. In Bocas del Toro it was not possible to compare climatic seasons because sampling was only carried out in the dry season in June.

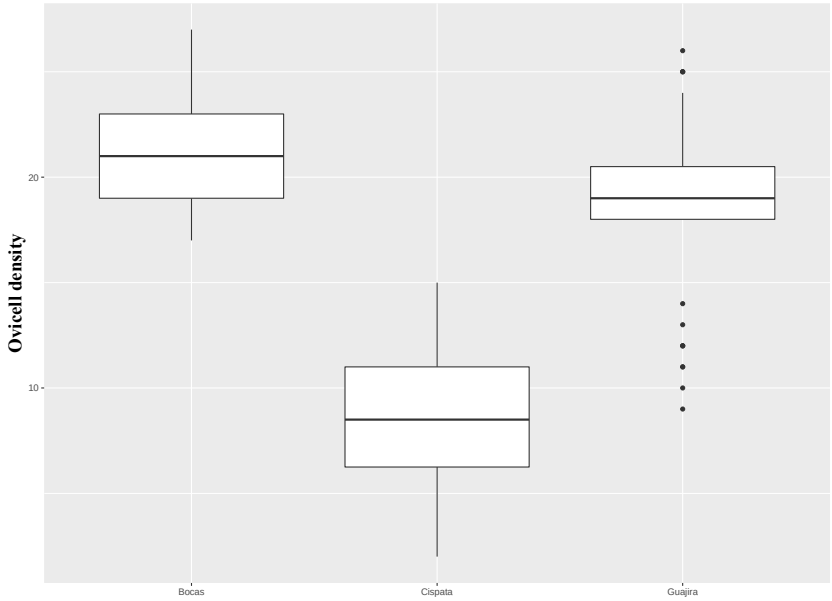

Figure 4. Differences between ovicell density per colony in the Caribbean areas analysed (Bocas, Cispatá and La Guajira).

The linear model showed that the interaction between temperature and salinity had a significative effect on the ovicell density in the Caribbean (Glm, $p<0.05$) (Figure 6), but no significant effect was observed on each factor independently (Table 5).

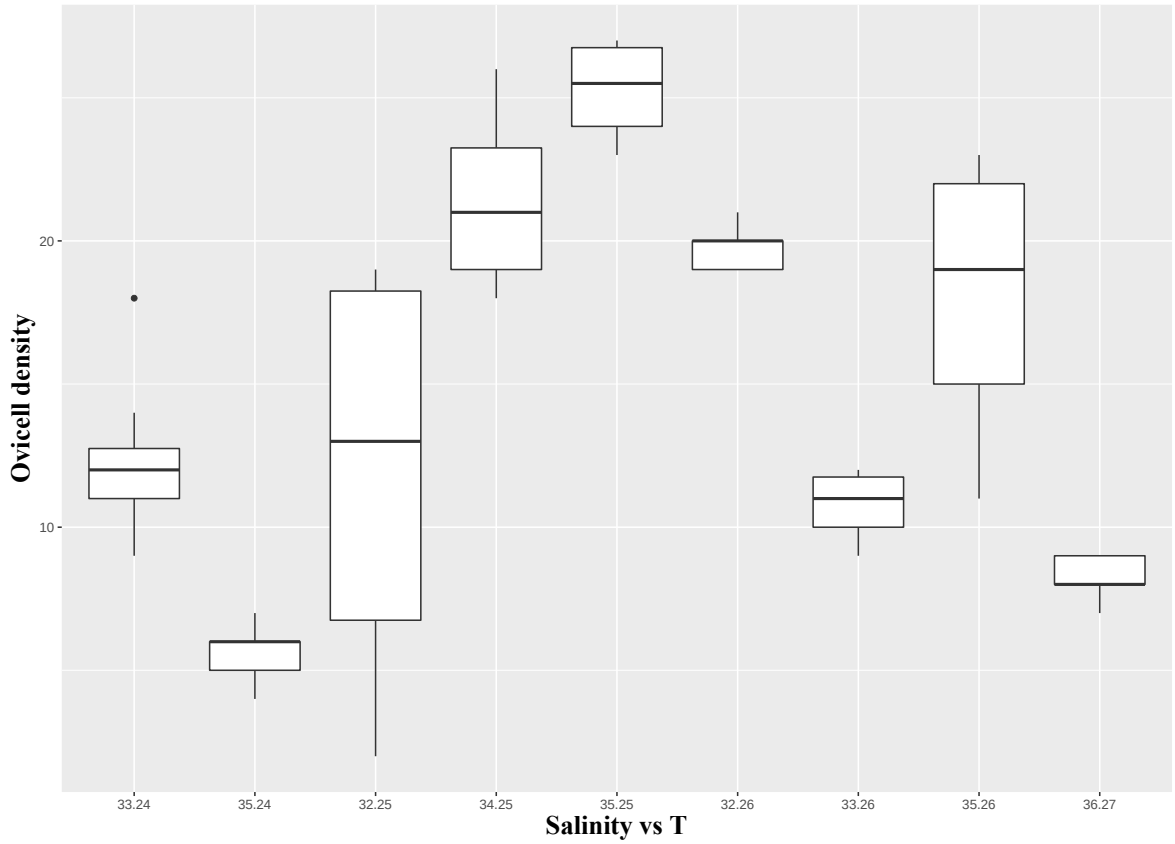

Figure 6. Effects of the interaction of salinity and temperature on the ovicell density in the Caribbean.

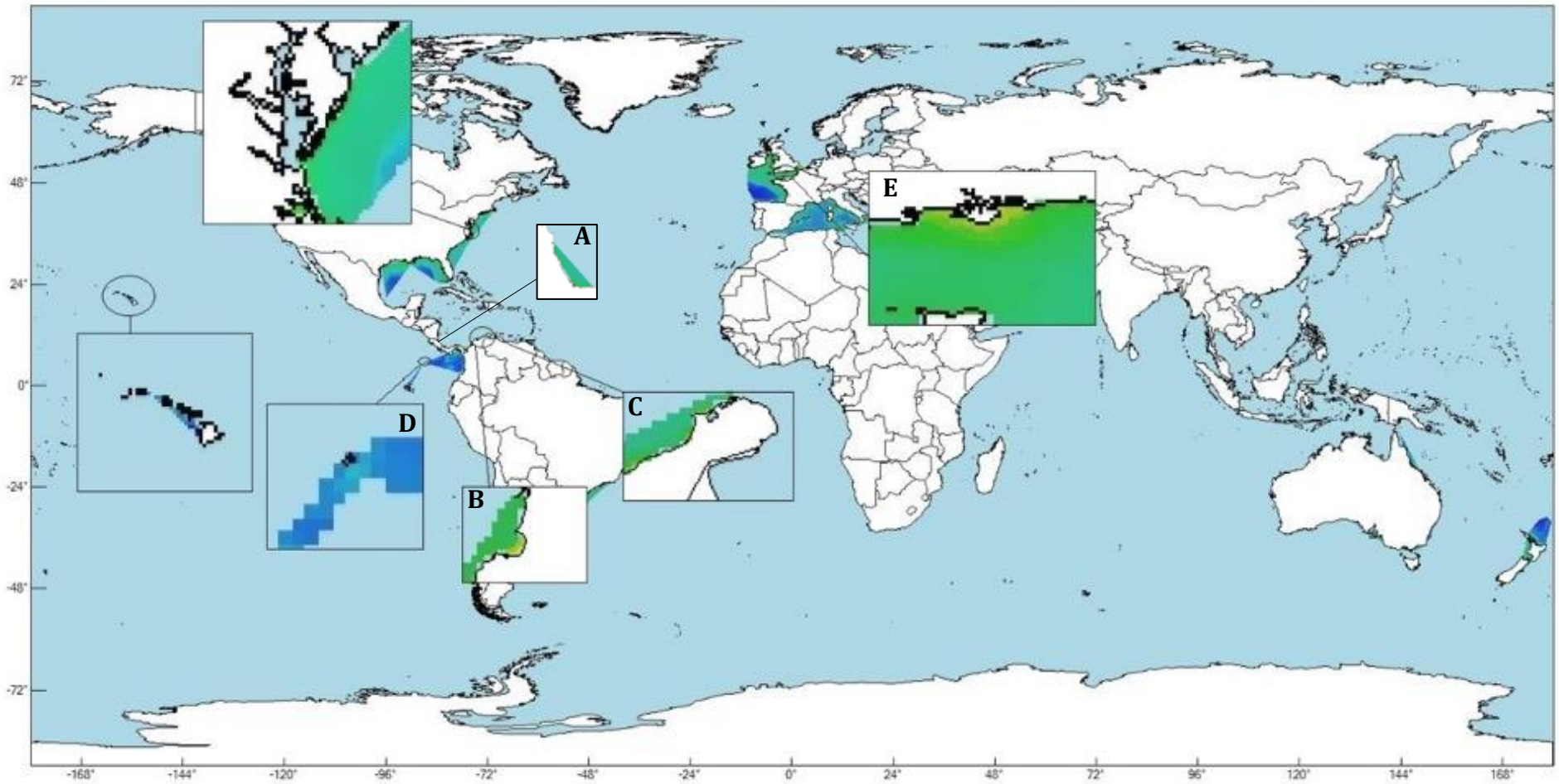

Figure 5: Species distribution model for *B. neritina* in Pacific and Atlantic Ocean, A. Bocas del Toro, Panamá; B. Cispatá, Colombian Caribbean; C. La Guajira, North Colombian Caribbean; D. SFF. Malpelo, Colombian Pacific; E. Portsmouth and Plymouth, South United Kingdom.

Colony size: The colony size is significantly different between the sampled areas (Anova, $p<0.05$), with the greatest difference between Cispatá and Portete (Tukey HSD, $p=0.002$) (Figure 7) and Bocas del Toro (Tukey HSD, $p=0.008$) (Table 5). Within stations, there were differences in the colony size between La Guajira and Cispatá Bay in both climatic seasons sampled. During the dry season colony size was lower in Cispatá (mean=3.5 cm) than in La Guajira (mean=7.8 cm), while during rainy season *B. neritina* samples in Cispatá were bigger (mean=5.4 cm) than in La Guajira (mean= 4.8 cm).

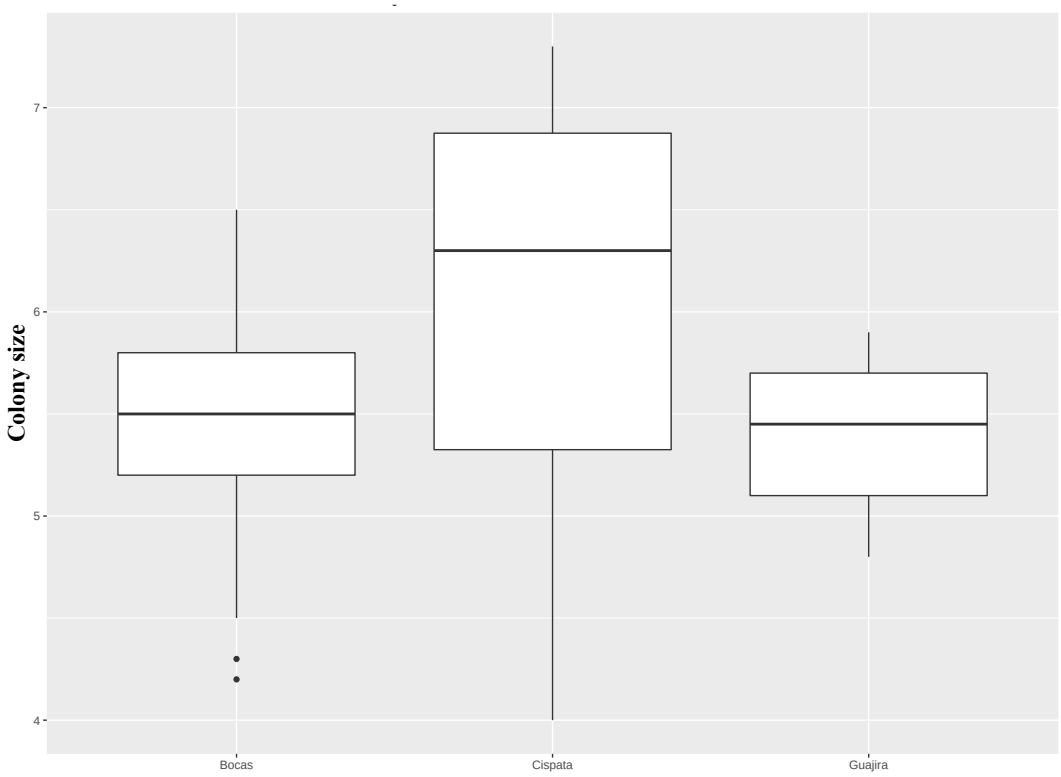

Figure 7. Differences between colony size in the Caribbean areas.

The linear model showed that the temperature (Glm, $p<0.05$) and the interaction between temperature and salinity had a significative effect on the colony size in the Caribbean ($p<0.05$) (figure 8), but no significant effect of salinity was observed on the colony size ($p=0.278$) (Table 5).

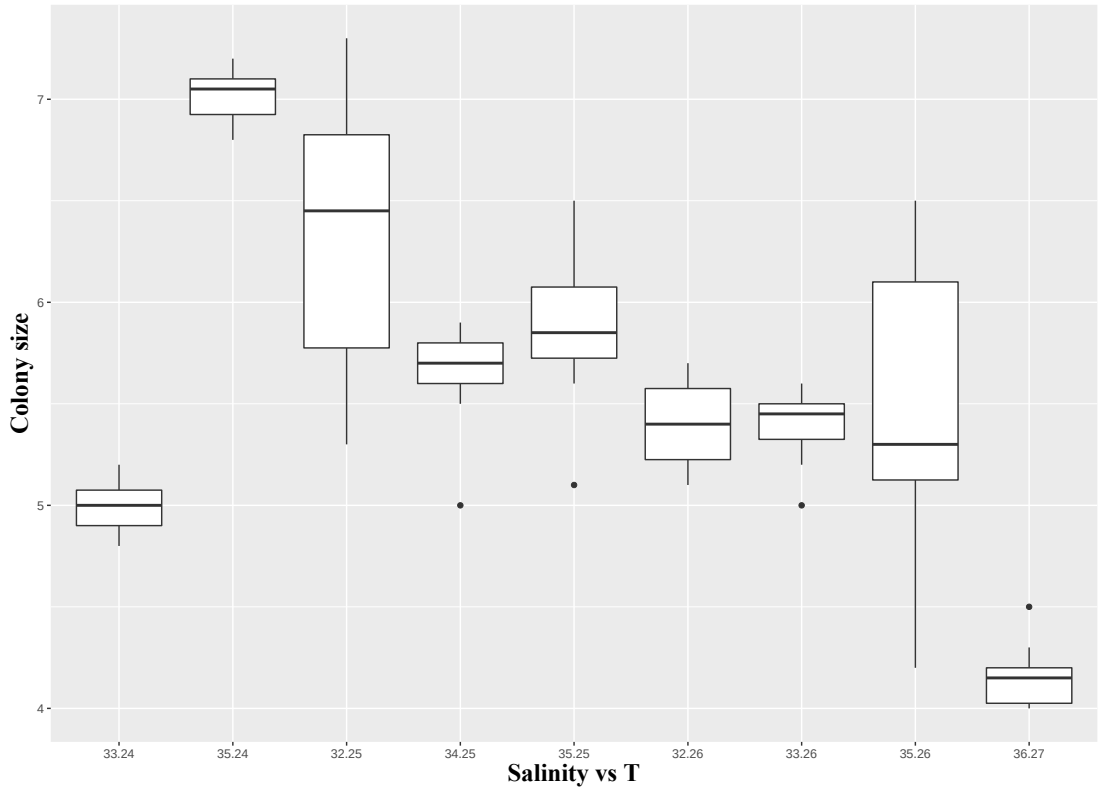

Figure 8. Effects of the interaction of salinity and temperature on the colony size in the Caribbean

Pacific

Ovicell density: The ovicell density is significantly different between the sampled areas (t-test, p=0.001). Ovicell density in Arrecife 1 (mean 21.8; SD 1.229) was significantly higher than that Arrecife 2 (mean 19.9; SD 0.994) (p= 0.001) (Figure 9; Table 5).

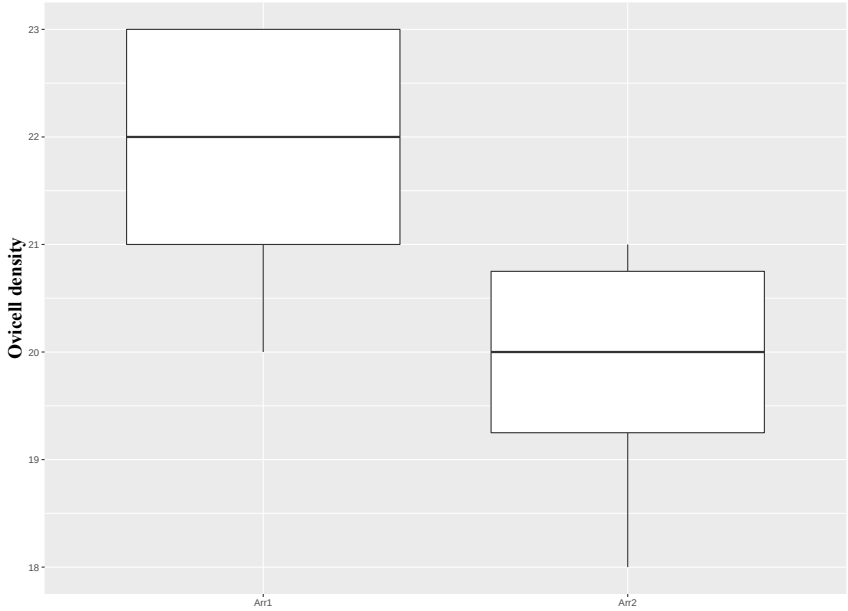

Figure 9. Differences between ovicell density in the Pacific areas.

The linear model showed that temperature had a significative effect on the ovicell density in the Pacific (Glm, $p<0.05$) (figure 10), but no significant effect was observed of the salinity or the interaction between temperature and salinity on the ovicell density (Table 5).

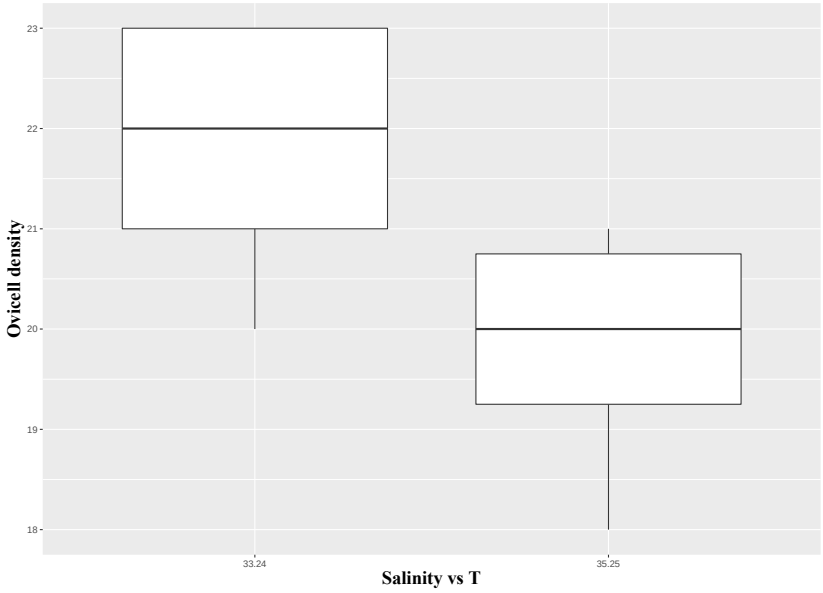

Figure 10. Effects of the interaction of salinity and temperature on the ovicell density in the Pacific.

Table 5. Interaction effects of temperature and salinity on the ovicell density and colony size

Caribbean	**Test**	**Ovicell density**				**Colony size**			
	ANOVA	**Df**	**MS**	**F**	**p**	**Df**	**MS**	**F**	**p**
	Caribbean	2	1972.3	158	p<0.01	2	4.177	7.314	p<0.01
	Tukey HSD	**diff**	**lwr**	**upr**	**p**	**diff**	**lwr**	**upr**	**p**
	Cis-Boc	-12.545	-14.322	-10.767	p<0.01	0.483	0.102	0.863	p<0.01
	Gua-Boc	-2.95	-4.8223	-1.076	p<0.01	0.07	-0.47	0.33	0.909
	Gua-Cis	9.595	7.817	11.372	p<0.01	-0.553	-0.933	-0.172	p<0.01
	GLM	**Df**	**Dv**	**F**	**p**	**Df**	**Dv**	**F**	**p**
	Temperature	1	54.01	0.812	0.369	1	57.410	64.053	p<0.01
	Salinity	1	53.12	0.161	0.689	1	56.970	1.186	0.2782
	T vs Salinity	1	45.32	10.934	0.001	1	46.140	29.462	p<0.01
South West UK	**Test**	**Ovicell density**				**Colony size**			
	ANOVA	**Df**	**MS**	**F**	**p**	**Df**	**MS**	**F**	**p**
	SW England	3	32.48	1.334	0.270	3	2.258	6.172	p<0.01
	Tukey HSD	**diff**	**lwr**	**upr**	**p**	**diff**	**lwr**	**upr**	**p**
	Ply 2 - Ply1	-1.65	-5.748	2.448	0.716	-0.725	-1.227	-0.222	0.001
	Port 1 - Ply 1	0.7	-3.398	4.798	0.969	-0.1	-0.602	0.402	0.953
	Port 2 - Ply 1	1.3	-2.798	5.398	0.838	-0.075	-0.577	0.427	0.979
	Port 1 - Ply 2	2.35	-1.748	6.448	0.438	0.625	0.122	1.127	0.008
	Port 2 - Ply 2	2.96	-1.148	7.048	0.24	0.65	0.147	1.152	0.005
	Port 2 - Port 1	0.6	-3.498	4.698	0.98	0.025	-0.477	0.527	0.999
	GLM	**Df**	**Dv**	**F**	**p**	**Df**	**Dv**	**F**	**p**
	Temperature	1	35.021	492.172	p<0.001	1	34.351	0.756	0.387
	Salinity	1	25.231	72.849	p<0.001	1	24.051	33.795	p<0.001
	T vs Salinity	1	24.132	108.938	p<0.001	1	23.163	2.914	0.091
Pacific	**Test**	**Ovicell density**				**Colony size**			
	t-test		**t**	**Df**	**p**		**t**	**Df**	**p**
	SFF Malpelo		3.8	17.247	0.001		-2.623	12.598	0.021
			SD	**mean**			**SD**	**mean**	
	Arr 1		1.229	21.8			0.274	4.48	
	Arr 2		0.994	19.9			0.125	4.73	
	GLM	**Df**	**Dv**	**F**	**p**	**Df**	**Dv**	**F**	**p**
	Temperature	1	18.05	14.44	0.001	1	0.3125	6.884	0.017
	Salinity	0	0			0	0		
	T vs Salinity	0	0			0	0		

Dv: deviance residuals; MS: mean square; Cis: Cispata; Gua: Guajira; Boc: Bocas del Toro; Ply: Plymouth: Por: Portsmouth: Arr: Arrecife

Colony size: The colony size is significantly different between the sampled areas (t-test, p=0.021). Colony size in Arrecife 2 (mean 4.73; SD 0.125) was significantly higher than that Arrecife 1 (mean 4.48; SD 0.274) (Figure 11) (Table 5).

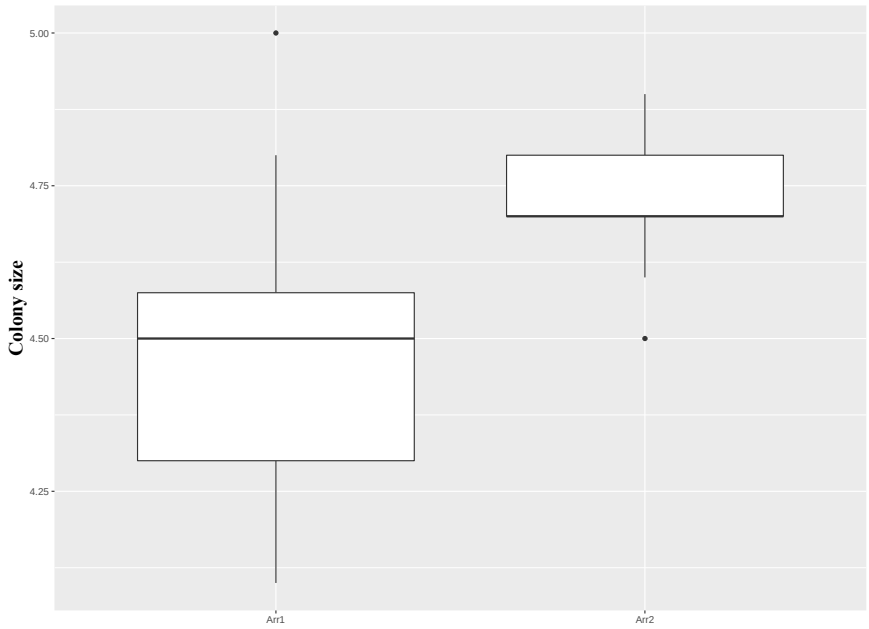

Figure 11. Differences between colony size in the Pacific areas analysed.

The linear model showed that the temperature (Glm, p<0.05) had a significative effect on the colony size in the Pacific, but salinity and the interaction between temperature and salinity do not have significant effect on the colony size (Figure 12) (Table 5).

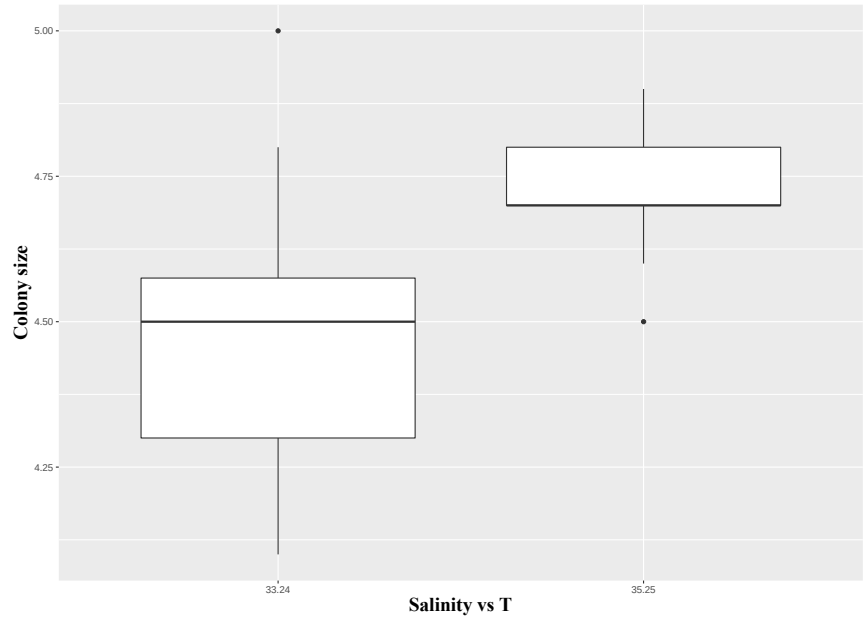

Figure 12. Effects of the interaction of salinity and temperature on the colony size in the Pacific.

South West England

Ovicell density: The ovicell density is not significantly different between the sampled areas (Anova, p=0.270) (Figure 13; Table 5). Within stations, there were no significant differences in the ovicell density between Plymouth and Portsmouth in both seasons sampled, Summer (Anova, p=0.235) and winter (Anova, p=0.069), however, the position of ovicells differed, during summer ovicells were found at the tips of the colony in Plymouth and around the middle in Portsmouth. While during winter no ovicells were found at the tip of the colonies analysed.

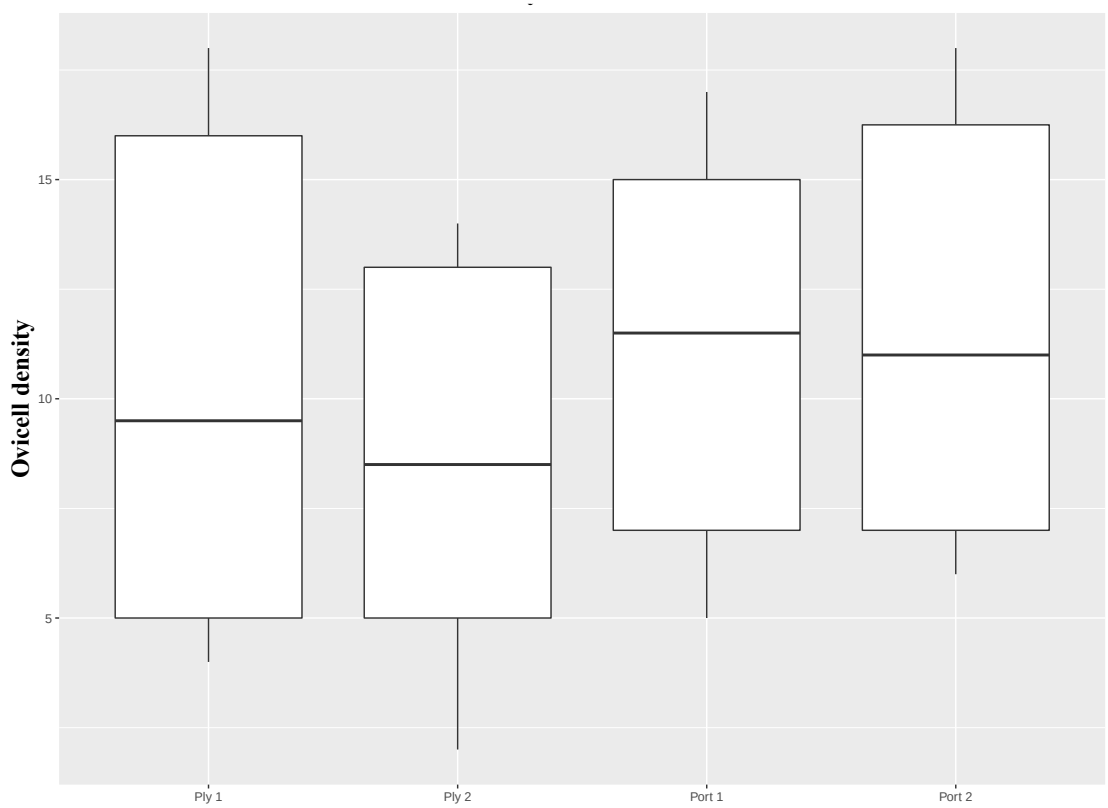

Figure 13. Differences between ovicell density in the South West England areas analysed.

The linear model showed that the temperature, salinity and the interaction them (Glm, p<0.05), have significant effect on the ovicell density in the South West England samples analysed (Figure 14) (Table 5).

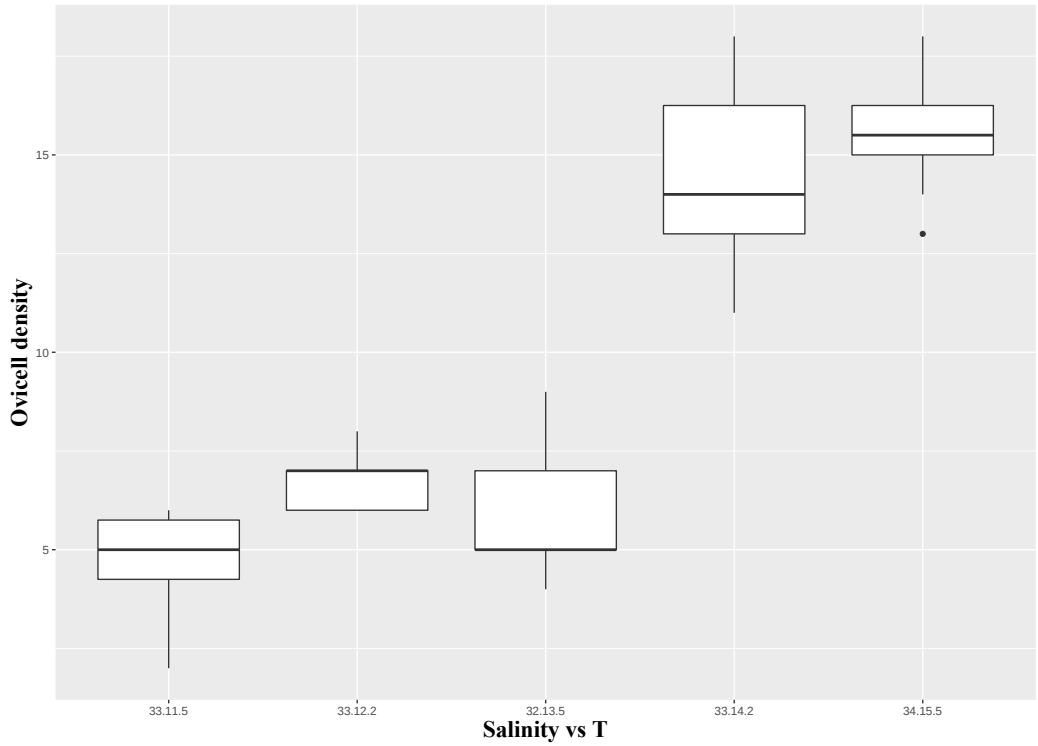

Figure 14. Effects of the interaction of salinity and temperature on the ovicell density in the SW England.

Colony size: The colony size is significantly different between the sampled areas (Anova, $p<0.05$), with the greatest difference between Plymouth 1 and Plymouth 2 (Tukey HSD, $p=0.001$) and Portsmouth 2 and Plymouth 2 (Tukey HSD, $p=0.005$) (Figure 15) (Table 5). Within stations, there were no differences in the colony size between Plymouth and Portsmouth in both seasons sampled. During winter colony size was slightly smaller in Portsmouth (mean=7.1 cm) than in Plymouth (mean=7.5 cm), while during summer *B. neritina* samples in Portsmouth were bigger (mean=7.8 cm) than in Plymouth (mean= 7.6 cm).

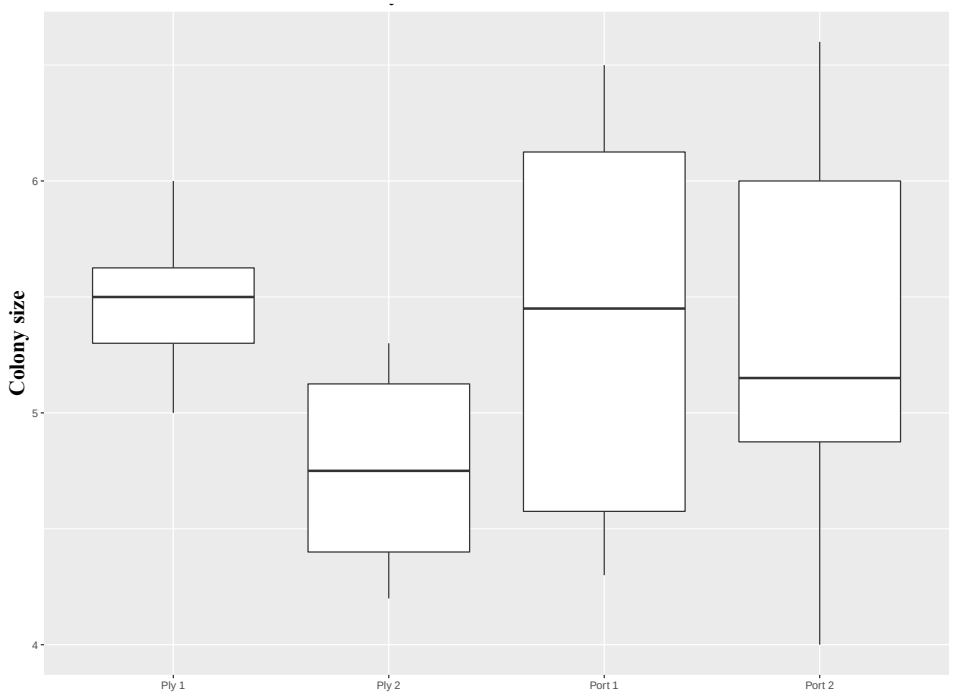

Figure 15. Differences between colony size in the South West England areas analysed.

The linear model showed that the salinity (Glm, $p<0.05$) had a significative effect on the colony size in the SW England, but temperature ($p=0.387$) and the interaction between temperature and salinity do not have significant effect on the colony size ($p=0.091$) (Figure 16) (Table 5).

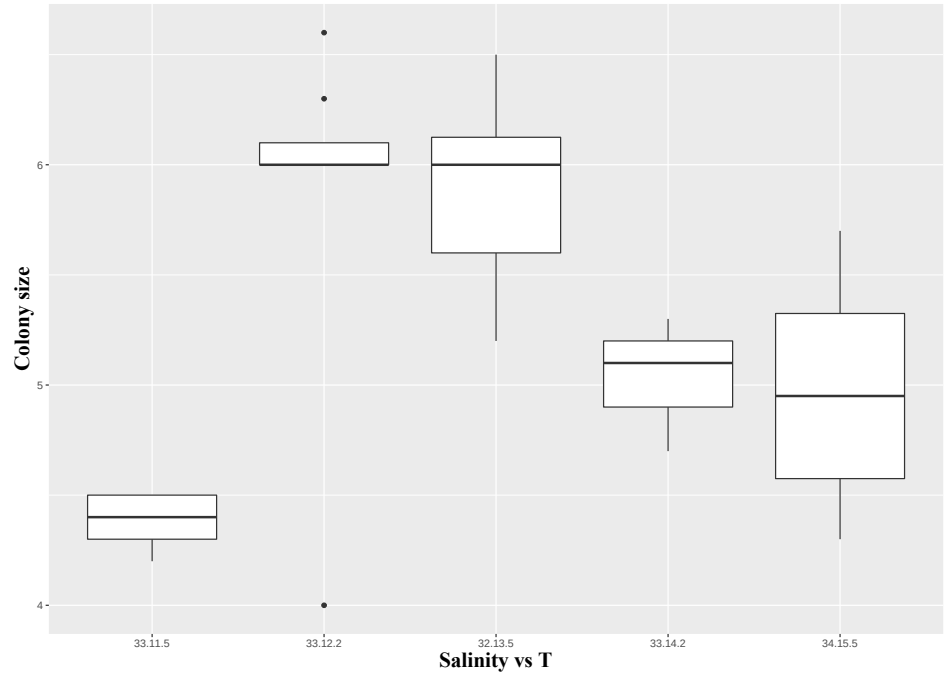

Figure 16. Effects of the interaction of salinity and temperature on the colony size in the SW England.

DISCUSSION

This study suggests a relationship between environmental factors, the number of mature zooids, ovicell density and the colony growth of *Bugula neritina* and a possible influence of those factors on the morphometry and the reproduction strategy performed. Temperature, salinity and nutrients derived from primary productivity are the factors influencing most strongly the switch from asexual to sexual reproduction and ensuring the survival and colonization of new habitats (O'Dea et al., 2008).

High fluctuations in salinity provoke a reduction of mature zooids and ovicell production and subsequently bigger colony size (asexual growth). These traits are also influenced by the temperature (Amui-Vedel et al., 2007; Okamura & Bishop, 1988) and oxygen availability (O'Dea & Okamura, 1999). Increasing bathymetry on the other hand, is related to a decrease in zooid size (Schopf et al., 1980), changes in the morphology (Pachut & Cuffey, 1991) and encourages branching growth patterns (Thomsen, 1977; Hillmer, 1979; Schopf et al., 1980; Smith, 1995).
Specimens in changing salinity and temperature environments such as Cispatá Bay in the Colombian Caribbean invest less in sexual reproduction but present bigger zooid and colony size than specimens in more stable environments with similar temperature patterns such as Bocas del Toro in the Panamanian Caribbean. Both environments consist of submersed *R. mangle* roots but differ in the origin and oceanographic factors that influence their ecosystem dynamics. Our samples were collected during the Panamanian rainy season and were considerably different in relation to the ovicell density and colony size to the ones collected during the same climatic season in Cispatá Bay and in La Guajira.

Cispatá Bay is directly influenced by the freshwater injections from the Sinú river which partially flows into it and the typical precipitations from rainy season which increase turbidity due to the shallow depth in the area (Invemar-CVS, 2010). These constant variations in salinity have created a seasonal semi-brackish environment which modulates the distribution of euryhaline species (Patiño & Flórez, 1993; Quiroz & Arias, 2013). During dry season, salinity is more stable, and animals shift from borderline to inner areas into the mangrove bay (Quiroz et al. 2017), however,

during rainy season, salinity dramatically drops and these specimens die off, resulting in most species found in the exposed areas of the bay to the open sea (Alvarez-Leon & Gutierrez-Bonilla, 2007) with bigger colony size and very scarce ovicells. Bocas del Toro on the other hand, consist of a mangrove area directly exposed to the Caribbean Sea with clearer and deeper waters, less turbid than Cispatá and holding greater species diversity (Guzman et al. 2005). During the rainy season salinity in the first water mass layer decreases as well as surface sea temperature (Seemann et al., 2018) and less remotion of bottom particulate organic matter is produced.

The colonies collected in Panamá also differed from the colonies collected in La Guajira which is another mangrove area in the Caribbean Sea, in regards the ovicell density, zooid and colony size. This could also be attributed to the location of La Guajira in a desertic zone and the environmental dynamics in which the primary productivity and available suspended food is higher than in the mangrove system in Bocas del Toro (Panama) due to the continuous upwelling phenomenon typical of this region (Castro et al., 2006; Pulgar et al., 2011; Nielsen & Navarrete, 2004). The temperature and salinity fluctuations in Panamá also differ from the natural fluctuations in that northern part of Colombia due to the action of constant trade winds that cool down the superficial sea temperature in La Guajira (Corpoguajira & Invemar, 2012). in addition, the turbidity and nutrient circulation in this area allows the establishment of an abundant rather than diverse fauna and could be determining the sexual reproduction strategy of *B. neritina* in the area. This type of reproduction requires higher energy consumption which can be compensated with the available particulate organic matter characteristic of La Guajira.

Our results indicate that colonies in Cispatá reproduce sexually during the dry season and during the rainy season young colonies at the exposed areas of the Bay switch to asexual reproduction by investing more energy in colony growth by budding. Also, the constant high primary productivity in La Guajira together with less temperature and salinity fluctuations represent an adequate environment for the continuous sexual reproduction of *Bugula neritina*, with slight reduction in ovicell densities during the rainy season. Finally, in Bocas del Toro, during the rainy season

the ovicell density indicates that the sexual reproduction seems unaffected by the effects of salinity and temperature decrease.

In the South West of England, samples from Portsmouth had low ovicell density than Plymouth, during both climatic seasons sampled, which correlates with the marine traffic activity in both areas and the influence of freshwater interaction in Portsmouth marinas in comparison with the marina in Plymouth which is exposed to the open sea. This reduction in the overall ovicell production, encourages asexual budding and colony growth (Hartikainen et al., 2014). In addition, our results suggest a possible trade-off between ovicell density and morphometry, colonies with fewer ovicells per branch presented larger zooid size. This finding contradicts previous studies in cupuladriids in which zooidal size was unresponsive to energetic allocation to reproduction (O'Dea & Jackson, 2002). In our study, the investment in ovicells reduces the energy for the production of large zooids in *Bugula neritina*.

Colonies collected in the Colombian Tropical Eastern Pacific cannot be compared against climatic seasons as those samples correspond to a single time collection in one zone of the SFF Malpelo during dry season 2016. However, differences were found within the sampling areas; in Arrecife 1 the greater ovicell density and smallest colony size was found, this station correspond to a protected area of the island in between rock walls. Arrecife 2 was characterized by colonies with reduced ovicell densities and the biggest zooid size, this station corresponds to the most exposed area with strongest marine currents. Although this sample could be used as an outgroup for the rest of the biological material used in our study. Colony growth is detrimental for the switch to asexual reproduction (Håkansson & Thomsen, 2001); increasing zooid size does not necessarily correlate to the colony size but is a good indicator of the reproductive strategy (Keough, 1989; Keough & Chernoff, 1987).

Changing environmental factors and disturbances encourage asexual reproduction in *Bugula neritina,* probably because the energy requirements for sexual reproduction are usually limiting in these situations and physical breakage in the colony caused by strong currents requires additional energy expenditure in recovery (Cheetham & Sanner, 2001; Håkansson & Thomsen 2001; McKinney & Jackson, 1989). This strategy also allows the asexual dispersal to more stable environments

(O'Dea, 2006), but reduces genetic diversity, which is detrimental in species permanency in time. Higher turbidity as a result of environmental disturbances, reduces bryozoan colony survival and their reproduction (Jackson & Coates, 1986). Although, some studies have reported that chlorophyll-a caused by high primary productivity does not affect individual zooids but the colony size (Jebram, 1977; Winston, 1976). The survival of *B. neritina* during high suspended particulate organic matter would depend on the larval performance under that environmental stress, their ability to undertake metamorphosis and velocity of colony growth, as well as the availability of nutrients and carbonate (Cocito et al., 2004).

This study has shown that the morphology and reproductive strategy of *B. neritina* can be significantly influenced by external stressors and may be an indicator of the environment quality (Smith, 2014; Guinotte & Fabry, 2008; Taylor, 2005; Smith, 1995). Our current knowledge on how the reproductive strategies of this species will respond to further climate change scenarios is still limited and requires future investigations.

CONCLUSIONS

This study concludes that temperature, salinity and primary productivity is related to the reproductive strategy in *Bugula neritina*; in the Caribbean, during the dry season, this species reproduces sexually in mangrove areas and colonies reduce their size during rainy season. Different populations of *B. neritina* in contrasting ecosystems and environmental conditions presented similar responses in the production of ovicells and colony growth indicating a possible species-specific pattern. Colonies that invested in ovicell production reduced asexual growth. Further studies should address the performance of ovicell density under multi-stressor scenarios to evaluate detrimental environmental factors for the survival and reproduction of *Bugula neritina*.

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Chapter 5: Complete evisceration in the tropical Ascidian *Polycarpa captiosa* (Stolidobranchia: Ascidiacea) is caused by environmental stress

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ABSTRACT

Ascidians are important ecosystem improvers; as filter feeders, they contribute to nutrient circulation and turbidity control, and can potentially act as bio-indicators of water quality and disturbance. We evaluated the behaviour of the tropical ascidian *Polycarpa captiosa* under turbidity conditions in mesocosms to determine their optimal and limiting tolerance to this natural disturbance. Treatments replicated the water chemistry conditions of their natural habitat (24°C, salinity 35ppt) and included, 0) control, 1) Low turbidity (80 NTUs), 2) Medium turbidity (250 NTUs), and 3) High turbidity (1000 NTUs). Our results found that *P. captiosa* performs complete evisceration (ejection of entire digestive tract) 12-hours post high turbidity exposure and within 24-hours of medium turbidity exposure. We evaluated recovery after evisceration and found that animals regenerate their digestive system within 20-days post evisceration when transferred to control conditions, however, when conditions remained unchanged, 100% mortality was observed. Finally, we evaluated if the animals with regenerated digestive systems could reach optimal filtration rates and, if exposed to high turbidity, would again perform evisceration. We found that *P. captiosa*, can eject its gut more than once after stress disturbance but their mortality rate increases with the number of eviscerations and their filtration activity declines. This study is the first of its kind reporting evisceration in Chordates as a consequence of environmental stress.

Keywords: High turbidity, Coastal ascidians, Stolodobranchia, Gut ejection, Filter-feeders, Gut recovery, Mesocosms.

INTRODUCTION

Coastal ecosystems are constantly influenced by environmental factors that control the distribution of species, their feeding adaptations and reproductive strategies (Widdows et al., 1979). Those changes are triggered by natural causes (Moodley et al., 1998; McLusky, 1993) or as a consequence of human interaction, the latter often leading to dramatic changes to ecosystem health (Warwick et al., 1990; Loya 1976).

Turbidity refers to the degree to which suspended particles disrupt the natural penetration of light in the water column. Turbidity may originate from natural processes such as, storms, rainfall, upwelling, the influence of rivers and wave energy (Johannes, 1972) or from man-made causes, usually due to poor coastal management (Wilber, 1983; Stephenson et aI., 1958). The total concentration of suspended organic or inorganic matter (TSS) determines the survival of light dependant organisms like corals, reduces the feeding rates and efficiency of filter-feeders, and limits the food availability as a consequence of a reduction in primary productivity (Aldridge et al., 1987; Page, 1983; Moore, 1977). When turbidity exceeds the level of particle suspension, it precipitates, increasing sedimentation rates, and potentially destroying the ecosystems by asphyxiating benthic organisms or forcing the free-living forms to migrate (Rowe & Dean, 2010; Thrush et al., 2004; Gray et al., 1997).

Nevertheless, high turbidity does not always translate into a lethal circumstance; some organisms like filter-feeders can play an important role in water clearance and considerably reduce the damaging consequences of turbidity (Newcombe & McDonald 1991). Therefore, understanding the responses of benthic fauna to turbidity is key for the understanding and conservation of coastal fauna (Akoumianaki et al., 2013).

Polycarpa ascidians are filter-feeders that have the ability to filter up to 1 gallon per day (Minamoto et al., 2010; Jacobi et al., 2017). Their main ecosystem function is clearing the water column, and thus, to increase sunlight penetration and allow light-dependant organisms to thrive (Armsworthy et al., 2001; Ryland, 1999; Randlov & Riisgard, 1979). They are also potential bio-indicators of the water quality (Papadopuolou, & Kanias, 1977). Studies regarding the performance of ascidians under environmental stress and high turbidity scenarios have concluded that high

levels of inorganic suspension affects their geographic distribution (Naranjo et al., 1996), reduces growth rates and increases mortality in Phlebobranchs (Kelmo, et al., 2006; Robbins, 1985;1983 Millar, 1971; Riisgard & Randlov, 1981), limits ingestion (Jorgensen, 1949) and filtration rates (Fiala-Medioni, 1979a; b) or induces a suspension of feeding activity (Petersen, 2007; Holmes,1973). To our knowledge no previous study has reported evisceration in ascidians as a consequence of environmental stress or turbidity.

Recently, a study demonstrated that the ascidian *Polycarpa mytiligera* eviscerates after mechanical manipulation (Shenkar & Gordon, 2015). However, the ejection occurred via the oral siphon within 5 seconds of contact, and the ejected guts where mostly broken as an involuntary rupture of the pharynx after the mechanical stress. Their findings stated that ascidians regenerated their pharynx within 19-days after evisceration and suggested this is an adaptative behaviour against predation, but no conclusive findings supported that affirmation (Shenkar & Gordon, 2015).

Some "old" taxonomic literature from late 19th century has reported ascidians (mainly *Polycarpa*) without branchial sacs in the Indian Ocean and Australia (Monniot, 2002; 1987; Monniot & Monniot, 2001; Monniot et al., 2001; Tokioka,1970; 1961; Kott, 1985; Herdman, 1906; Sluiter, 1898; 1895; 1885; Willey, 1897) and two ascidians were wrongly named as *Styeloides abranchiata* (Sluiter, 1885) and *Styeloides eviscerans* (Willey, 1897) after this finding. However, none of these studies have reported the biological reasons for this trait, and no recent research has looked into this behaviour in ascidians to date.

Evisceration is a form of self-induced amputation or autotomy of essential organs and tissue. It has been well documented in Echinodermata (Asteroidea, Ophiuroidea, Crinoidea, Holothuria and Echinoidea) in which animals eject their digestive systems and other organs naturally, enabled by a fast softening of the connective tissue followed by strong muscle contractions that induce a rupture of their introvert, and detachment of the organs (Zhang et al., 2017; Garcia-Arraras & Greenberg, 2001; Bai 1971) to subsequently eject them through a rupture in the cloaca (Jespersen & Liitzen, 1971). This phenomenon may occur either seasonally or after an external stimulus (Andriyono et al., 2016; Byrne, 1985; 1982; Emson &

Wilkie 1980; Smith & Greenberg, 1973). After this, animals regenerate the lost organs and tissue, starting with their digestive track (Thorndyke et al., 1999).

Regeneration capacity is limited in Chordates (Jaźwińska & Sallin 2016; Seifert et al., 2012; Poss, 2010). Although, no studies have focused on the understanding of the biological triggers for evisceration in ascidians, there is a great number of studies on the regenerative ability of these animals, especially on the colonial *Botrylloides* spp, in which the whole colony shares blood vessels and remains embedded in the tunic (Blanchoud et al., 2018; Kassmer et al., 2016; Voskoboynik & Weissman 2015; Kürn et al., 2011; Brown et al., 2009; Tiozzo et al., 2008). These studies represent the starting point for the understanding of the regenerative pattern in *Polycarpa* ascidians.

This study seeks to investigate evisceration and regeneration in *Polycarpa captiosa* after high turbidity exposure, and to determine if their filtration efficiency is affected by the evisceration events. Animals were exposed to several levels of turbidity to evaluate the optimal turbidity tolerance and survival. This study reports for the first-time evisceration in ascidians after environmental stress.

METHODS

Study organisms

We analysed 50 adult individuals (4.0 ± 0.5cm length; 2.5 ± 0.5cm wide; 6 ± 1.5g) of the Stolodobranchia solitary ascidian *Polycarpa captiosa* (Sluiter, 1885). These animals have a geographical distribution restricted to the Indo-Pacific oceans (Lee et al., 2013; Shenkar & Swalla,2011; Rocha et al., 2012). These animals have a complex pharynx formed by four folds on each side of the branchial sac, with 8 stigmata per mesh and no para-stigmatic vessels. The stomach is rounded with Ca. 15 internal folds per side, and together with the U-shaped intestinal loop, occupies one third of the left posterior region of the body. Our samples were collected by local traders in Singapore and transported to United Kingdom through an aquarium supplier (Cheshire Aquatics). Animals have a leathery tunic, pale orange to brown coloured (Figure 1). Once in the laboratory, animals were detached from the rocks they came on and cleaned of epibionts.

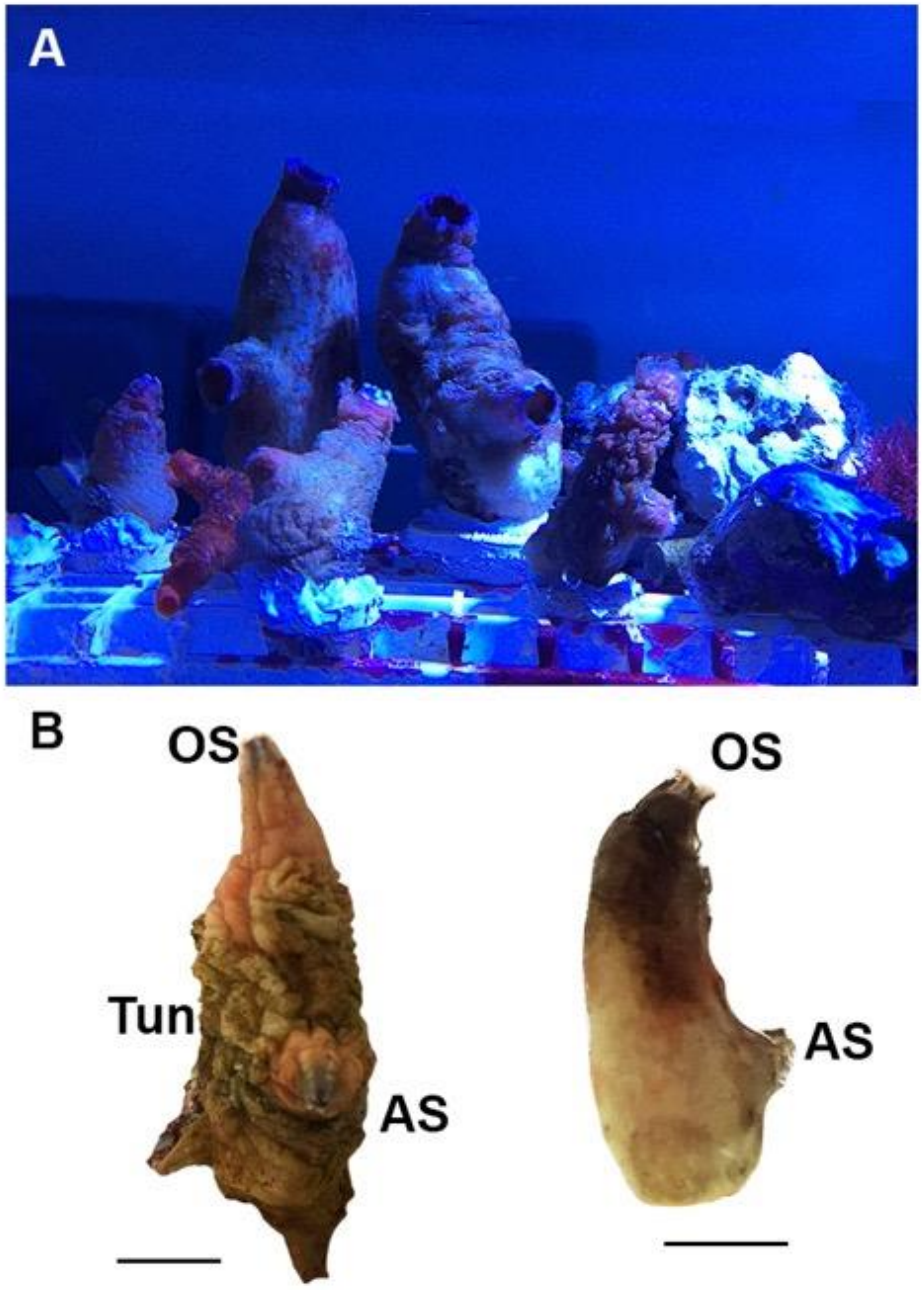

Figure 1. Specimens of *P. captiosa.* A. Adult ascidians in their acclimation tank with opened siphons. B. View of the squirted tunic (Tun) (*left*) and the body (*right*) of the ascidian showing oral siphon (OS) and atrial siphons (AS). Scale bar: 1cm..

Acclimation time

Ascidians were glued on a ceramic plug and kept in a 375L tank with a salinity of 35ppt and 24±0.5°C for three weeks prior to experimentation (Figure 1A). The tank was maintained with a double filtration system, a temperature chiller, a thermostat and artificial lighting, replicating light patterns at their natural habitat. We fed animals three times a week with a solution of lab-cultured phytoplankton (*Dunaliella salina, Nannochloropsis oculata* and *Tetraselmis* sp.) vitamins and amino acids (Red Sea supplements). In addition, every other week, 10% water changes were performed. At the end of the acclimation time, we separated animals that did not perform well and labelled the chosen specimens for experimentation and kept an individualised record of their weight, performance and health.

Experimental design

A cost-effective, space-optimisation system was designed and built to evaluate the effects of high turbidity in ascidians (Figure 2). We created a controlled turbidity mesocosms in a separate 330L tank, in which we placed a set of 40 glass beakers (1L each) as our experimental units, on a stair-like structure with four platforms equidistant from each other (10 beakers per platform).

The tank was filled at its maximum volume and all beakers on their platform were submersed and sharing the exact same water chemistry conditions with similar light intensity patterns. Then, we introduced one *P. captiosa* individual in each of them. To ensure all units would get similar water flow, we connected two wave makers to both sides of the tank (Figure 2). Each platform corresponded to a different treatment with 10 experimental units each, 0) control 1) Low turbidity 2) Medium turbidity and 3) High turbidity (Figure 4). Control beakers were kept at the bottom of the tank.

We cultured three phytoplankton species with different cell size and mobility (*N. oculata* (3µm)*, Dunnaliella salina* (10µm - flagellated) and *Tetraselmis* sp. (14 µm)) in laboratory conditions to create turbidity, and prepared stock solutions to achieve each turbidity concentration per experiment. Turbidity was measured in Nephelometric turbidity units - NTUs using a Beckman Coulter counter, Low turbidity

(Lt) was set at 80NTUs, medium turbidity (Mt) was set at 250NTUs and high turbidity (Ht) was set at 1000 NTUs (Figure 4).

Ascidians were not fed three days prior the treatments to optomise their feeding activity and evaluate their clearance rate and absorption efficiency. For each experiment, we lifted the first row of beakers halfway above the tank's water surface, to isolate them from the rest of the tank system. This way, we obtained 10 independent experimental units to perform experimentation. Then, a specific volume of algal stock solution was added to each lifted beaker to set the turbidity concentration desired; we ran one treatment at the time around mid-day to ensure maximum light intensity and energy per ascidian. In order to maintain non-mobile algae (*N. oculata, Tretaselmis* sp) suspended, we introduced a small air diffuser to each 1L beaker with a gentle flow.

Experiment 1: Optimal filtration activity

We collected water samples at the beginning, 30 mins after starting and at the end of the experiment and checked their concentration using a Neubauer chamber, to subsequently evaluate their clearance rate and absorption efficiency. The turbidity treatment lasted an hour, during which visual censuses were made on the filtration activity (squirting, open siphons, faeces production, length of squirting time) and video recording were made on the ascidian's behaviour using GoPro 7 submersible cameras. At the end of each turbidity exposure, three ascidians per treatment were left in the turbid exposure for two days. For the rest, the experimental water in each beaker was changed and ascidians were put back in the system for one-month recovery (Figure 4). Treatments were performed a week away from each other. Animals were video-monitored for 24-hours post exposure and all faeces produced were also collected.

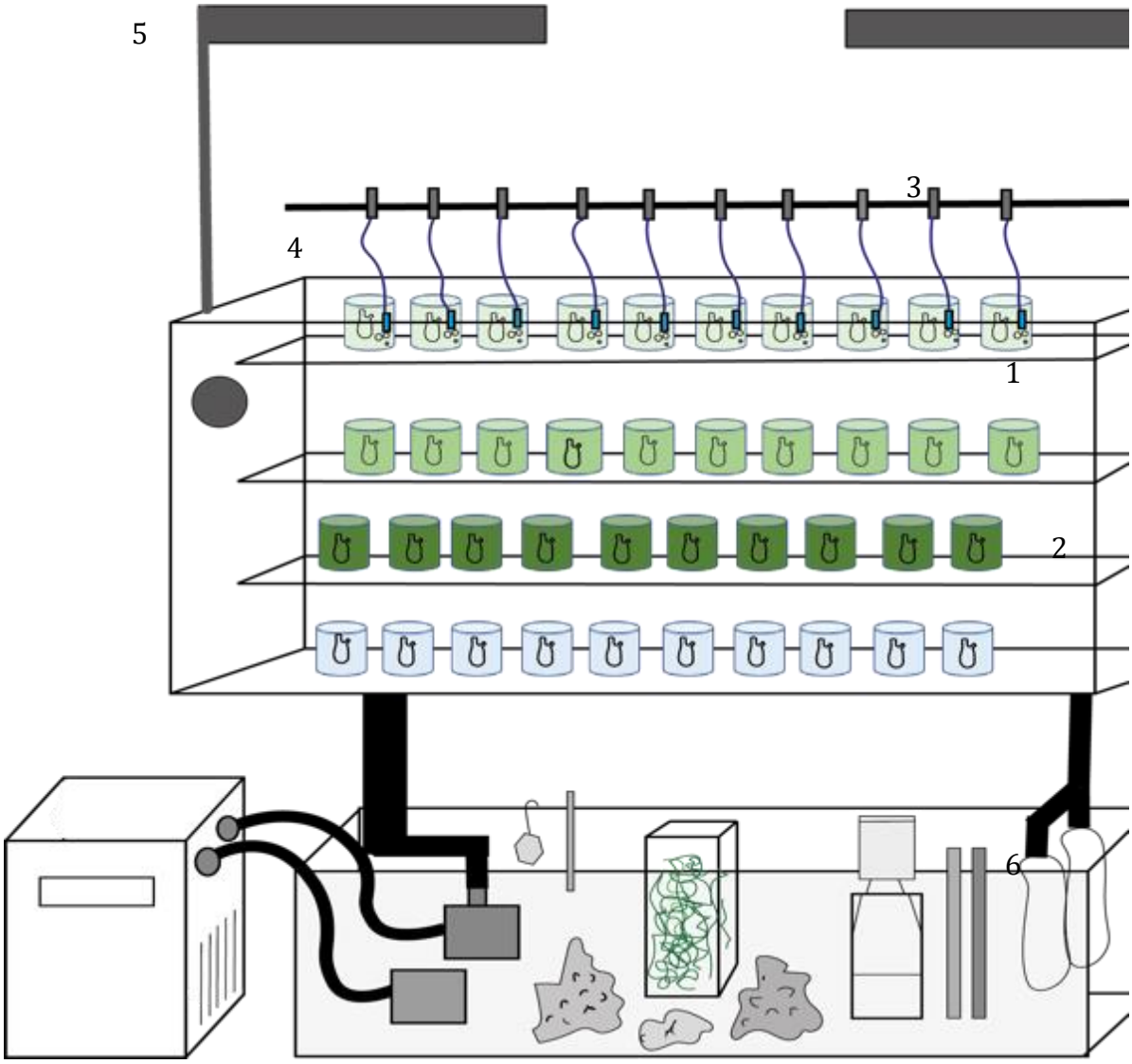

Figure 2. Drawing of the mesocosm set up for different turbidity levels. The colour intensity in the beakers represent the different treatments at which each row of beakers was exposed to. Turbidity was added only when lifted above the water surface. 1 wake maker, 2. Thermostat, 3. Airline connected to air pump, 4. Air diffuser in each beaker, 5. Artificial lighting, 6. High-quality filtration system.

Determination of the filtration capacity

After performing one-hour visual censuses and analysing one-hour video recordings per treatment we calculated the filtration activity of *P. captiosa* (No. of times open and close siphons; No. of squirts; Length of open and close siphons; Length of squirting) to examine differences between treatments.

We calculated the filtration rates per treatment; Clearance rate (CR) was measured as the total volume of cleared of algal cells per ascidian gram per unit time based on Armsworthy et al. (2001) and Macdonald & Ward (1994) following the equation, CR=((cr-Ce)/cr)gr animal, where, cr is the total cell concentration of the algae mix at the beginning of the experiment and Ce is the mean cell count at the end of the experiment.

Absorption of nutrients

At 24-hour post experimentation, all faecal pellets were collected from the seven ascidians that were left for recovery, with a glass pipette and filtered (Whatman) using a vacuum pump, then rinsed with 1M isotonic ammonium formate (NH_4HCO_2) to eliminate sea salts, subsequently dried at 37°C for one-hour and dry-weighed in order to estimate the absorption efficiency (AE).
AE was calculated based on Conover (1966) as the total weight of algae added at the beginning of the experiment minus the weight of faecal pellets after an hour of measurements, using the equation, AE= 100-(TPM-fw/ TPM) x 100, where fw is the faeces dry weight after the experiment, TPM is the total seston weight at the beginning of the experiment.

Experiment 2: Recovered gut filtration activity

After one-month of recovery, we selected five ascidians per each treatment that had recovered successfully and regrown their guts. We exposed them to turbidity (80NTUs) to evaluate if their already-known optimal clearance rate (from Exp. 1) was achieved after recovering their guts. The experiment consisted in lifting 10 new beakers with the specimens recovered per groups. A) 5 from T1 and 5 from T2 and B) 5 from T3 and 5 control (Figure 3).

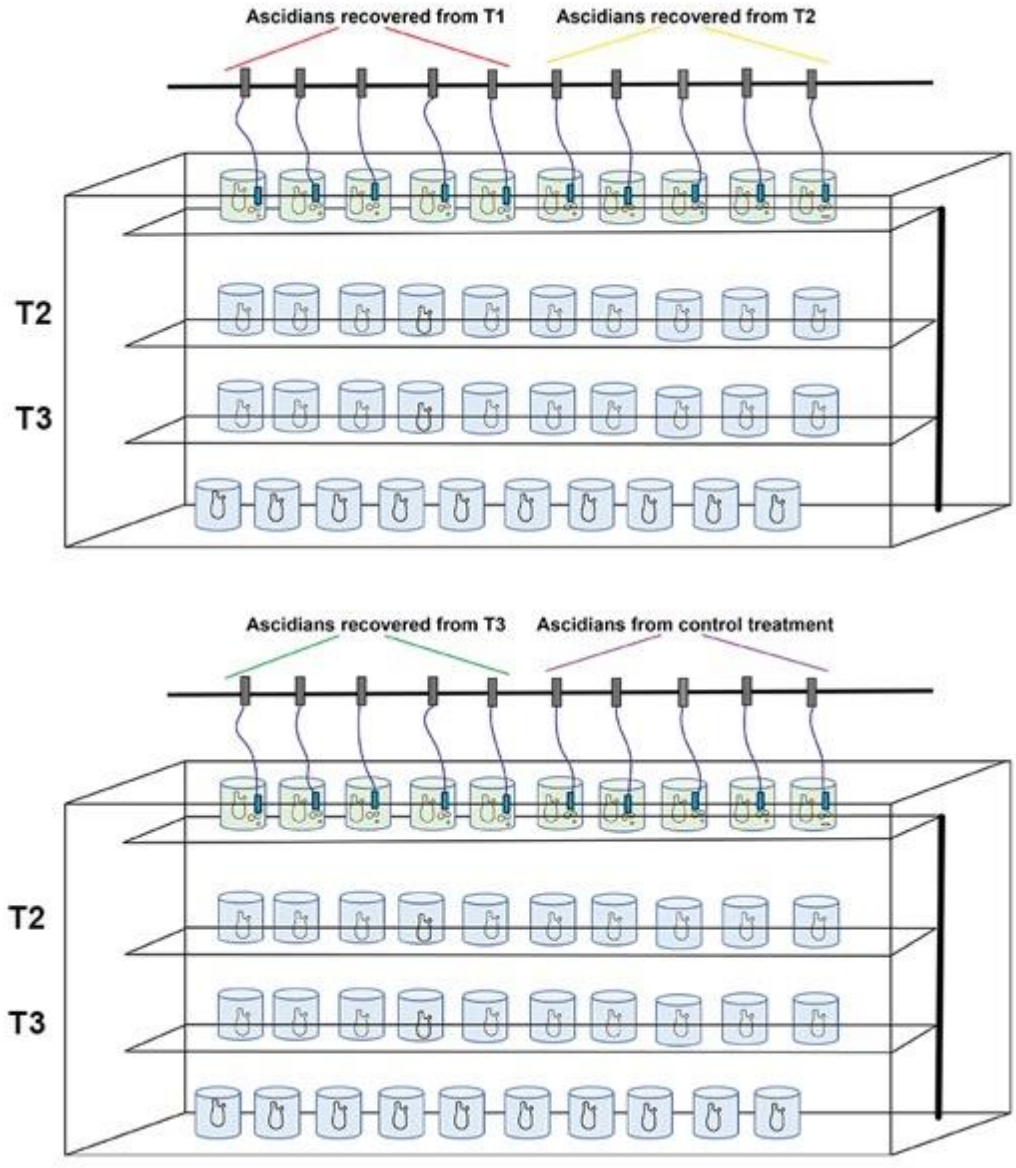

Figure 3. Drawing of the mesocosm set up for experiment 2 at the low turbidity 80NTUs. The remaining recovered tunicates were untouched here.

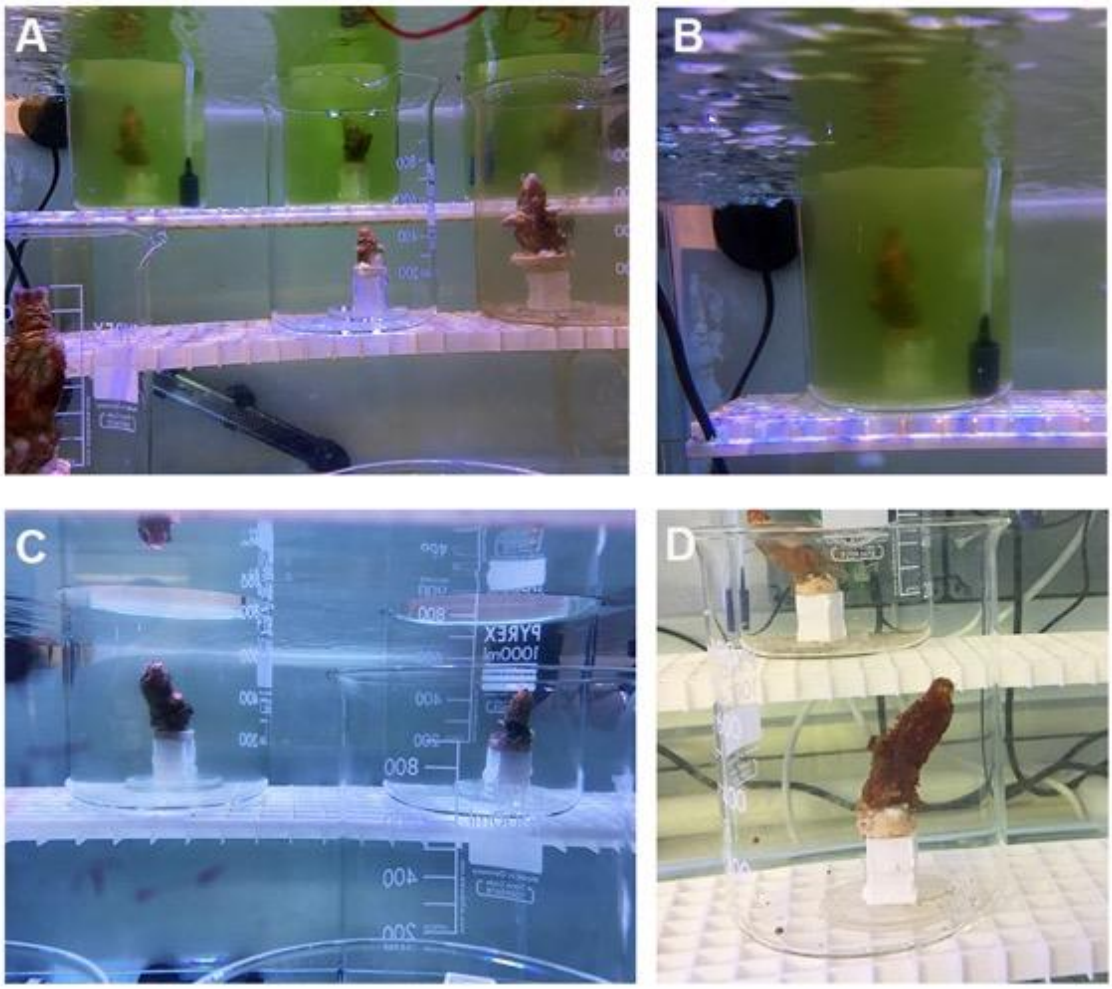

Figure 4. High turbidity experimentation for *P. captiosa*. A. Specimens being treated in lifted beakers with an air diffuser. B. Detail on one individual under high turbidity exposure. C. Animals left for recovery in their beakers and positions to keep tract of their recovery behaviour. D. Detail of an individual performing filtration during recovery time.

Statistical analysis

Filtration activity. To analyse differences in the filtration activity and the treatments, a one-factor ANOVA was performed, we considered statistically significant if $p<0.05$. All analyses were performed using the statistical software R.

Clearance rate and absorption efficiency. A repeated measures analysis was performed using general linear models (glm), our model included treatment, length of exposure and their interaction to understand the effect of the turbidity level and the exposure time on the filtration rates.

Evisceration. We performed a one-factor Anova to determine if there were significant differences between the gut ejection and the treatments. Also, to evaluate if gut ejection and recovery were affected by the turbidity treatment, and the length of exposure we ran a linear model (glm).

RESULTS

Experiment 1: Optimal filtration capacity

Filtration activity

From the censuses and video analyses we identified three main activity behaviours in the ascidians during the turbidity treatments, Siphon opening, Squirting and siphon closing (Table 1). In addition, our results showed that there are significant differences in the filtration activity among turbidity treatments (ANOVA, P=0.002) (Figure 5). The activity was reduced from T2 and increased in T1 compared to the control.

Table 1. Filtration activity (means) for each ascidian during the three turbidity treatments and the control.

Activity	Control	T1	T2	T3
N	10	10	10	10
No. Times OS	6±1	10±2	5±1	4±1
Min. time OS (sec)	20±1	35±3	120±5	180±9
Max. time OS (min)	16±4	28±2	32±1	38±3
No. Squirts	2±1	1±0.5	4±0.5	5±1
Min. time CS (sec)	15±0.4	2±0.4	5±0.3	6±0.4
Max. time CS (min)	10±2	3±1	21±1	23±0.5

OS: Open siphons; CS: Closed siphons; T1: Low turbidity 80NTUs; T2: Medium turbidity 250NTUs; T3: High turbidity 1000NTUs

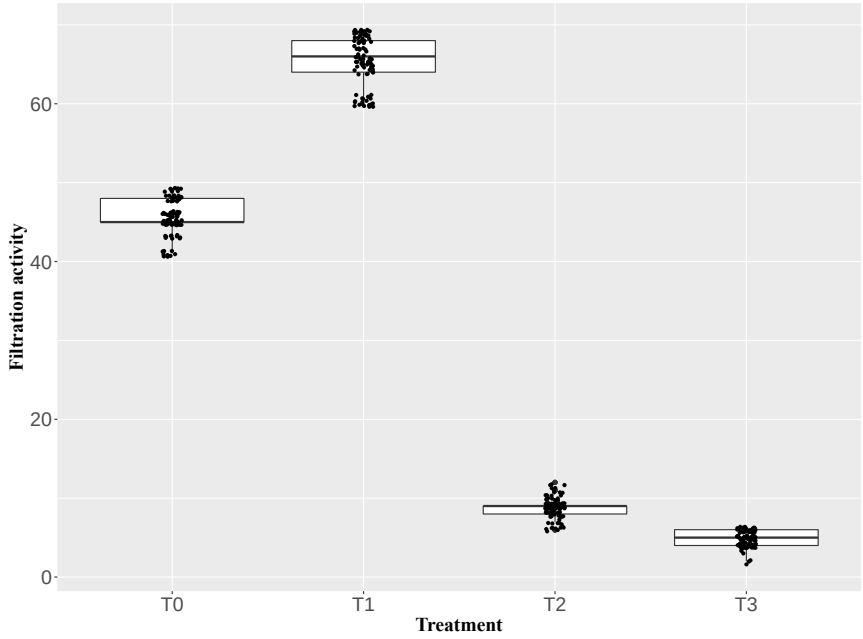

Figure 5. Differences between the filtration activity and the turbidity treatments. Activity was greatly reduced from T2 but significatively low in T3 compared to the control. T1 showed the highest activity.

Clearance rates

Our results showed that there are significant differences between the treatments for clearance rate ($p<0.05$). Optimal clearance rate was observed at the low turbidity treatment (80NTUs) (Figure 6).

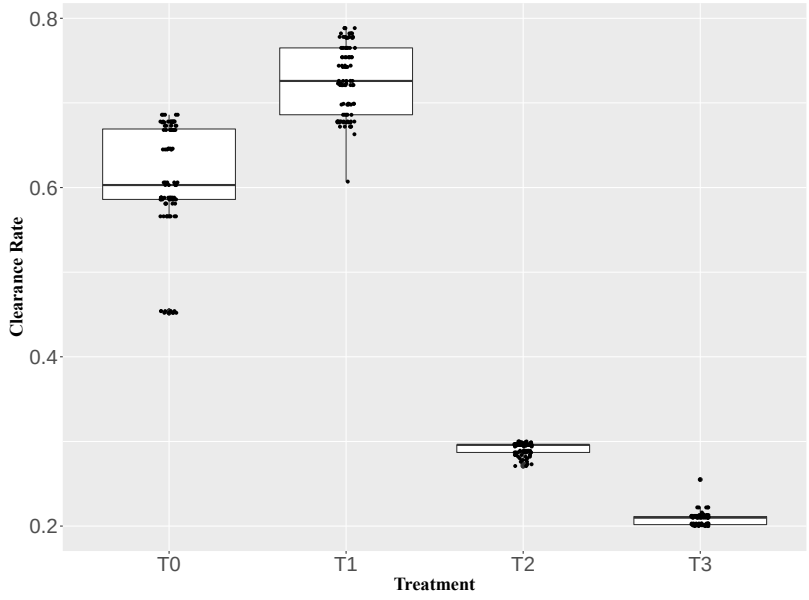

Figure 6. Differences between the treatment and the Clearance rate. T1 presented the highest rate.

There is also an interaction of turbidity treatment and gut ejection on the clearance rate ($p<0.05$) (Figure 7).

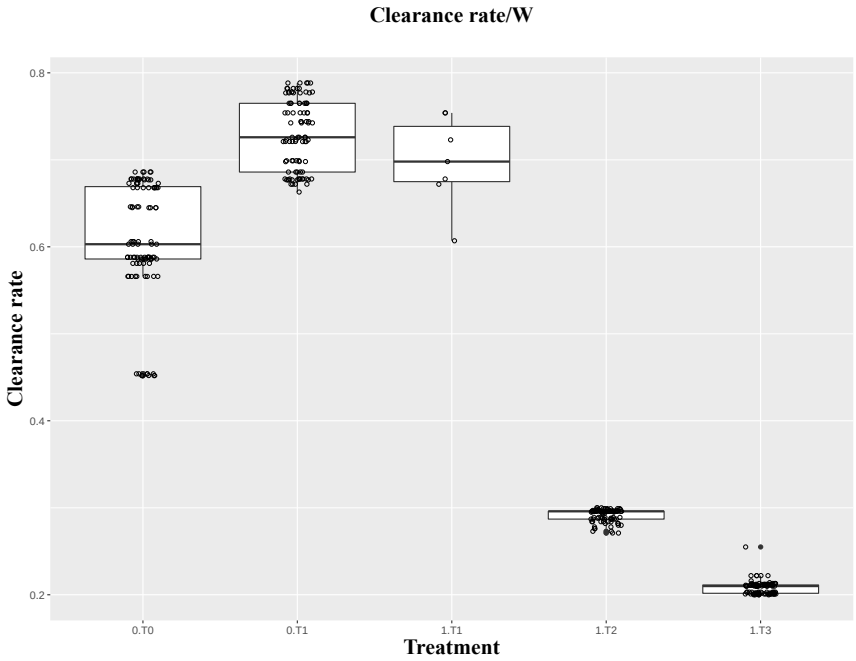

Figure 7. Effect of the turbidity treatment and the gut ejection on the clearance rate.

Absorption efficiency

We found that, similarly to the clearance rate, the efficiency at which ascidians absorbed nutrients was affected by the turbidity treatments ($p<0.05$) (Figure 8).

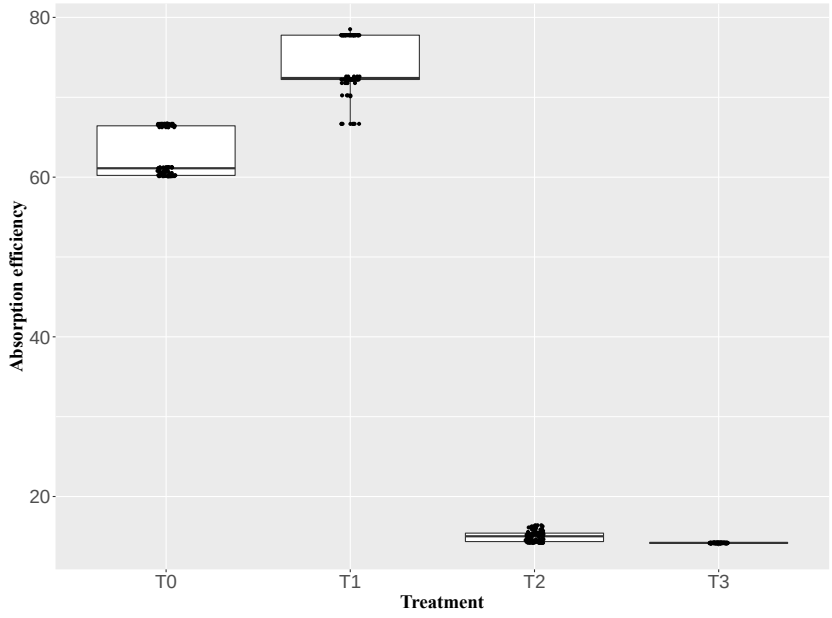

Figure 8. Effect of the turbidity treatment on the Absorption efficiency.

Evisceration process

We found significant differences between the gut ejection across turbidity treatments (ANOVA, $p<0.05$); ascidians exposed to T2 and T3 were more likely to eject their guts than those in T1 (Figure 9). This behaviour occurred at different times after exposure depending on the treatment ($p=0.003$). In general evisceration occurred about 12-hours post turbidity treatment, ascidians performed total evisceration in about 35 minutes via the atrial siphon (Figure 10). The evisceration process is energetically expensive and complicated, as it requires constant contraction of the body to detach it. After evisceration, ascidians remained with open siphons for a 30-minute period (Figure 11) and then squirts for over a 10-hour period. Within 12 days post evisceration, individuals slightly open their siphons, which we hypothesise that this is to create water interchange. The gut regeneration time depend on the turbidity level ($p<0.05$). Ascidians exposed to T2 and T3 recovered their guts within 20 days after exposure while ascidians exposed to T1 that performed evisceration, regenerated their guts within 15-days (Figure 12).

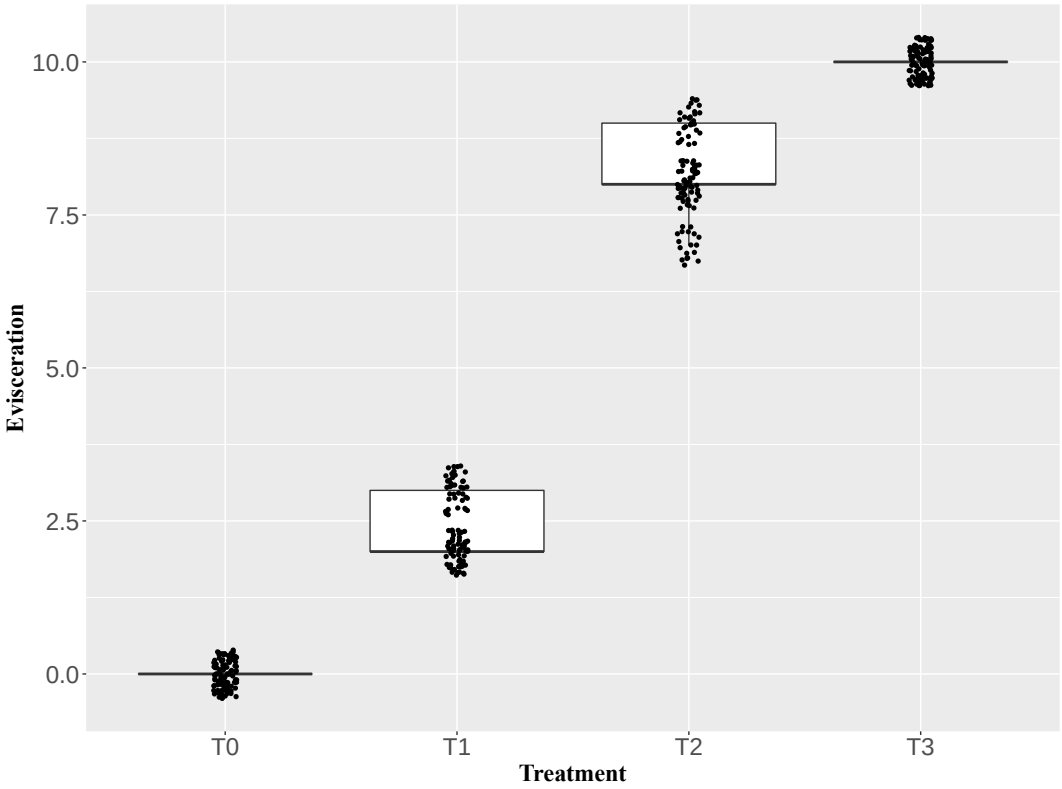

Figure 9. Differences between the evisceration and the turbidity treatments.

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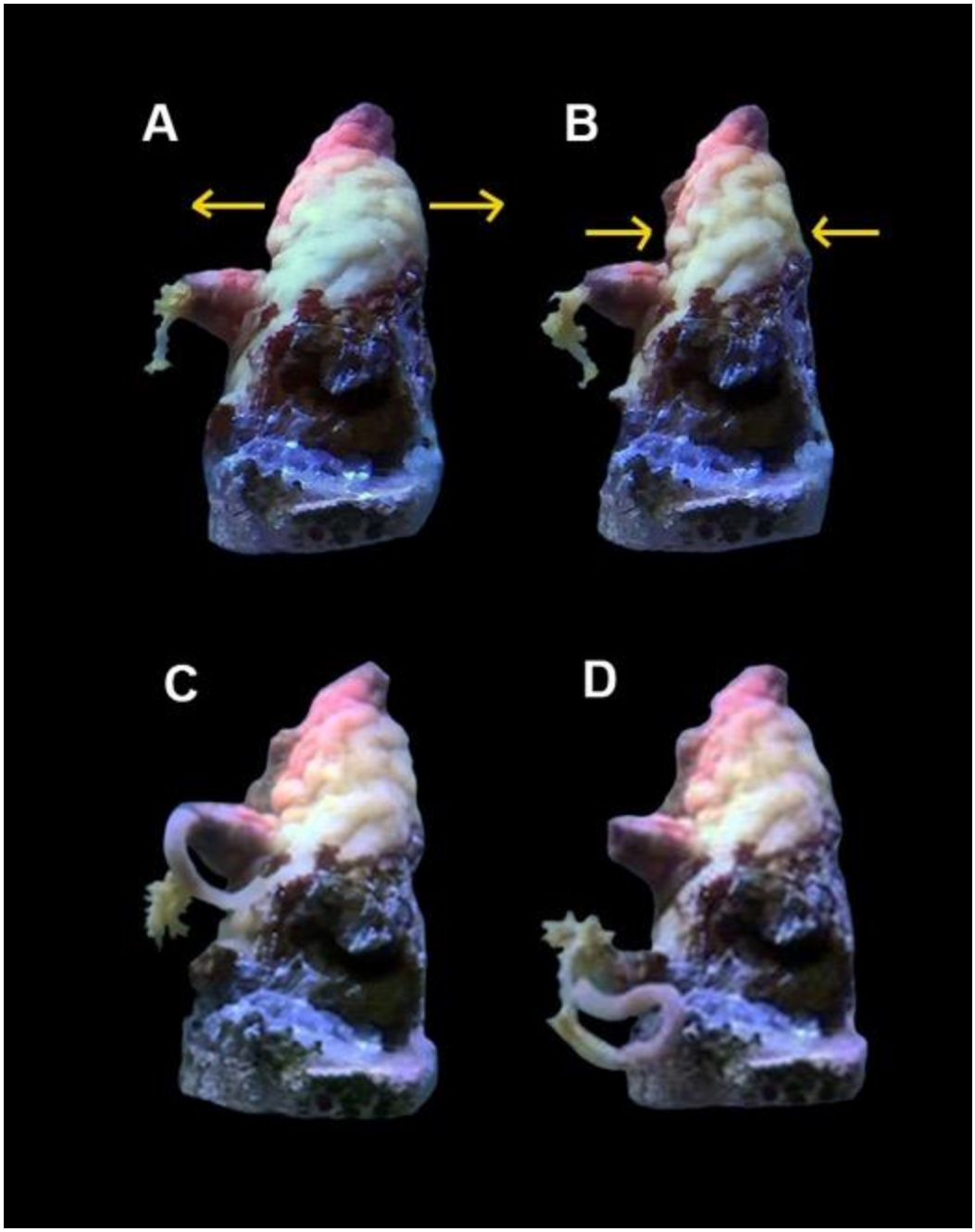

Figure 10. Evisceration process in *Polycarpa captiosa* after turbidity treatments. A. 12-hours post exposure, part of the pharynx is observed coming out the atrial siphon. Ascidian shows regurgitant-like movements, expanding and contracting the body. B. Elapsed 10 minutes, the pharynx has come out 10% more and ascidian continues contracting musculature. C. About 8 minutes after 60% of the gut is hanging from the atrial siphon, contracting behaviour is less active. D. Finally 10 minutes later, the entire gut has been eviscerated and the animal remains with open siphons for Ca. 30 minutes.

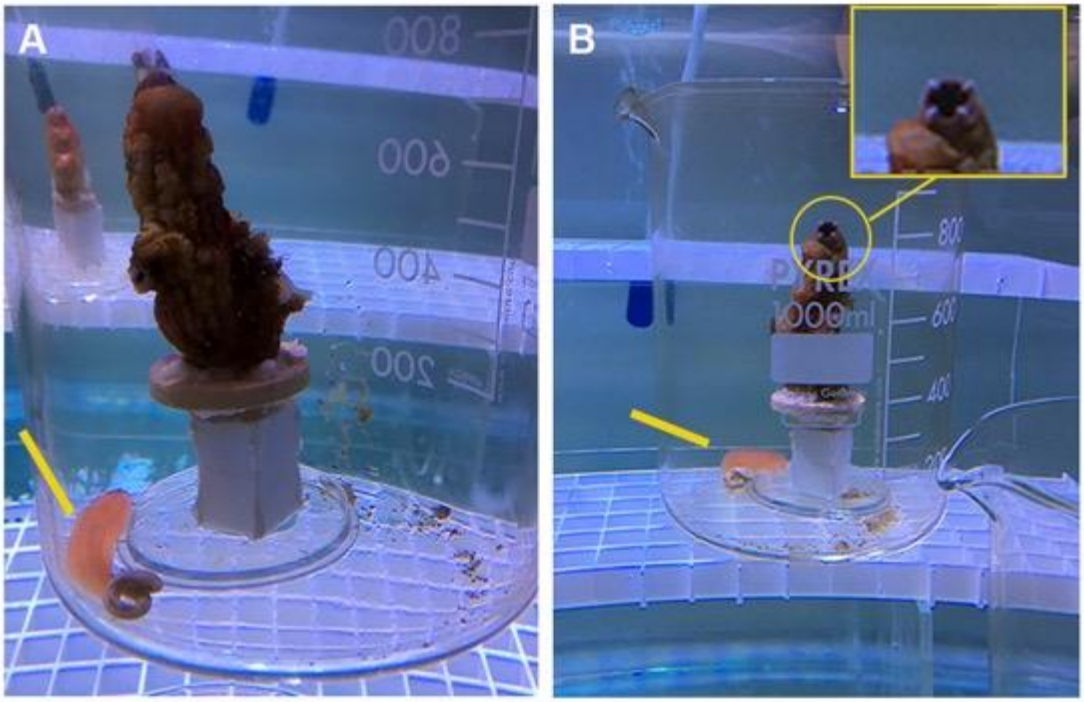

Figure 11. Behaviour of P. captiosa after evisceration. A. Individual eviscerated elapsed 12-hours after the low turbidity experiment, gut ca be observed at the bottom of the beaker and its siphons are slightly open. B. Individual remains with open siphons for about 30-minutes after evisceration, gut can be observed at the bottom of the beaker

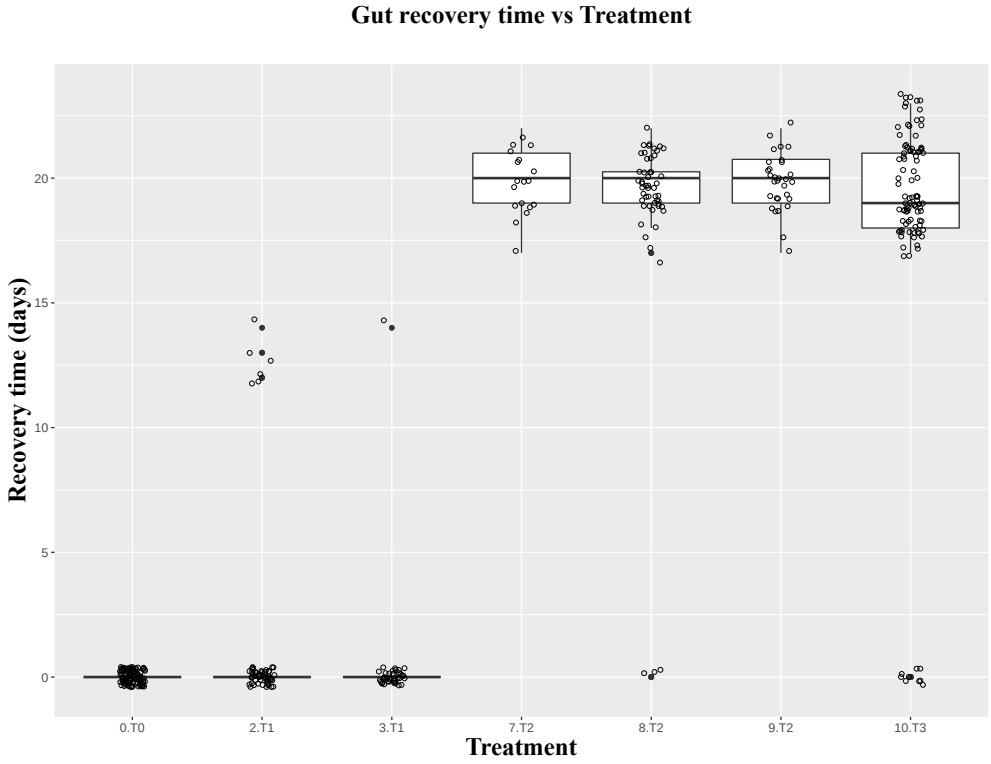

Figure 12. Effects of the treatment and gut ejection on the recovery time

After evisceration, guts were gently removed from the beaker and examined under the microscope (Figure 13). At this stage they were peach coloured, and the pharynx was strong enough to be held by soft tweezers. We weighed, measured and compared them with the tunicate body measurements. Then, we dissected the stomach and intestine with the purpose of finding any gut blockage and could have led into the evisceration. However, we could not find great accumulation of organic matter in those organs. We observed that fresh-ejected pharynx had small red-pigmented dots in between the rows of stigmata's (Figure 15). Half of the eviscerated guts were preserved in molecular grade ethanol and the other half in liquid nitrogen for further molecular analysis. After preservation guts turned pale white coloured. Animals that were intentionally left under the turbidity treatments ejected their guts but did not recover and died after the two-days unchanged exposure.

Figure 13. Eviscerated gut of Polycarpa captiosa. Showing a peach colour and good apparent health. Scale bar: 0.5cm.

Experiment 2: Recovered guts filtration activity

After one-month of gut recovery, we selected and exposed 15 ascidians to a low turbidity exposure (80NTUs) as we have determined this is the optimal turbidity tolerance for *Polycarpa captiosa*.

Our results showed a reduction of the clearance in comparison to the previous experiment ($p<0.05$). Ascidians that were previously treated in T1 significantly decreased their optimal clearance rate (Figure 14).

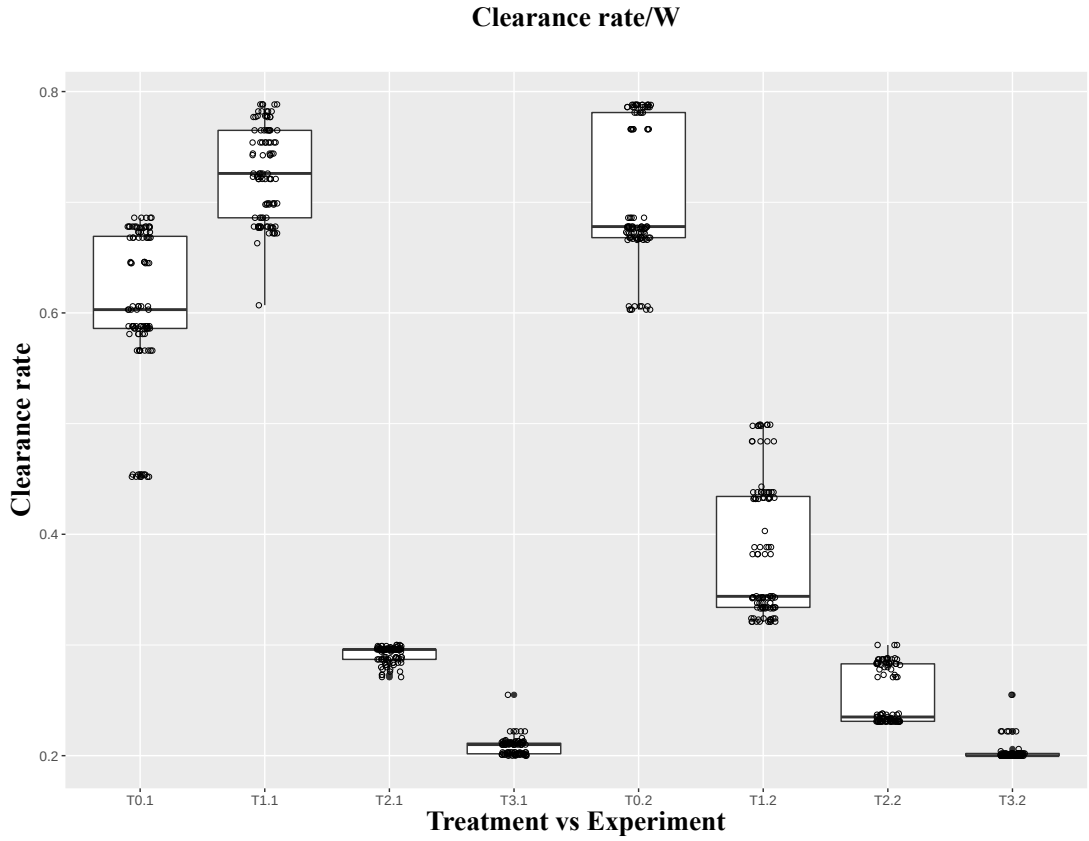

Figure 14. Effect of the previous treatment at which ascidians were exposed to (T0-T3) and the experiment (1,2) on the Clearance rate.

In addition, eight ascidians re-eviscerated their previously recovered guts. When we examined the new ejected pharynx, we noted that the red-pigmented dots in between the stigmatas were not present in the second guts (Figure 15). Finally, after another month of recovery, we dissected an individual that has performed evisceration twice and had recovered. We noticed that the pharynx had a pale-yellow colour different to the untreated pharynx from the control treatment (Figure 16).

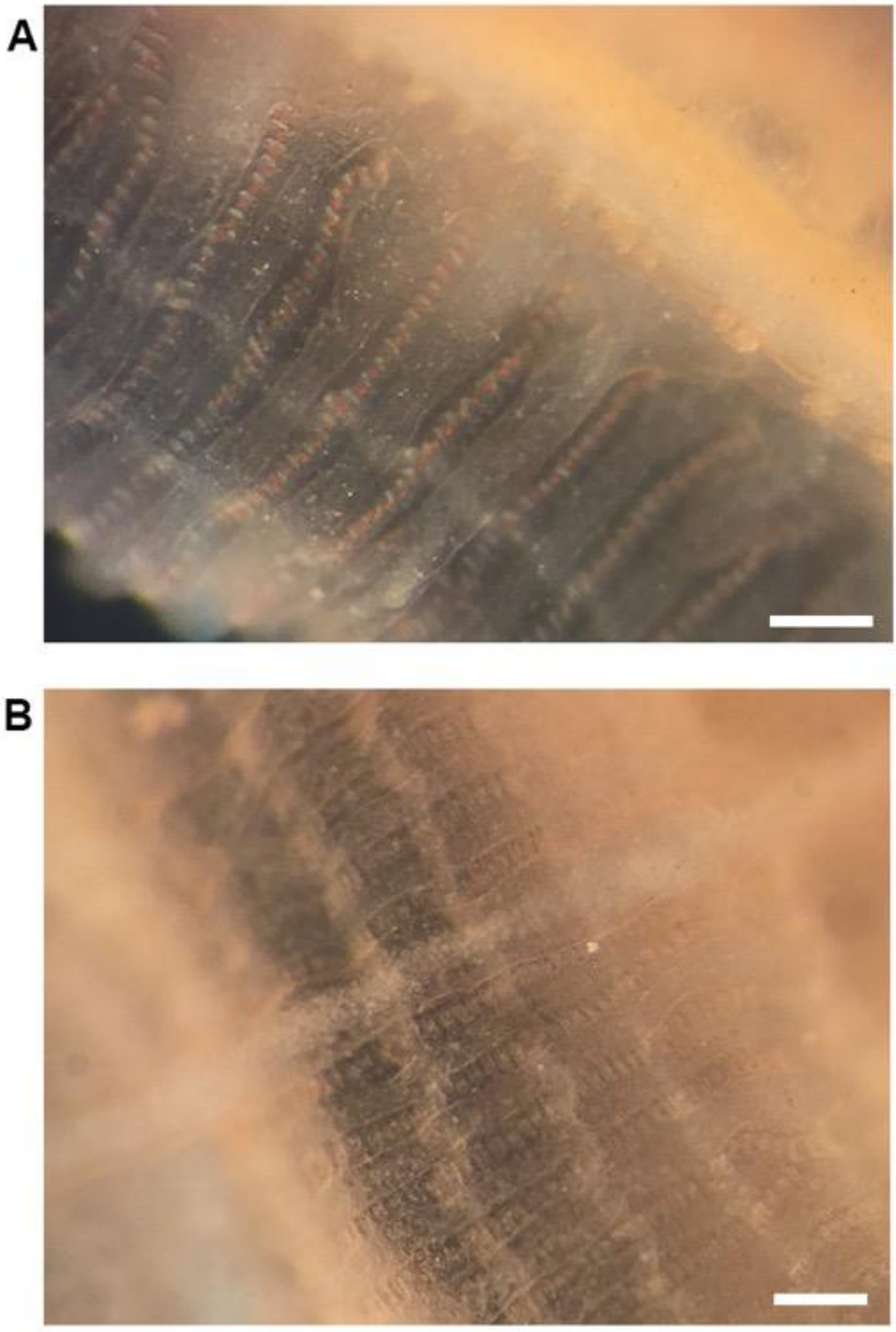

Figure 15. Eviscerated pharynx of *Polycarpa captiosa*. A. Pharynx after first evisceration. Red pigmented dots can be observed in between the row of stigmatas. Scale bar: 0.2cm. B. Second Pharynx eviscerated; no red dots are observed. Scale bar: 0.1cm.

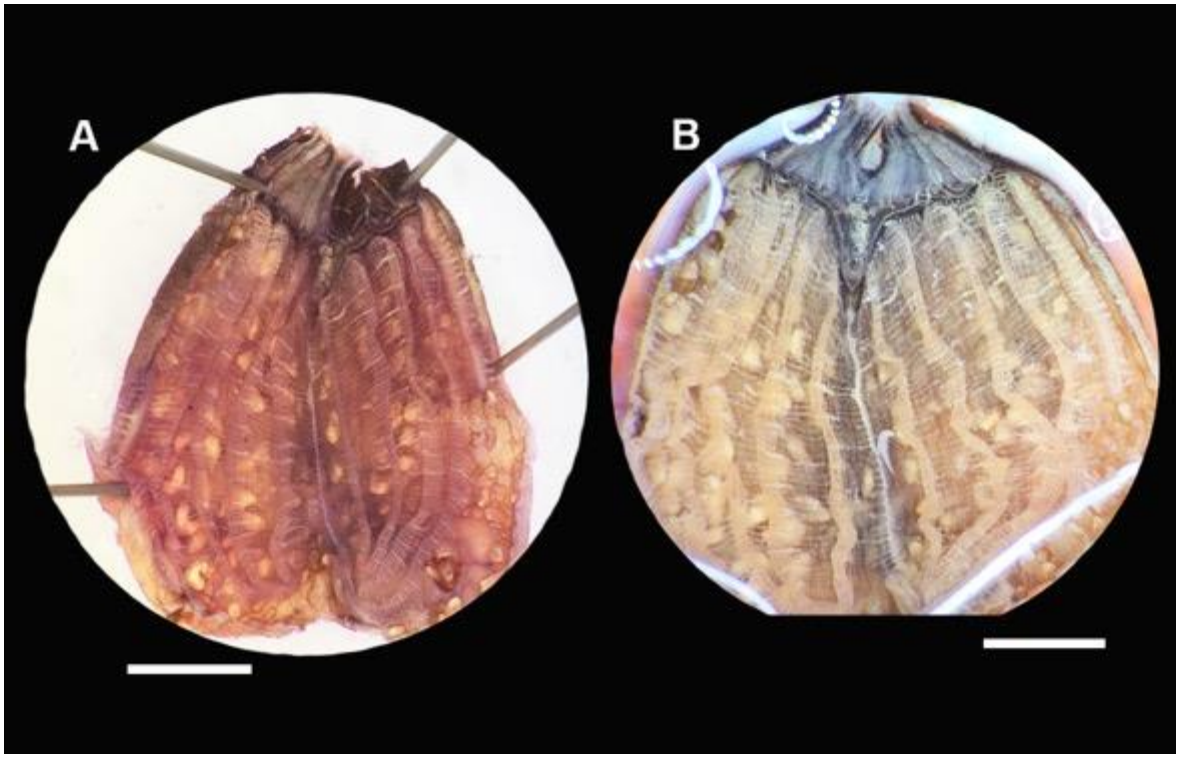

Figure 16. Butterfly dissection of *Polycarpa captiosa*. A. Individual from the control treatment. Pharynx has a peach-pink colour and gonads are bright yellow underneath the pharyngeal mesh. Scale bar: 0.5cm. B. Individual recovered from experiment 2. Second Pharynx has a pale-yellow colour and pale gonads can be spotted from behind it. Scale bar: 0.5cm.

DISCUSSION

Effect of turbidity on the filtration rates of P. captiosa

Our results demonstrated that the optimal turbidity tolerance for *P. captiosa* is 80NTUs or below, at this concentration, animals were more active than in the other treatments and produced a larger number of faeces, which is an indication of ingestion and nutrient absorption. Treatments set at higher turbidity treatment challenged the ascidian's clearance rate and the efficiency of nutrient absorption. Filtration activity showed that ascidians were less active under higher turbidity levels, we hypothesise that the excess of suspended food outside their tolerance range induce a feeding shock in which animals are not capable of filtration and remain with siphons opened but no pumping activity (Hoyle, 1953). In nature, eutrophic ecosystems, were the suspended organic matter is higher, the ascidian's absorption efficiency is expected to increase, however, we demonstrated that environmental stressors such as high turbidity can modify the ingestion rate at which the food is captured and affect the efficiency of absorption of particulate material.

Polycarpa captiosa is an Indo-pacific ascidian that inhabits shallow coral reef areas (Lee et al., 2013). Due to its location within reef forming organisms, this species potentially contributes to the water transparency and nutrient recycling that benefits a deeper penetration of light into the water column to allow other light-dependant organisms such as corals to thrive (Burge et al. 2016). Our findings are important for local coastal management and conservation strategies as prolonged high turbidity events might limit the ecosystem functions of this species and for instance the survival of its dependant organisms.

Significance of evisceration in ascidians

Evisceration is an energy-expensive physiological response to environmental stressors which in the short term affects the animal capability to perform filtration and to adapt with the surrounding environment affecting their vulnerability to predation and reproduction but in the long term could dramatically reduce their populations. After 4-months experimentation with *P. captiosa* individuals, 40% of the animals deceased and the remaining representatives reduced their filtration

rates compared to the control specimens. In addition, the gut health was severely affected, ascidians presented more delicate and less irrigated pharynx after double evisceration.

Ascidians are unique among chordates for presenting an exoskeleton or tunic composed by proteins and other macromolecules such as tunicin (Blanchould et al., 2018). The tunic is capable to elongate along with the animal growth and undergo regeneration after physical damage (Rinkevich et al., 2007a; 2007b; Voskoboynik et al., 2007; Brown et al., 2009). Environmental stress affects the normal filtration of the ascidian and promotes the gut ejection events. Animals regenerate their guts within 20 days after evisceration. During this time, they carried on gas and water exchange by occasionally opening their siphons. This research contains novel findings on the stress responses of Chordates to environmental stress.

CONCLUSIONS

Ascidians perform evisceration after environmental stress, our results indicated that this behaviour could occur more than once depending on the severity of the disturbance., but optimal clearance rate and gut health is affected by the evisceration events.

ACKNOWLEDGMENTS

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Chapter 6: The effects of ocean acidification and warming on the biological functions of coastal marine filter-feeders

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ABSTRACT

The resilience of marine filter-feeders under environmental disturbances caused by climate change, and the impact of those changes on their biology and ecosystem functions represent a real concern for coastal management and conservation strategies. Using a short-term study, we evaluated the biological responses of the tropical ascidian *Polycarpa captiosa* to ocean acidification and warming (OAW). Treatments included (0) control: ambient pCO2 (±400 ppm) at 25°C, (1) ambient pCO_2 (±400 ppm) and high temperature 30°C, (2) elevated pCO_2 (±1,000 ppm) at 25°C, and (3) elevated pCO_2 (±1,000 ppm) at 30°C. The responses of ascidians were assessed by analysing their resilience (survival, resilience), and clearance, absorption and ingestion rates. We found that these levels of disturbance significantly affect the resilience and performance of ascidians at the individual level. Animals reacted by skin shedding and gut ejection after all high temperature and elevated pCO_2 treatments, and 20% mortality was observed in ascidians that could not recover from the environmental stress.

We presume that ascidians increased their metabolic rates under warm temperature treatments, as clearance and ingestion rates increased by 50%, and absorption efficiency was not significantly affected. In contrast, under higher pCO2 conditions, a significant reduction of the ingestion rates and clearance rate was registered. Our results could be considered crucial for the future of the coastal managements and conservation strategies of those important ecosystem improvers.

Keywords: *Polycarpa captiosa*, Climate Change, Ecosystem Functions, Gut Ejection.

INTRODUCTION

The atmospheric gas emissions created as a consequence of human industrialization are considered the main cause of the increase of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). By 2004, the concentration of CO_2 had increased from 250ppm in preindustrial times to 440ppm (Dickson et al., 2007; IPCC, 2005; 2013). Modelling studies (RCP8.5 scenario) have concluded that by the end of the century, atmospheric CO_2 will increase to 1000 ppm, ocean pH would decrease by 0.3-0.4 units and carbon ion concentration would decrease by 30% compared to preindustrial levels (Feely et al., 2004; Dickson, 2010; IPCC, 2007a). In the oceans, seawater is weakly buffered with respect to hydrogen ions [H^-], allowing CO_2 to be dissolved and produce carbonic acid [H_2CO_3]. This leads to decreased pH and calcium carbonate saturation [Ω_{cal}], increased dissolved inorganic carbon (DIC/C_T) and decreased carbonate ion concentration [CO_3^{2-}] (Dickson, 2010; Sabine et al., 2004).

The question of how much of the anthropogenic CO_2 has penetrated the ocean and how it has modified ocean chemistry, together with the understanding of the effects of those changes on the nature at ecosystems and species level, are foci of research worldwide (Dickson, 1990; Seibel & Walsh, 2003; IPCC, 2007b; Gattuso & Lavigne, 2009; Kroeker et al., 2011; Godbold & Solan, 2013). Ocean acidification (OA) is one of the major threats to ecosystem health because of the negative impacts on marine organism physiology and ecology. This is especially true for calcified organisms whose skeleton formation is affected by the decrease of carbonate saturation under OA (Lombardi et al., 2011; Kroeker et al., 2013; Lane et al., 2013).

The simultaneous increase of mean ocean temperature of up to 6°C since preindustrial times represents another area of concern for marine climate change research (Pörtner & Farrell, 2008; IPCC, 2012; 2018). Ocean warming, as well as ocean acidification, affect species' distribution, metabolism and thus, ecosystem health (Hale et al., 2011; McCulloch et al., 2012). High temperature negatively affects processes at the cellular and system level by taking organisms outside their thermal tolerance ranges (Bishop & Brand, 2000; Pörtner, 2008; Wood et al., 2010). Warming also results in a decreasing phytoplankton biomass in the oceans

(Sommer et al., 2012) which represents a limiting factor for filter feeding organisms like ascidians (Kim et al., 2019; Rodolfo-Metalpa et al., 2011). Warming then, could worsen the ocean acidification impacts on marine fauna, resulting in a complex effect when both stressors are combined (Anthony et al., 2008). Considering this, analyses that evaluate both stressors are needed to understand the role of ocean warming on the biological response to low pH by considering multiple influencing factors (Kroeker et al., 2013).

Feeding by filtration of the water column is an important ecosystem function performed by many marine species including ascidians. Rates of filtration and ingestion depend directly on the efficiency of metabolic activity and are key in the species' survival because they directly influence growth, distribution and reproduction (Pörtner, 2008). Understanding how these activities would cope with Ocean Acidification and Warming (OAW) is one of the main foci of economic-species research, mainly because of its direct impacts on their ecosystem services (Donohue et al., 2012; Calosi et al., 2013). Most studies have found contrasting responses from species (Maas et al., 2012; Melatunan, 2012; Lemansson et al., 2018; Calosi et al., 2013) to phylum level (Hendriks et al 2010; Kroeker et al 2010). On one hand, a notable reduction of the metabolic activity under short term ocean acidification has been observed in molluscs and coral larvae (Reipschläger & Pörtner, 1996; Michaelidis et al., 2005; Rosa & Seibel, 2008; Beniash et al., 2010; Melatunan et al., 2011; Nakamura et al., 2011), in contrast, some bivalves and crustaceans seemed to show no evident change in metabolic activity under short term elevated pCO_2 conditions, but a reduction in filtration rates and growth under long term exposure to combined OAW (Marchant et al., 2010; Donohue et al., 2012; Thomsen & Melzne, 2010).

This suggests that organisms could adapt to short exposures by reducing the energy spent in basic biological functions such as feeding (Guppy, 1999); but that in the long term this would influence survival, growth and fitness (Calosi et al., 2013). It should be noted that metabolic reduction is not necessarily a negative response as it could lead to adaptation and phenotypic plasticity in the long term (Ghalambor et al., 2007; Thomsen & Melzner, 2010), which would allow species with naturally lower metabolic rates and reproduction strategies to thrive.

Organisms like ascidians are potential indicators of the water quality due to their filter-feeding habits and a good model system to evaluate the effects of OAW because, their larval stages are environmentally vulnerable to lower pH and warming (Gosselin & Qian 1997), which influences their dispersal and survival rates (Dupont & Pörtner, 2013; Dupont et al., 2010; 2012; Byrne, 2011; 2012 Pechenik, 1999). Also, their ability to filtrate up to 1 gallon of water per day under normal conditions allows research to evaluate their performance under disturbances in short-term experimentation. Finally, these organisms are common in all marine ecosystems and a conspicuous component of coastal systems playing important roles in the ecosystem dynamics and processes, understanding their possible threats would predict the health of the ecosystems they inhabit.

The organisms used in this research were solitary ascidians from the genus *Polycarpa*. This genus has a reported cosmopolitan distribution, but we focused on the effects of ocean acidification and warming on the biological response of the tropical Indo-Pacific *Polycarpa captiosa* under current tropical ocean temperature (25°C) and pCO2 (±400 ppm) and under values predicted for the end of the century (30°C and pCO_2 ±1,000 ppm). We evaluated effects on the performance of their ecosystem functions by analysing their resilience, and clearance, absorption and ingestion rates of particulate matter in the water column.

METHODS

Specimens used

In this study, adult individuals of *Polycarpa captiosa* (Figure 1) were used. Taxonomic identification was confirmed using Rocha et al. (2012) and Lee et al. (2013). Animals were collected in coral reefs ecosystems in Indonesia between 1 and 5 m depth and obtained through an aquarium supplier (Cheshire Aquatics, UK).

Phylum Chordata Haeckel, 1874

Subphylum Tunicata Lamarck, 1816

Class Ascidiacea Blainville, 1824

Order Stolidobranchia Lahille, 1886

Family Styelidae Sluiter, 1885

Genus *Polycarpa* Heller, 1877

Polycarpa captiosa (Sluiter, 1885)

Figure 1. Specimens of *P. captiosa* A. Adult ascidian showing the oral siphon (Os), atrial siphon (As) and its leathery tunic (Tun). B. Body of the ascidian showing the musculature (M). C. Butterfly cut of the ascidian body showing morphological key features, Endocarps (En), Anus (An), Stomach (St) and Intestinal loop (In). Scale 0.5 cm.

Description. *Polycarpa captiosa* is a solitary ascidian of ovoid form often attached to coralline substrate from its posterior end. Tunic is tough, colour either pink or reddish-brown, slightly lumpy and leathery. The atrial siphon opens at half the distance down the body. Both siphons have a proximal purple ring in which the lobes have four white protrusions marks. Body is brown and has transverse muscles. Branchial tentacles are simple, dorsal tubercle is heart-shaped, dorsal lamina is smooth and continuous. There are four non-overlapping folds on each side of the branchial sac, with 6-8 stigmata in each mesh. No para-stigmatic vessels were present. The stomach is elongated with internal folds, and together with the U-shaped gut loop occupies the 1/3 of the left posterior region of the body. Elliptical endocarps are found attached to the body wall from the pre-pharyngeal band to the gut loop.

Ecological features. These ascidians can filter up to 1US gal per day, adapting their metabolic activity to circadian rhythms (Jacobi et al., 2017; Lee & Lambert, 2013; Minamoto et al., 2010; Ryland, 1990;). Some of their ecosystem functions include nutrient recirculation and purification of the water column (Shenkar & Swalla, 2011). They can be considered as pioneer or foundation fauna, creating available substrates and habitat for other species, filtering the seawater from an excess of suspended particulate material and improving light penetration in the water column, which is a key requirement for light-dependant organisms to thrive.

Acclimation process

For this study, adult specimens (4.5 ± 0.5 cm in length, 2.7 ± 0.3 cm wide and 7 ± 1.1 g) were cleaned from other epibionts with a soft brush, measured, weighed and attached from the base to a ceramic plug using reef glue and placed in an upright position on an egg crate tray in a 300L acclimation tank.

Animals were maintained at a salinity of 35ppt at 25±0.5°C for one-month prior to experimentation, in a 300L recirculating tank conditioned with a protein skimmer (V2 Skim Pro 450), temperature chiller (D-D Model DC-750), a thermostat and artificial lighting replicating natural 12-hour day and night patterns. Water parameters were maintained as Calcium (375-450 mg/L); Total alkalinity (2.13±0.32 mol/kg); Nitrite (0-0.2 mg/L); Nitrate (0-0.2 mg/L); Phosphates (0-0.03 mg/L); Magnesium (1250-1350 mg/L); pH (8.0-8.2); Salinity (35 ppt - 1.025 sg). Animals were fed three times a week with a solution of live phytoplankton (*Dunaliella salina*, *Nannochloropsis oculata* and *Tetraselmis* sp.), concentrated zooplankton suspension (copepod) (Gamma Nutraplus), vitamins and aminoacids (Red Sea supplements). Partial water changes of 10% were performed every other week and at the same time tank walls were scrubbed and faeces and precipitated organic material were siphoned out.

Experimental design

This study was designed to replicate the expected scenarios for the future OAW through CO_2 enrichment in aquaria (DOE, 1994; Gallo et al., 2019). We set up a laboratory-controlled set of mesocosms following the internationally accepted guidance and analytical parameters accepted in ocean acidification research

(Dickson et al., 2007), which in our case included Total alkalinity (µmol kg^{-1}), pH (Total scale) and pCO_2(µatm), along with seawater temperature and salinity.
We built a semi-independent system of nine experimental units. Every treatment shared the same source of seawater and was exposed to the same conditions to avoid water chemistry bias (Hurlbert, 2013) and ensuring no interdependence of replicates in the same treatment (Cornwall & Hurd, 2015). For this, prior to experimentation, tanks were acclimated, and desired water chemistry mixed in the system. When the conditions were stable in the system, individual 30 L tanks (n=9) were isolated, at which point each tank was treated as an independent replicate (Figure 2).

One adult specimen of *Polycarpa captiosa* was placed in each experimental tank (9 in total). Controls were maintained at ambient pCO_2 (±400 ppm) at 25°C for the total length of the experiment (12 weeks). Treatments were run sequentially in the following order: Treatment 1 (T1): ambient pCO_2 (±400 ppm) and at high temperature 30°C for two weeks, then ascidians were left into an adaptation/recovery period of three weeks under control conditions. Following this, ascidians from T1 were exposed to Treatment 2 (T2): elevated pCO_2 (±1,000 ppm) at 25°C for two weeks, and subsequently were left in an adaptation/recovery period under control conditions. Finally, these specimens were placed under Treatment 3 (T3): elevated pCO_2 (±1,000 ppm) at 30°C for another two weeks.

Although the ocean chemistry predictions do not consider tropical coastal areas because of the regional oceanographic variability and natural fluctuations that currently take place (due to coastal processes such as high dissolved organic material, high productivity and nutrients levels, turbidity and geological erosion) (Melendez & Salisbury, 2017), we replicated the oceanographic conditions offshore in laboratory to avoid those coastal processes bias and we used the OAW conditions predictions for 2100 made by Dickson, (2010).

For instance, the scenarios tested do not strictly replicate the coastal predictions at the native location of *P. captiosa* for 2100 but represent an approximation to the expected predictions for this period in the open ocean (IPCC, 2013). This mesocosm experiment ran for twelve weeks in total, including the recovery periods. During the

experiment, the water parameters were regularly measured to allow conditions to be kept stable (Supplement 1) and observation on the behaviour and filtration activity of tunicates were recorded.

Mesocosm set-up

The ocean acidification and warming experimental system was inspired and modified from Guo et al. (2015) and Lemasson et al. (2018). We created two pCO_2 concentration treatments ±400 ppm, (current concentration of CO_2) and ±1000 ppm (predicted CO_2 by the end of the century) following Gattuso & Lavigne (2009), and two temperature treatments 25°C and 30°C. Each experimental treatment took place in a 400L system of tanks connected to each other and to a 100L sump with live rock, a protein skimmer (100L/min flow rate), *Chaetomorpha* sp. as a nutrient-control algae, two filter socks (64µm), a temperature chiller, an evaporation/salinity control system (using reverse osmosis water) and a 4000slh flow rate pump to constantly recirculate the water to each tank (Figure 2).

To achieve accurate chemistry in our tanks, we purchased filtered natural sea water (64µm), and once in laboratory it was UV sterilised (Vecton 300 V2 - 15 Watt) for 48 hours prior adding it to the systems. The seawater was left running without ascidians in the system for a month to reach bacterial community maturity and monitored daily for fluctuations of water chemistry.

Subsequently, we mixed CO_2 (90%) and dry air at different ratios using two calibrated mass flow controllers (Omega ®) to generate CO_2-enriched air with ±1000 pCO_2. The air was obtained using an air compressor and then filtered with a disc-like airline filter (Gelman Acro 50) to eliminate water and unwanted particles, before passing it through a mass flow controller with a pressure regulation valve (FMA 5400A/5500A -10 SLM) to ensure a stable air flow could be maintained. Pure CO_2 was from a cylinder (BOC) and regulated by a pressure valve and a needle valve (3 bar) to produce a stable gas flow and then passed through a calibrated mass flow controller (FMA 5400A/5500A - 10 SCCM).

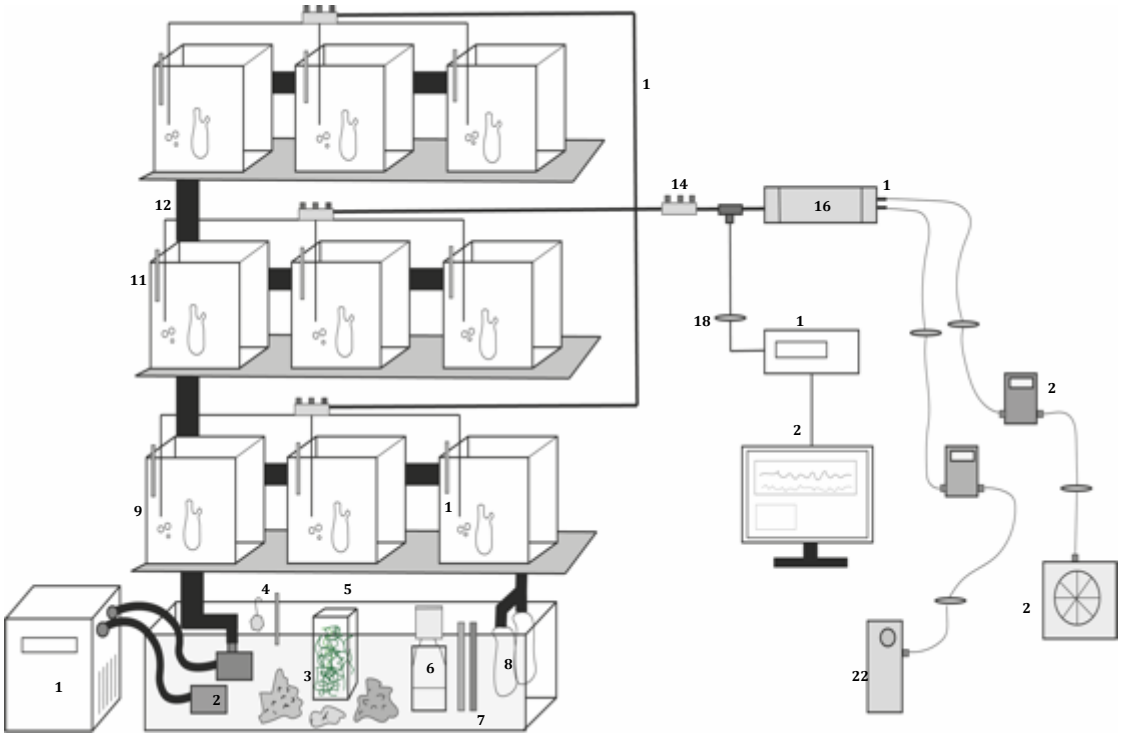

Figure 2. Drawing of the mesocosm set up for high acidification system. 1.Temperature chiller, 2. Flow pump 4000L min^{-1}, connected to the chiller and to the vertical system. 3. Live rocks for natural filtration, 4. Auto top-up system to control evaporation. 5. *Chaetomorpha* sp. living algae with an attached submersible LED light for optimal algae growth. 6. Protein skimmer (200L min^{-1}), 7. Thermostats set at 26°C. 8. Filter socks (64µm). 9. Experimental tank. 10. Bubbling airline, 11. APEX system. 12. Connecting tubing. 13. Main bubbling airline. 14. Gas control tap. 15. T connector, 16. Gas mixing capsule, 17. Output tubing, 18. Airline filter, 19. LiCOR, 20. Computer. 21. Mass flow controller. 22. CO_2 cylinder. 23. Air compressor.

Ambient air and CO2 were mixed in a sealed white PVC capsule resulting in a CO_2 concentration of ±1000 ppm. A small proportion of gas mixture (±1L) was analysed and monitored with a CO_2 analyser (Li-830, LI-COR) and the remaining gas was bubbled into each of the high pCO_2 experimental tanks (Figure 3). This system was placed in a room in which environmental temperature was controlled and monitored daily, environmental carbon dioxide was monitored daily with a CO_2 sensor and alarm.

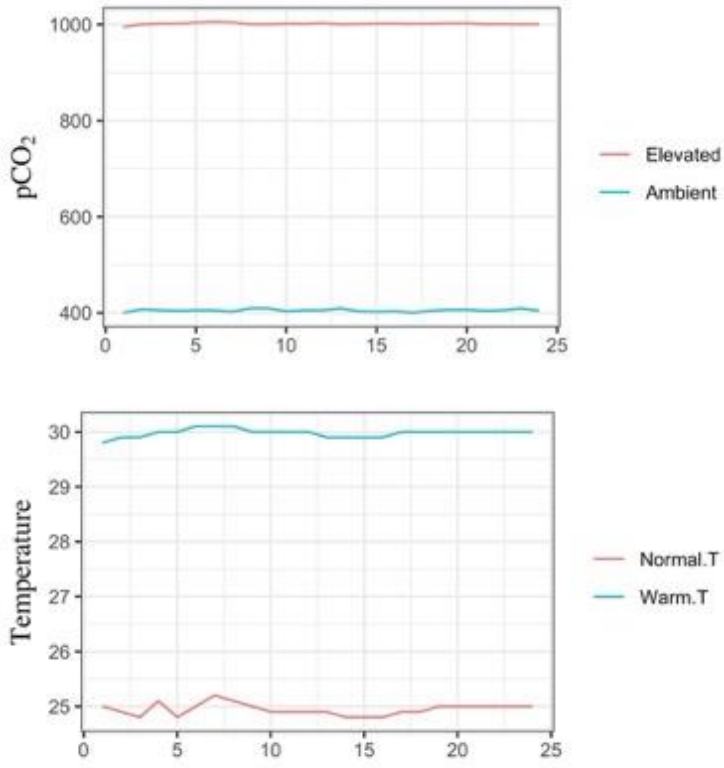

Figure 3. Ocean acidification and warming seawater chemistry variability over 24-hours during experimental time. *Top*. pCO_2 fluctuations for the Elevated (±1000ppm) and Ambient (±400pm) treatments. *Bottom*. Temperature fluctuations for the Normal (25°C) and Warm (30°C) scenarios.

Measurement of seawater chemistry

Salinity was measured daily using both a manual refractometer and a conductivity meter (Thermo Scientific); temperature was measured using both a digital thermometer (Fisher Scientific) and a continuous measurement system (APEX) which had a fluctuation alarm. For marine chemistry measurements, duplicate 500 ml samples were collected and stored in borosilicate air-tight bottles for pH and total alkalinity analysis. For total alkalinity, samples were poisoned with 200µl saturated $HgCl_2$ solution (0.02%) and kept in the dark until measurement, following the GOA-ON Standard operating procedures - SOP1 (Dickson et al., 2007).

Total scale seawater pH was measured using a temperature-controlled (±0.1°C) spectrophotometer (Thermo Scientific TM Orion AquaMate 7000) and plastic cuvettes in a temperature-controlled laboratory. To achieve reading accuracy, samples were dyed using pH adjusted (7.9 ± 0.1) *m*-cresol purple solution (2mmol L^{-1}). Total alkalinity was measured from poisoned samples by titration following

SOP3b (Dickson et al., 2007). Partial pressure of carbon dioxide (pCO_2), aragonite concentration (Ω_A) and calcite concentration (Ω_C) were calculated at the end of each experimental week using CO_2sys.xls (adapted from Lewis & Wallace, 1998), with the Lueker et al. (2000) constants fitted to total scale pH and the KSO_4 dissociation constant (Dickson, 1990) (Table 1). To ensure accuracy and reduce human bias in the seawater chemistry measurements, only one person performed the titrations and spectrophotometric pH records.

Table 1. Seawater chemistry measured and calculated for each system during each scenario.

*P*co$_2$ vs temperature	N	T (°c)	Sal	Ph	At	*P*co$_2$	Ω_a	Ω_c
C: ambient vs control	12	24.9±0.2	34.9±1.1	8.01±0.10	2.13±0.32	597.2±146.1	1.70±0.32	2.64±0.50
T1: ambient vs warm	4	25.4±0.3	35.3±1.2	8.02±0.10	2.32±0.29	669.7±155.9	2.02±0.31	3.11±0.47
T2: elevated vs control	4	24.8±0.2	34.9±1.2	7.65±0.10	2.13±.032	1174.6±420.9	0.99±0.22	1.53±0.34
T3: elevated vs warm	4	24.6±0.2	35.3±0.7	7.72±0.16	3.00±0.16	1053.6±223.3	1.98±0.37	3.07±0.57

Ambient (400ppm), elevated (1000ppm), control (25°c), warm (30°c), n (number of measurements) sal(ppt), ph (total scale), at (µmol kg^{-1}), pco$_2$(ppm), ω_a (aragonite concentration), ω_c (calcite concentration)

Physiological measurements

To evaluate filtration rates throughout each experiment, ascidians were fed three times a week with a diluted solution of 0.06mg live phytoplankton (20ml mix of *N. oculata*, *Dunnaliella salina* and *Tetraselmis* sp.) to obtain a concentration of approximately 10^8cell/L^{-1} for each experimental tank. After each measured day, faeces were siphoned using a glass pipette, then filtered using 25mm Whatman filters, rinsed with 1M isotonic ammonium formate NH_4HCO_2 to eliminate trace salts, dried at 40 °C for two hours and weighed in order to calculate the total ingestion and absorption rate per animal.

Resilience (R). Survival, gut ejection and recovery time were used as a measure of resilience under OAW scenarios. Observations on the ascidian behaviour and tunic appearance were made daily for one hour. Ascidians that performed gut-ejection, were marked, removed from the experiment and recovery time was recorded including the transition behaviour. These ascidians were included in the analysis as

a negative response to the treatment. Every ascidian that ejected its gut was replaced for the following treatment with a new individual. Ascidians with tunic damage or shedding the outermost membrane layer were also marked and tissue recovery time was also recorded as an adaptation behaviour to ocean acidification and warming but were not separated from the experiment.

Clearance rate (CR). Several studies have performed different calculation methods for the clearance rates in suspension feeders, most of them express it as dependant on the respiration, metabolic rate and food consumption (Jorgensen, 1949; Fiala-Medioni, 1978a; 1978b; Holmes, 1973; Klump, 1984; Kowalke, 1999; Petersen & Riisgard, 1992; Randlov & Riisgard, 1979; Robbins, 1983; 1984; Hughes et al., 2005; Sanders et al., 2013).

In this study, we calculated clearance rate as the maximum water transparency reached after the experimental time by counting the remaining algal cells in each experimental unit, adjusted to the animal body weight in order to provide an accurate estimation of each individuals' filtration capacity. This allowed a direct assessment of this biological function under environmental disturbances such as ocean acidification and warming. Here we expressed the ingested weight as a measure of the organic absorption rate and feeding efficiency.

Clearance rate was calculated as the volume of water that has been cleared of cells per ascidian per unit of time based on Armsworthy et al. (2001) and Macdonald & Ward (1994) following the equation,

$$CR=fr(cr-Ce)/cr$$

where *fr* is the mean flow rate of pumped water by the ascidians in the experiment (L min^{-1}), cr is the total cell concentration for all the algae cell sizes (Number of particles ranging from 3μm to 14μm) at the beginning of the experiment and Ce is the mean cell count at the end of the experiment. Clearance rate adjusted for the ascidian body weight (CR W) was ultimately used in the analysis and was calculated following the equation,

$$CR.W= Bwe/CR$$

Where, Bwe is the body weight in g after the experimentation, and CR is the clearance rate.

Standardized ingestion rate (IRs). In this study, we calculated the ingestion rate adjusted to each ascidian's body weight, as the dry weight of algal cells ingested per hour per miligram of animal. Because as for clearance rate, the ingestion potential is related to the body size and weight; we called this adjustment, standardized ingestion rate following Armsworthy et al. (2001). Studies focused on evaluating the ingestion rate in ascidians are scarce and the majority are part of the "old" literature (Riisgard & Randlov, 1981) and to our knowledge, the assessment of the standardized ingestion rate of tropical stolidobranchs under climate change scenarios and its effects on the provision of ecosystem functions is understudied.

Understanding how much of the suspended organic material is ingested by a filter feeder also determines the amount of fragmented nutrient that is put back into the system to be absorbed by other, smaller organisms, which represents an important biological function for the survival of microbiota and larvae.
Here we calculated it as the dry weight of the phytoplankton ingested per unit time by 1mg of animal and it was calculated based on Bayne et al. (1985) using the equation,

$$IRs=CR.W \times TPM$$

Where TPM is the total weight of the algae cells used to create the turbidity in g L^{-1} and CR.W the adjusted clearance rate based on body weight.

Absorption Efficiency (AE%). Studies on the absorption efficiency in suspension feeders demonstrate that it is related to the increase of food concentration (Thompson & Bayne, 1972; Riisgard & Randlov, 1981). Here, we calculated it based on Conover (1966) as the total weight of algae added at the beginning of the experiment minus the weight of faecal pellets after an hour of measurements, using the following equation,

$$AE= 100-(TPM-fw/ TPM) \times 100$$

Where fw is the faeces dry weight after the experiment, TPM is the total seston weight at the beginning of the experiment.

Statistical Analysis.

All data were tested for normality using a Shapiro-Wilk test and analysed for homogeneity of variances using Bartlett's test; where significant, data was

transformed using square root and logarithm base 10 (Log10). If still not significant, data was analysed using non-parametric tests such as Wilcoxon signed-rank test. When normal data was found, a one-factor ANOVA was performed, we considered statistically significant if $p<0.05$. All analyses were performed using the statistical software Rstudio.

Resilience. To evaluate if survival, gut ejection and recovery after evisceration were affected by temperature and pCO_2 conditions, we ran a linear model (glm) and an analysis of deviance type 2 test. We considered statistically significant if $p<0.05$.

Clearance rate, absorption efficiency, standardized ingestion rate. Because of the experimental design employed in this research, a repeated measured analysis was performed using general linear models (glm), our model included temperature, pCO_2 and their interaction to understand the effect of the interaction of temperature and pCO_2 on the clearance rate, absorption efficiency and standardized ingestion rate. We considered statistically significant if $p<0.05$.

RESULTS

Resilience

Overall, 90% of the specimens remained alive and performing filtration after each treatment. There were significant differences in the outer membrane shedding between the pCO_2 treatments (n= 60; Anova, $p<0.05$) but no significant difference between temperature treatments ($p=0.065$). There was a significant interaction between pCO2 and temperature with 95% of individuals shedding under the combined high treatment ($p<0.05$). Ascidians did not shed their outer membrane under T1(400ppm, 30°C) or control.

Under high pCO_2 exposure, 90% of the ascidians ejected their guts. The linear model revealed a significant effect of pCO_2 on gut ejection (n= 60; Anova, $p<0.05$). There were no significant effects of the temperature ($p=0.470$) or the interaction T vs pCO2 on this behaviour (n= 60; Anova, $p=0.825$) (Figure 4). In T3 (1000ppm, 30°C), ascidians performed the greatest gut ejection. No ascidians ejected their guts in the control system. Animals that performed gut ejection, recovered at different

rates depending on the treatment. During T1 (400ppm and 30°C), gut recovery took 10 days approximately, after T2 (1000ppm, 25°C) total gut recovery was observed after 15 days while on T3 (1000ppm, 30°C), gut recovery was achieved in a 50% after 19 days post-exposure.

During T1(400ppm and 30°C) and T3(1000ppm, 30°C) there was an increase on faeces production significatively different than the control treatment (Anova, $p<0.05$), indicating a possible increase of their metabolic rate; in contrast, under the temperature treatments the faeces production was not significatively different ($p= 0.955$).

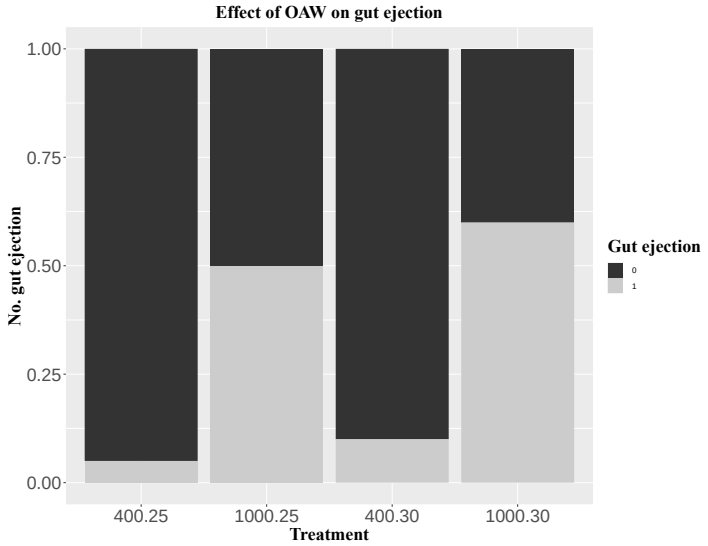

Figure 4. The effect of ocean acidification and warming on gut ejection in *Polycarpa captiosa*.

Clearance rate based on body weight (CR. W)

There were significant effects of the temperature and the pCO_2 (n= 60; Anova, $p<0.05$) treatments on the clearance rate based on body weight of ascidians, but no significant effect of the interaction of both conditions (Anova, $p=0.539$) (Figure 6). The rate at which algae cells were removed from the experimental tanks decreased proportionally on the treatment (Table 2) in comparison with the control (Figure 5). Algae cell counts after T1(400pmm, 30°C) was lower than the control but, after T2(1000ppm, 25°C) and T3(1000ppm, 30°C) cell count was higher evidencing a direct impact on the filtration capacity proportional the acidification. The reduction in

clearance rate observed in T3 (Figure 6), indicates a possible reduction of their metabolic rates as less faeces was produced under those conditions.

Table 2. Mean and standard deviation of the feeding physiology measurements for all ascidians per treatment.

Measurement	Control	T1	T2	T3
Clearance rate cr [ml min^{-1}]	2.47 ± 0.01	2.42 ± 0.01	2.28 ± 0.01	2.21 ± 0.01
Clearance rate based on body weight cr.w [g l^{-1} min^{-1}]	0.64 ± 0.16	0.55 ± 0.02	0.48 ± 0.01	0.45 ± 0.01
Standardized ingestion rate irs [mg h^{-1}]	38.39 ± 1.0	33.32 ± 1.5	29.03 ± 0.22	27.26 ± 0.26
Absorption efficiency (ae, %)	72.5 ± 2.0	69.72 ± 2.5	22.00 ± 0.46	20.98 ± 0.46

Control (25°c, 400ppm), t1 (25°c, 1000ppm), t2 (30°c, 400ppm), t3 (30°c, 1000ppm)

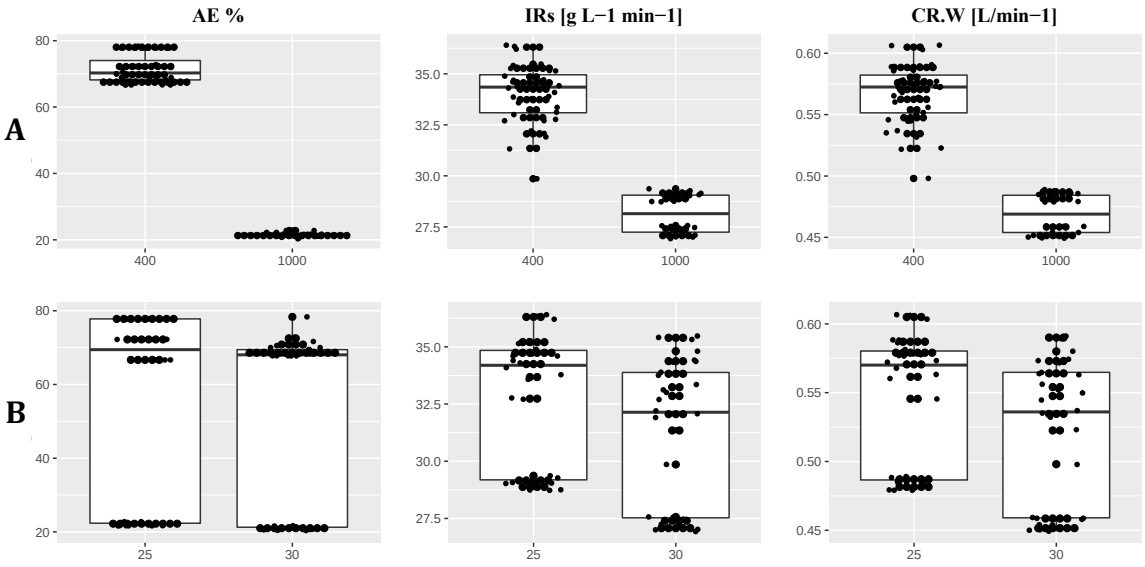

Figure 5. Independent effects of A) pCO_2 (400ppm, 1000ppm) and B) temperature (25°C, 30°C) treatments on the absorption efficiency (%), standardized ingestion rate (g $L^{-1}min^{-1}$) and clearance rate base on body weight (L min^{-1}) of *Polycarpa captiosa*.

Standardized ingestion rate

The standardized ingestion rate was affected by both OAW scenarios (Figure 5). The linear model showed significative effects of temperature and pCO2 (Anova, $p<0.05$) on the standardized ingestion rate of *Polycarpa captiosa*. But no effect was evident between the interaction of both conditions on the feeding behaviour ($p=0.550$) (Figure 6).

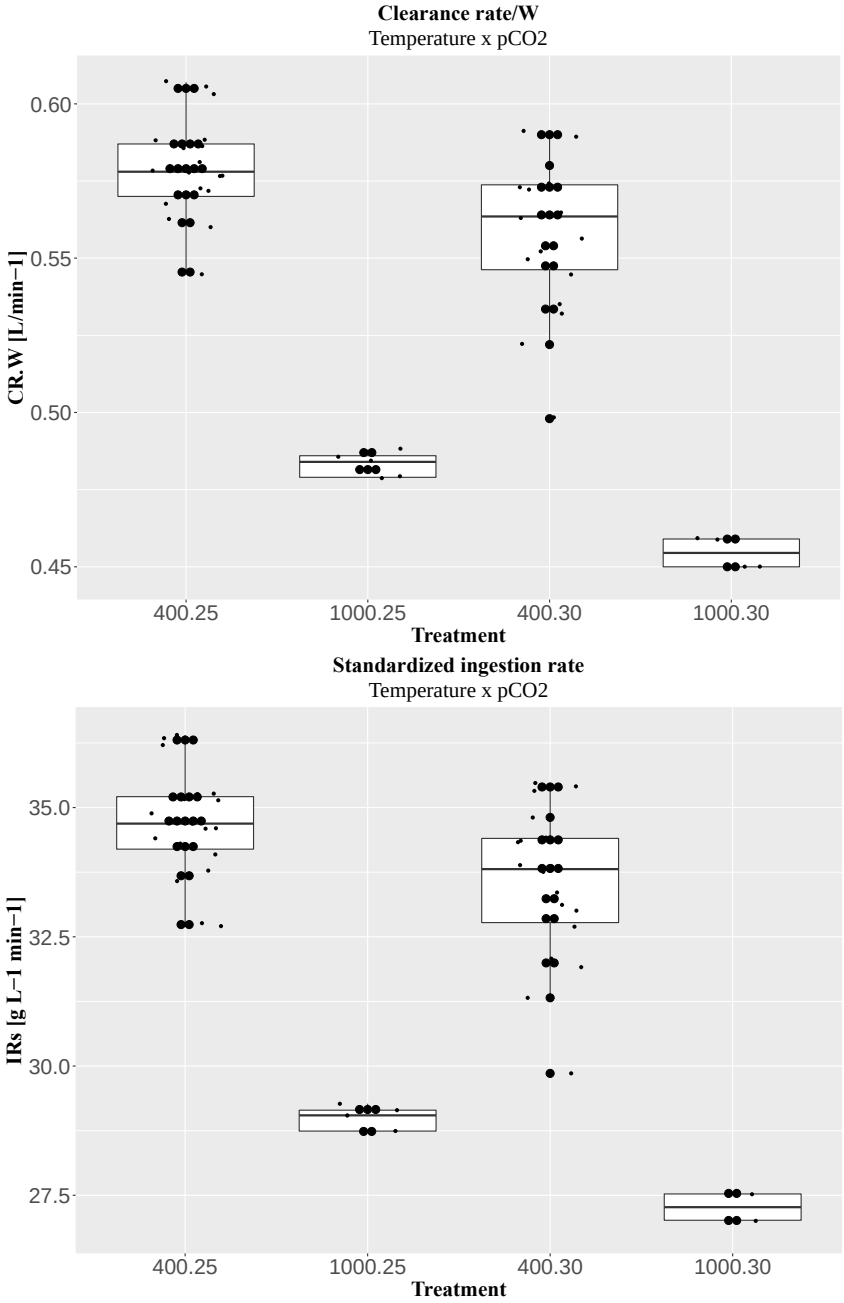

Figure 6. Effects of the interaction between temperature and pCO_2. *Left:* on the clearance rate based on body weight (L min^{-1}). *Right*: on the standardized ingestion rate (g L^{-1} min^{-1}) of *Polycarpa captiosa*.

Absorption efficiency

The linear model showed a positive effect of warming ($p<0.05$) on the absorption efficiency of *Polycarpa captiosa* (Figure 5), and a negative effects of ocean

acidification ($p<0.05$), but no effects in the interaction of both conditions ($p=0.250$) (Figure 7).

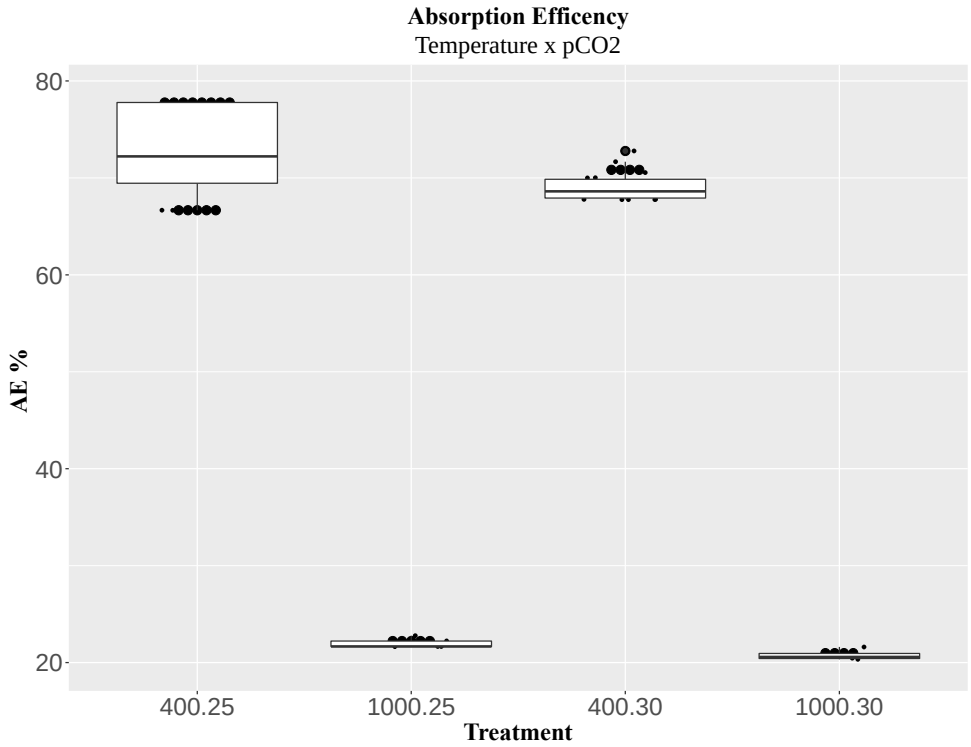

Figure 7. Effects of the interaction between temperature and pCO_2 on the absorption efficiency (%) of *Polycarpa captiosa*.

DISCUSSION

Polycarpa captiosa is a model representative of marine suspension feeders and plays an important role in coastal ecosystems. Our results showed important negative effects of ocean acidification and warming on their clearance rate, ingestion rate and absorption efficiency as well as on their resilience and adaptation under multistressor scenarios predicted for the end of the century, potentially affecting their ecosystem functions. We predict that under OA scenarios, they would be restricted to colonise new substrates and ecosystems, leading to eventual population reduction and more dramatically, species extinction (Solan et al., 2004).

Resilience

After 12 weeks of treatment, 90% of the ascidians remained alive. Despite combined environmental stressors, and some energetically expensive gut ejection, few individuals died. Gut recovery time differed among treatments providing an important insight about the influence of environmental conditions on the

regeneration of essential tissue in *Polycarpa* species. In addition, all the animals exposed to high acidification treatments performed total shedding of the outermost tunic skin, presumably as an additional stress response to prolonged seawater chemistry changes. Total skin recovery was observed after the third week of exposure and could be explained as a microbiome recovery of this species' tunic and a full recovery to the disturbances.

Tunicates are distinguished among chordates for presenting an exoskeleton or tunic composed of proteins and other macromolecules such as tunicin. These tunics are capable to elongate during animal growth, so ecdysis is not a common behaviour in ascidians. However, they have the unique ability to undergo whole body regeneration after physical damage (Rinkevich et al., 1995; 2007a; 2007b; Voskoboynik et al., 2007; Brown et al., 2009; Blanchould et al., 2018) and partial organ and system-level regeneration (Shenkar & Gordon, 2015) a trait rare in other chordates. Our results showed an effect of ocean acidification and warming on the skin shedding of *Polycarpa captiosa* as only under the elevated pCO2 conditions this was observed. During the recovery time it was observed that new skin is produced within two weeks post-exposure and the previous shed skin remained attached to the animal for a period of 20 days after which it naturally detached. We suggest this behaviour might allow ascidians survival under environmental disturbances.

Clearance rate based on body weight.

Our results revealed a positive effect of warm temperature (30°C) on clearance rate, during T1 (400ppm, 25C), as ascidians removed 10% more algal cells than in the control system, this could be a case of an enhanced metabolism and high energetic demand as a consequence of the thermal disturbance which has been previously observed in other suspension feeders like bivalves (Lemasson et al., 2018l). However, under T3 (1000ppm, 30C), the lowest clearance rate was observed, indicating that the combined effect of both warming and acidification reduced the ascidians capacity to perform optimal filtration in the short-term exposition.

Clearance rate is linked to the complexity of the pharynx (it has a filtration area of four non-overlapping folds on each side of the body), the normal functioning of this filtration system depends on the optimal environmental conditions at which cells can

work (Petersen & Riisgård, 1992). Our finding suggests that if the increase of water temperature exceeds the thermal tolerance in ascidians in the long term, their physiological activity would be severely affected, and their ecosystem function reduced. Without this important function many light dependant organisms would decline leading to other changes in the habitat provision for larvae, juveniles and, adult economically important invertebrates and fishes (Petersen, 2007).

Under high pCO_2 conditions, a negative effect was observed on the clearance rate as it slowed down in comparison to the control treatment (400ppm, 25C) and provoked an increase of gut ejection cases in both elevated pCO_2 treatments which lead to a reduction of the feeding behaviour. Under T2 (1000ppm, 25C), ascidians reduced their clearance rate in comparison to the control conditions at the same temperature (Figure 6), but the feeding behaviour (indicated by open siphons) remained similar, which we interpret as a feeding shock, in which the nervous control of the water-pumping cilia (Hoyle 1953) is affected by the environmental disturbances and for instance, a reduction of the suspension feeding process is experienced (Holmes 1973; Fiala-Medioni 1978c; Robbins 1983; Petersen & Riisgard, 1992; Armsworthy, et al 2001). This physiological behaviour would subsequently have a negative effect on the ecosystem functions of *P. captiosa*. Under short term OAW conditions, water viscosity decreases, and the ciliary activity in the ascidians pharynx reaches an optimal rate preserving a constant clearance rate that allows the animal to obtain the maximum food intake despite the thermal stressor, however, in the long term this ability is affected.

Standardized ingestion rate

Our results evidenced an effect of both ocean acidification and warming treatments on the standardized ingestion rates of *P. captiosa* but not a significant effect on the interaction of both stressors. These results resemble those obtained for clearance rate as both measurements are directly related to the environment, but their impacts on the animal physiology are different. While clearance rate takes into account the volume of water filtrated, the standardized ingestion rate provides insights on the amount of organic food obtained through clearance per animal weight.

The rate at which an animal ingests available food positively reflects its level of nutrition and energy budget and determines the degree of adaptation to its

environment. For instance, it is expected that a reduction of the ingestion rate is related to a lack of environmental coping and stability (Petersen, 2007). Under T1(400ppm, 30C), the amount of algal cell ingested per animal, was higher, evidenced by an increase of faeces produced post-treatment. As in clearance rate, the metabolic activity was likely increased under higher thermal exposure and the lower water viscosity stimulated the muco-ciliary activity in *Polycarpa captiosa* enhancing their ingestion rate. This metabolic increase can potentially mitigate the adverse effects of warming caused by ocean acidification compensating the energy cost of adaptation to the environmental stressors as it has been reported for other suspension feeders (Sanders et al., 2013).

On the other hand, both pCO2 treatments (T2 and T3) had a negative effect on the standardized ingestion rates of *P. captiosa* evidenced by a decrease on the ingested algal cells found in faeces during the short-term exposure to ocean acidification. As happened with clearance rate, elevated pCO_2 and high temperature can affect the ingestion of organic matter due to a deterioration of key physiological structures and evisceration in ascidians. This response negatively alters the animal resilience under these scenarios and influences their mortality in the long term as has been demonstrated in other organisms like bivalves (Lemasson et al., 2018; Melzner et al., 2009).

Absorption efficiency

Our results demonstrated that the absorption efficiency was significatively affected by warming and acidification (Figure 7). The optimal absorption was achieved at ambient pCO_2 and 25C in the control treatment, followed by T1(400ppm, 30C) in which the absorption decreased 10%. The lowest absorption was found at elevated pCO_2 regardless of the temperature in comparison to T1 and the control, but it was particularly inefficient in T3 (1000ppm, 30C) in which both high temperature and pCO2 were interacting. Possibly due to a poor adaptation to the short-term exposure to the stressors and a feeding shock observed similarly in the ingestion and clearance rate. When faeces weight was similar to the initial algae weight, we considered absorption efficiency as reduced because it reflects the animals' inefficiency to obtain energy from food. The quantity and weight of faecal ribbons obtained during all the treatments was consistent and directly related to the water clearance rate.

We predict that the ineffective absorption of nutrients under OAW predicted conditions for the end of the century would reduce ascidians ability to provide competent ecosystem services and their lack of energy acquisition from food would dramatically reduce their populations which for instance would reduce the survival rate of dependant economical important organisms.

Even though, coastal ecosystems are not expected to dramatically decease in extent under OAW scenarios by the end of the century, the synergy between environmental and biological variables might affect the normal processes within them and severely change them (Agostini et al., 2018).

CONCLUSIONS

Our results demonstrate that ocean acidification and warming have an impact in the provision of biological services in tropical ascidians. The feeding behaviour of *Polycarpa captiosa* under warming scenarios showed an increase of their metabolic rates, impacting positively on their water clearance function. However, multi-stressor scenarios that involve the addition of elevated pCO_2 in the water chemistry affect negatively the absorption of nutrients from filtration feeding and affects resilience and survival of these organisms. This suggests a likely reduction of their populations in the wild by the long-term exposure to elevated pCO_2 values and high temperature. The metabolic cost of adaptation to prolonged exposition to OAW for non-economical important species like ascidians suggests an exponential reduction of their absorption and ingestion of particulate organic material. This will negatively affect other important biological functions such as sexual reproduction, provision of habitat complexity and control of inorganic pollution that plays a key role in supporting ecosystem health.

The native geographical range of *P. captiosa* includes important coral reef ecosystems, typically known for presenting low turbidity levels, and high diversity of commercially important invertebrates and fishes. If our predictions reflect a reality for the future physiological behaviour of ascidians, their ecosystem services for the region are likely to be negatively impacted leading to the reduction of water transparency, nutrient availability and light penetration in the water column.

Gut ejection and shedding of the outermost tunic membrane are other energy-expensive physiological responses to environmental stressors that in the short term

compromise the animal's ability to cope with the surrounding environment and affect their feeding and reproduction and increase their vulnerability to predation. Further studies should evaluate how microbiome in ascidians gut is affected by ocean acidification and warming scenarios and the extent to which gut ejection is a result of disturbed digestive bacteria in *Polycarpa* species at different environmental disturbance ratios.

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Supplementary material I: "24 hours variability in pCO_2 and temperature injected in experimental units"

Supplementary material II: "pH (Total scale) calculated in 24 hours treatment"

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Supplementary material I: “24 hours variability in pCO_2 and temperature injected in experimental units”

Hour	Elevated	Ambient	Pressure (kPa)	CO2 Absorption	Flow Rate
1	995.364	400	107.114	0.1243	0.752592
2	1000.013	407	107.058	0.1189	0.752463
3	1001.985	405	107.06	0.106	0.75348
4	1002.304	404	107.032	0.1063	0.752961
5	1004.133	405	107.014	0.1069	0.753442
6	1005.762	405	107.006	0.1082	0.753961
7	1004.962	402	106.993	0.1153	0.754433
8	1000.466	409	106.97	0.1154	0.754109
9	1000.354	409	106.941	0.1115	0.754223
10	1001.853	403	106.898	0.1094	0.753422
11	1001.353	405	106.876	0.1087	0.753517
12	1003.377	405	106.815	0.1081	0.754083
13	1000.402	409	106.741	0.1078	0.753376
14	1001.066	403	106.662	0.1075	0.751006
15	1001.943	402	106.535	0.1076	0.753152
16	1002.312	403	106.446	0.1075	0.752189
17	1001.587	400	106.404	0.1076	0.7516
18	1001.851	404	106.312	0.1084	0.756246
19	1002.828	406	106.232	0.1082	0.757771
20	1002.955	406	106.188	0.1091	0.753834
21	1000.529	404	106.106	0.1085	0.753204
22	1000.888	405	106.056	0.1083	0.749266
23	1000.413	409	105.925	0.1079	0.747607
24	1000.616	404	105.744	0.1078	0.745344

Supplementary material II: “pH (Total scale) calculated in 24 hours treatment”

Hour	*Ambient*	*Elevated*
0	8.21	8.21
1	8.03	7.73
2	7.94	7.55
3	7.92	7.56
4	7.91	7.56
5	7.91	7.56
6	7.91	7.57
7	7.90	7.57
8	7.91	7.57
9	7.91	7.57
10	7.91	7.57
11	7.91	7.56
12	7.90	7.55
13	7.91	7.55
14	7.90	7.56
15	7.91	7.57
16	7.91	7.57
17	7.91	7.57
18	7.91	7.57
19	7.90	7.56
20	7.91	7.59
21	7.91	7.56
22	7.90	7.54
23	7.88	7.55
24	7.88	7.55

Chapter 7: Ingestion of microplastic fibres cause severe gastro-intestinal damage in tropical ascidian *Polycarpa captiosa* (Tunicata: Ascidiacea)

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ABSTRACT

The impacts of microplastic pollution on marine fauna represent a major concern for conservation due to the increasing abundance of these polymers in almost all marine ecosystems. Here, we evaluated the ingestion of polyester, polyvinyl and polypropylene microfibres in the tropical Indo-pacific ascidian *Polycarpa captiosa* and their effects on its biology and filtration activity. Microfibers were collected from launderettes, to replicate a common contamination pathway. Individuals were exposed to a high concentration of these microplastics for 12-hour, 24-hour, 48-hours and to a control with no fibres for reference. Results showed that 90% of the ascidians eviscerated very damaged guts around 15-hour post microplastic exposure, from which only 20% recovered. Deceased specimens presented decomposed organs and gonads after 15 days of exposure. In addition, we found significant differences in the microplastic accumulation in different digestive organs in relation to the exposure time. Ascidians exposed for longer, reduced their filtration activity, clearance rate and absorption efficiency and presented the biggest accumulation of fibres in the pharynx suggesting a reduction in their pumping activity. This study constitutes the first records of lethal impacts of microplastic fibres on chordates and suggesting direct impacts on ascidian's ecosystem functions.

Keywords: Marine pollution; Gut ejection; Tunicates; Filter-feeders; Coastal research.

INTRODUCTION

Plastic pollution is a concerning marine environmental issue (Thompson et al., 2009). Plastic breakage into smaller particles of less than 5mm are considered a primary source of microplastics (Bosker et al., 2019; Arthur et al., 2009), other sources include their intentional production for purposes including clothing and the cosmetic industry, contaminating water systems worldwide (Yang et al., 2015; Costa et al., 2018). Depending on the specific gravity of those polymers, they can be found at different depths or even within sediment (Vered et al. 2019; Andrady, 2011; Avio et al., 2017; Thompson et al., 2004).

Since the estimated number of microplastics (MPs) in the marine environment is approximately 51 trillion microparticles (Plastics Europe, 2015; Desforges et al., 2014), controlling them represents a great challenge for marine conservation (Eriksen et al., 2014; Ryan et al. 2009; Derraik 2002). This issues are the entrance pathways of polymers into the sea, accumulation of toxins in essential tissue (Passarelli et al., 2018), affects in food webs (Auta et al., 2017; Hurley et al. 2017; Houle, 2015), and the biological response (physiological, behavioural, reproduction and health) to accumulation of microparticles (Balbi et al., 2016).

Of all types of microplastics found globally in the oceans, fibres represent one of the most common in coastal waters (Mathalon & Hill, 2014; Lusher et al., 2014; Dekiff et al., 2014), and have been found in the gastro-intestinal system of many organisms including mussels, annelids, crustaceans and fishes (Watts et al., 2014; Haṁer et al., 2014; De Witte et al., 2014; Fossi et al., 2014; Besseling et al., 2013; Murray & Cowie, 2011). The ingestion of these particles can potentially affect non-selective filter-feeder organisms causing deleterious impacts on their health (Capolupo et al., 2018; Détrée & Gallardo, 2018; Cole et al., 2015; Moos, et al., 2012), including gut blockage, essential tissue damage, false satiation and subsequently starvation (Ziajahromi et al., 2018; Germanov et al. 2018; Wright et al., 2013). Damage to filter feeders threatens their important ecosystem functions and survival (Kolandhasamy et al., 2018; Phuong et al. 2017; Pollock et al., 2014).

Biological features such as mouth size and feeding rate are key for the study of the response of benthic filter-feeders to MPs (Galloway et al., 2017) as they represent the first control over food consumption (Scherer et al., 2017). Animals with a bigger mouth size like solitary ascidians, would accidentally ingest larger amounts of MPs which constitutes a disadvantage over smaller fauna and a threat to their integrity and normal functioning (Goncalves et al., 2018).

Ascidians (Tunicata: Ascidiacea) are marine filter feeders with important ecosystem roles. They can filter several litres daily (Layman et al., 2014; Draughon et al., 2010; Petersen, 2007) and retain small microparticles in their digestive systems (Tzafriri-Milo et al., 2019). Their feeding contributes to nutrients recirculation, allowing the survival of smaller organisms and fishes (Burge et al., 2016; Ribes et al. 2005). Studies of the impacts of MPs on these ecosystem functions are scarce and have mainly focused on potential bioaccumulation and on early development (Messinetti et al., 2019; Vered et al. 2019; Dewar-Fowler, 2019; Zega et al., 2009; Kelmo et al., 2006; DeVantier et al., 1998). However, ascidian potential to adapt to changing environments (Stabili et al., 2016; Shenkar & Swalla, 2011; Knott et al., 2009; Lohrer et al., 2006) and improve environmental quality, makes them model species for the study of biological response to microplastics (Messinetti et al., 2018; Su et al., 2013; Newcombe & Macdonald, 1991). Additionally, due to their phylogenetic position, they represent an ideal model system for the understanding of the response of higher taxa to environmental stressors, unlike molluscs, crustaceans or annelids (Delsuc et al., 2006).

In chapter 5 we found that some *Polycarpa* ascidians respond to environmental stress by skin shedding or evisceration. Here, we tested the effects of polyester, polyvinyl and polypropylene microfibres (1±0.5 mm) on the filtration activity of *Polycarpa captiosa* individuals under laboratory conditions at different exposure times (12, 24 and 48 hours). We examined responses in their filtration performance, biological functions and survival (Aim 3).

METHODS

Study species

Polycarpa captiosa is a tropical Indo-pacific ascidian (Lee et al., 2013; Monniot, 2010). Its natural habitat includes shallow and clear water with a sea surface temperature between 24-26°C. Our specimens were collected by local traders in Singapore from coral reef areas at ca. 5m depth and transported to the United Kingdom through an aquarium supplier (Cheshire Aquatics). We used adult individuals of 5±1cm length and 3±1cm width and around 10g wet weight (Figure 1). In total 80 individuals were used for this study, 20 individuals per treatment including the control.

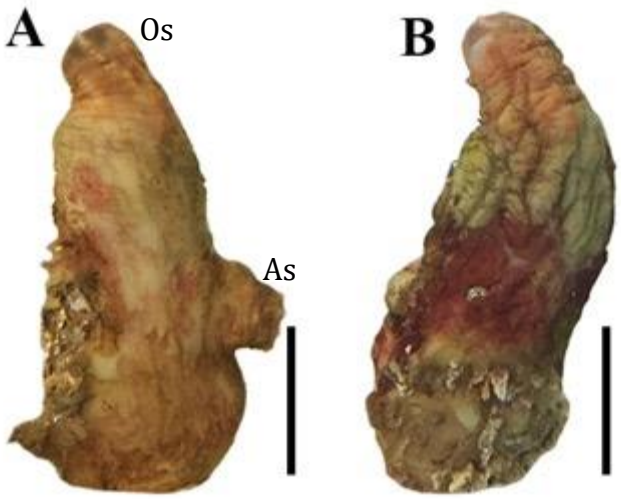

Figure 1. Specimens of *Polycarpa captiosa* A. Right side. Adult showing the oral siphon (Os) and atrial siphon (As) B. Left side. Detail of leathery tunic. scale bar: 1.5 cm.

Prior to experimentation, each ascidian was, measured, weighed and glued onto a labelled ceramic plug using reef glue and placed onto acrylic stands in the aquaria. All animals were kept in a 300 L tank for acclimation for three weeks under controlled environmental conditions and using artificial sea water (AquaForest). Normal conditions were set as; Salinity 35ppt, SST 25°C, Alkalinity 8.5 dKH, pH 8.4, pCO2 440ppm; Mg 1320 mg/L, and Ca 420 mg/L. Animals were fed three times a week with a solution of live phytoplankton (*Dunaliella salina*, *Nannochloropsis oculata* and

Tetraselmis sp.), zooplankton suspension (copepod), and vitamins and aminoacids (Red Sea).

Microplastic selection and quantification

To replicate a common pathway of microfibre contamination, we collected waste lint and filtered wastewater from launderettes (Figure 2). Lint was rinsed in distilled water for three days to rinse off detergent chemicals. Ten grams of fibres were then separated and cleaned from other non-plastic components via flotation using 1L of saturated saline solution (1.2g/mL^{-1}) overnight in a sealed and clean glass beaker (Li et al., 2015). Subsequently, fibres were filtered in a 0.45µm filter paper (Whatman AE98) using a vacuum pump, rinsed in ultrapure water, counted and measured (1±0.5mm). A subsample of fibres was verified using Carl Zeiss V8 stereo microscope and identified using Raman spectroscopy (Araujo et al., 2018; Zada et al., 2018), all obtained wavelengths were compared with the literature to verify the polymer type (Su et al., 2017). We identified Polyethylene terephthalate (Polyester) (60%), Polyvinyl alcohol (20%) and Polypropylene (10%) in our fibre mix.

Figure 2. A. Waste lint collected in local launderettes. B. Microfibres cleaned.

The micro-fibres were dried and weighed (7g) and diluted again in 5L artificial sea water (35ppt) using a magnetic stirrer to create a microplastic stock solution with a concentration of around 2000 microfibres per litre to ensure ingestion was likely to occur in our treatments and to be comparable the literature (Lee et al., 2013; Ogonowski et al., 2016; Van Cauwenberge et al., 2014).

Experiment 1: Microplastic ingestion over different exposure times

In total 80 ascidians were used in this study; 20 per treatment, 0) the control (no MP), 1) 12-hour exposure, 2) 24-hour exposure, 3) 48-hour exposure.

Two days before MP experimentation, ascidians were fed as usual to avoid disturbances in the feeding pattern and to ensure animals were hungry. We used ten individual ascidians (replicates) per time per treatment and replicated the experiment a week after with another ten animals per treatment (40 replicates per replicate time, 80 animals in total).

Ascidians were each placed in a 2L glass beaker with 1500 ml of 0.2µm filtered artificial seawater mixed with 200ml of the MP stock solution. Each beaker had a magnetic stir bar at the bottom and was placed on a magnetic stir plate (100 rpm) to keep micro-fibres in suspension during the experiment. Animals were placed in an upright position on top of a grid platform positioned ca. 4cm above the bottom of the beaker to allow circulation of MP (Figure 3). To ensure temperature and salinity remained unaltered, treatments ran in a closed laboratory with constant temperature and a 10h day using artificial lighting.

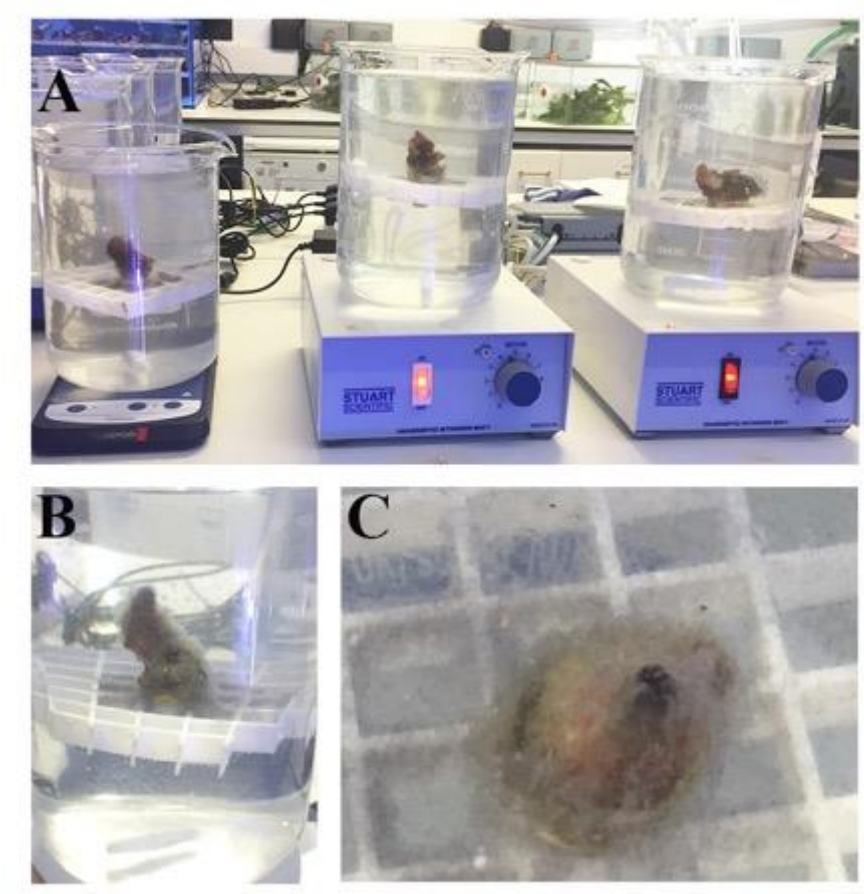

Figure 3. A. MP exposure experimental set up. B. *P. captiosa* during experimentation time. C. Detail of filtration activity, open oral siphon.

To record filtration activity (the number of times animals opened their oral siphons, squirting time, faeces production and the pumping activity), one-hour observations was made of five individuals per treatment. In addition, two GoPro 7 cameras were attached to each ascidian treatment tank for the duration of each treatment. In total 20 hours video recordings and 20 visual censuses were made of filtration behaviour of *Polycarpa captiosa* during MP exposure.

After completion of each treatment, ascidians were rinsed with 45µm filtered artificial sea water to remove any fibre from the surface of the tunic. Ascidian were placed in a 30L tank with normal conditions for 6 hours to re-acclimatise, during this time ascidians were fed with 10ml of phytoplankton mix and 5% water change was performed every 2 hours to avoid re-ingestion of any suspended fibres. Faeces were collected from each individual using a glass pipette and stored in pre-washed polyethylene universal tubes at 10C for subsequent analysis.

Experiment 2: Effects of MP on the clearance rate and absorption efficiency

Subsequently after experiment 1, each ascidian was put in a 1L glass beaker inside a 300L tank for an hour with set normal water chemistry conditions to achieve a slow transition between experiments and avoid additional stress to the animals (Figure 4). After this time, we lifted each beaker above the tank surface to isolate each ascidian and added 20ml solution of saturated phytoplankton mixture to achieve a concentration of 10^8cell/L^{-1} which we have previously assessed as the optimal concentration for clearance and absorption rate for this species under normal conditions. One-hour visual censuses were made and two-hour video recordings of the filtration activity of *P. captiosa* per each treatment. Observations finished after two-hours. in undisturbed conditions, total clearance is achieved.

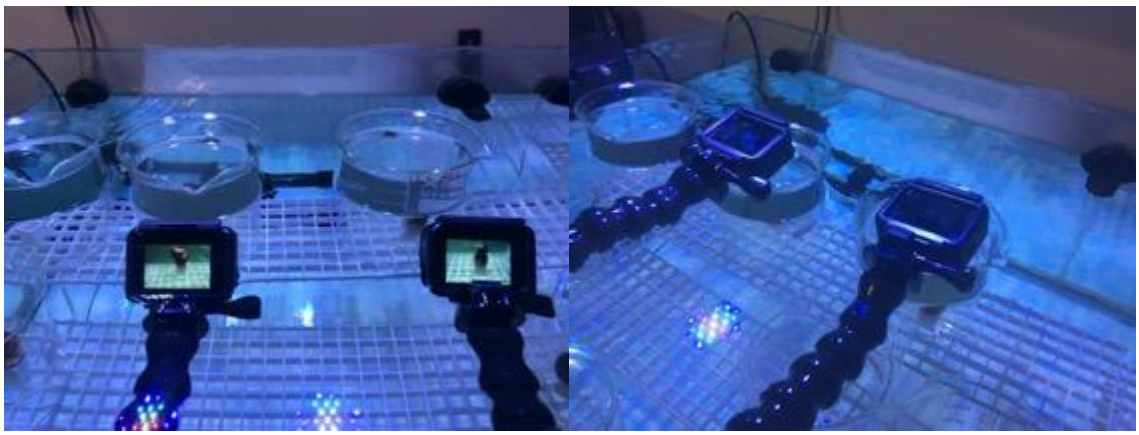

Figure 4. Experiment 2 set up for the clearance and absorption rate measurements.

Clearance rate was measured as the total volume of cleared of algal cells per ascidian g per unit time based on Armsworthy et al. (2001) and Macdonald & Ward (1994) following the equation, CR=((cr-Ce)/cr)gr animal, where, cr is the total cell concentration of the algae mix at the beginning of the experiment and Ce is the mean cell count at the end of the experiment. The absorption efficiency was calculated based on Conover (1966) as the total weight of algae added at the beginning of the experiment minus the weight of faecal pellets after an hour of measurements, using the equation, AE= 100-(TPM-fw/ TPM) x 100, where fw is the faeces dry weight after the experiment, TPM is the total seston weight at the beginning of the experiment.

Quantification of MP fibres ingested

Around 15-hours after experimentation, 70% of ascidians from the 12-hour treatment and all ascidians from the 24 and 48-hour treatments performed total evisceration. The digestive system was then collected and analysed for MP ingestion. This procedure allowed us to avoid sacrifice of all the animals, and instead we dissected only five individuals per treatment to evaluate microplastic accumulation in the skin and the degree of the digestive damage. In addition, any faeces excreted prior to gut ejection were also collected and separated in universal tubes.

The ejected digestive systems of *P. captiosa* were photographed, dissected and inspected using a Carl Zeiss V8 stereo microscope, to identify accumulation of MP fibres. Subsequently, each organ (pharynx, stomach, intestine) was cut (Figure 5), labelled and placed in 2.5ml Eppendorf tubes with an alkaline solution of KOH 10% and left to digest in a rocking heated (35°C) platform to extract all the MP fibres accumulated per organ, this process does not degrade the polymers while disintegrating the essential tissue (Hurley et al, 2017; Karami et al., 2017; Dehaut et al., 2016); total digestion was achieved after 15 minutes. Subsequently, samples were transferred to 1.5ml Eppendorf tubes and centrifuged for 10 minutes at 14000 rpm, and supernatant was discarded to separate microfibres.

Those fibres were then rinsed in ultrapure water and vacuum filtered through filter 0.45µm papers (Whatman AE98) and oven-dried in glass dishes at 40 °C for 2 hours. After this, manual counts of the number of fibres per organ were performed using a Zeiss Lumar V12 microscope with an AxioCam 20X integrated.

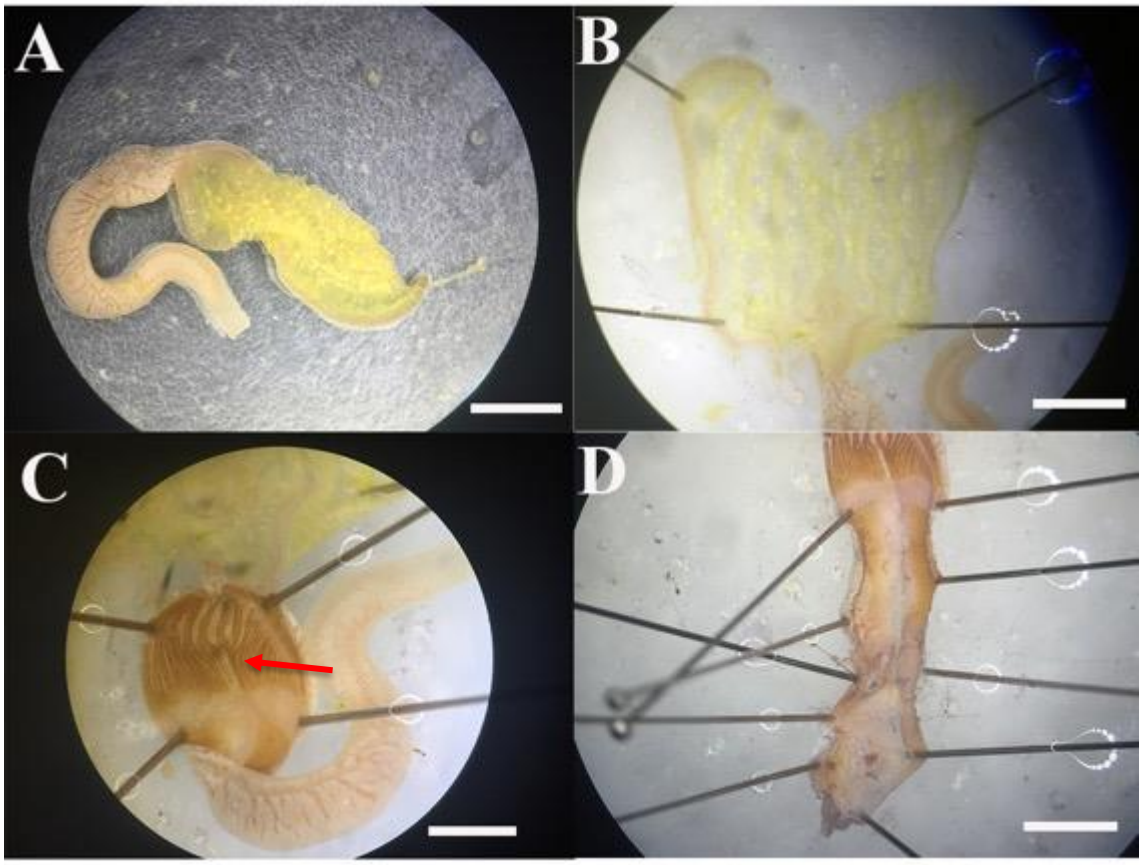

Figure 5. Eviscerated digestive system of *P. captiosa* after microplastic and feeding experiments. A. Complete ejected gut, scale bar: 0.5 cm. B. Pharynx, scale bar: 0.2cm. C. Stomach, detail of folds, scale bar: 0.2 cm. D. Intestine dissection, scale bar: 0.1 cm.

A similar procedure was performed with the excreted faeces. In order to determine if there was distinction between the polymers accumulated in each organ, samples were re-identified using Raman spectroscopy.

Statistical analysis

All the statistical analysis was performed using R Studio (Version 3.3.2). One-way Analysis of variance (ANOVA) test was carried out to establish any significant differences in MP among organs and faeces on each treatment, followed by a Tukey's HSD (homogenous variances) to compare the abundance of microplastics in each digestive organ per treatment. Significance was accepted if $p<0.05$. To evaluate if there was an effect of the exposure time and the filtration activity (clearance rate, absorption efficiency), general linear models (glm) were performed.

RESULTS

Experiment 1: Microplastic ingestion at different exposure times

Our results found significant differences between the microplastic fibres accumulated in faeces among the different exposure times (P=0.0003). Mean length of MP in the gut was 359.85µm (SD+301.19) and mean Length of MP in Faeces 152.87µm (SD+220.29) (Figure 6). More fibres were found in the faeces on the 12-hour experiment than in the other two treatments (25±2). The number of microplastic fibres was also significantly different among organs (n=60; p<0.05). In the 12-hour exposure treatment, most fibres were found in the stomach (35 ±1 fibres) (Figure 7) whereas in the 24 (21±2) and 48-hour (40±2) treatments most fibres were found in the pharynx (n= 60; p<0.05). The type of microplastic found in each individual organ did not show significant differences (p=0.324) (Figure 9), all polymer types were found in all the organs and faeces analysed.

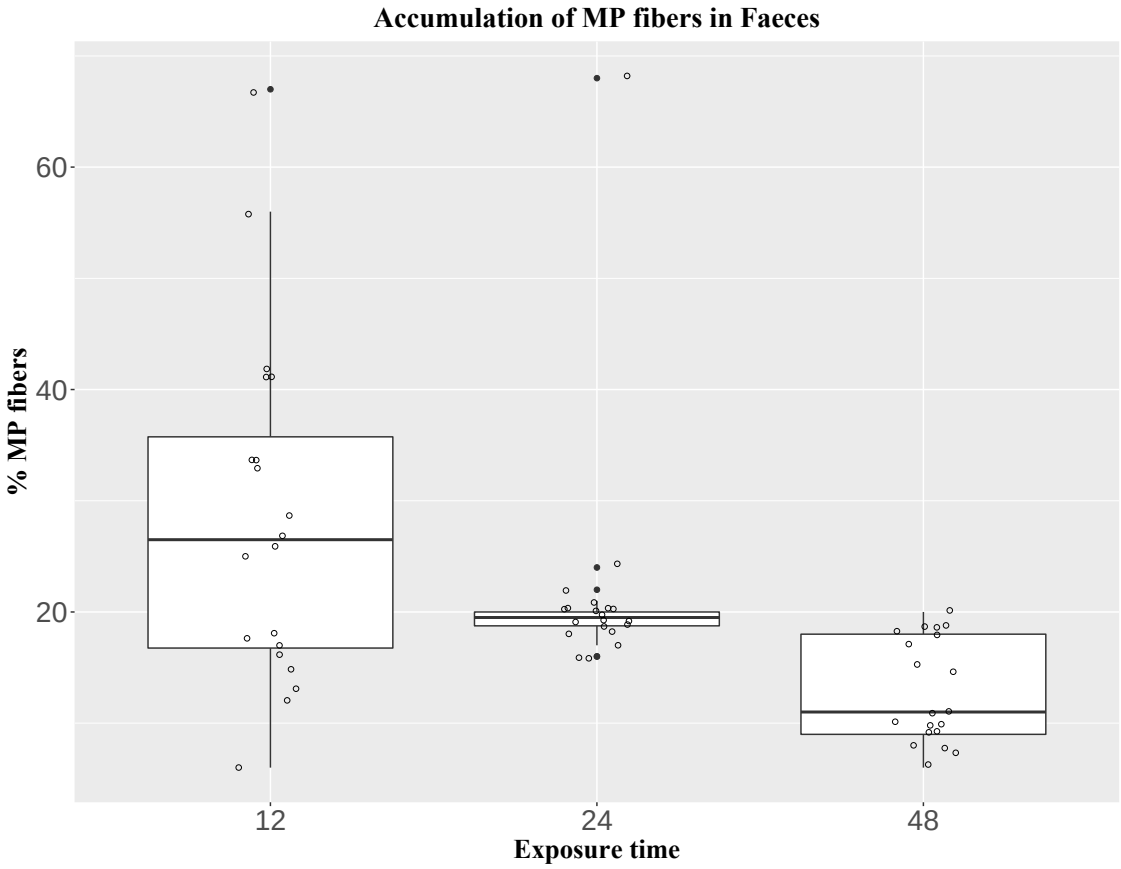

Figure 6. Accumulation of microplastic fibres in the faeces produced after each treatment (12, 24 and 48-hours).

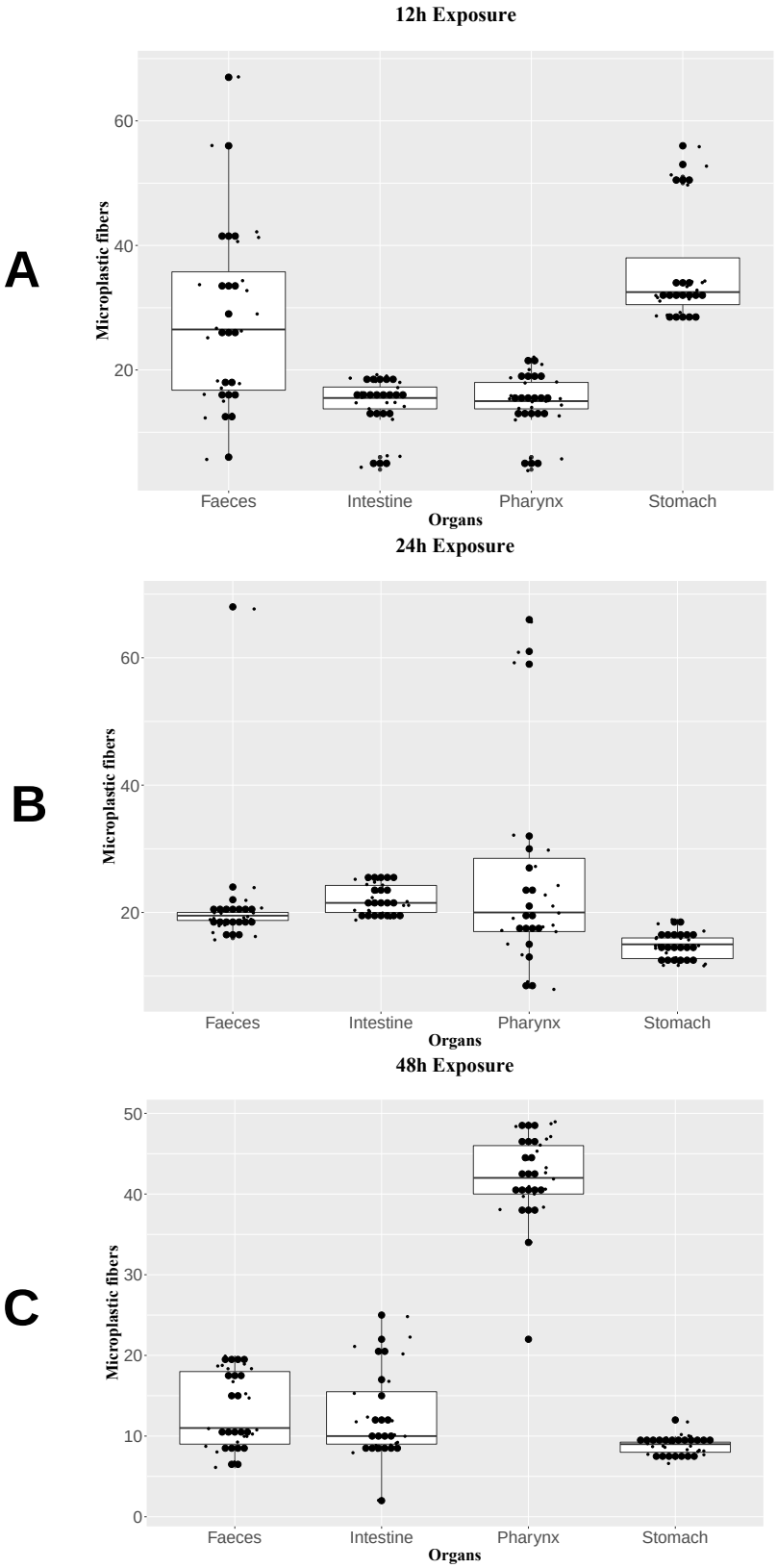

Figure 7. MP fibres found in each ascidian organ after each treatment. A) 12-hours, B) 24-hours, C) 48-hours.

Filtration behaviour

Filtration after MP exposure showed significant differences among treatments (n= 60; $p<0.05$), optimal filtration activity was observed in the control as expected, but a considerable reduction was observed from the 12-hour exposure treatment (Figure 8). The filtration behaviour, expressed in the number of times the oral siphons opened, was considerably inefficient after the 24 and 48-hours exposure treatments, ascidians reduced the number of times they opened oral siphons and increased the length of squirting time. Faeces production during the longer exposure was also different compared to the control (n= 60; $p<0.05$).

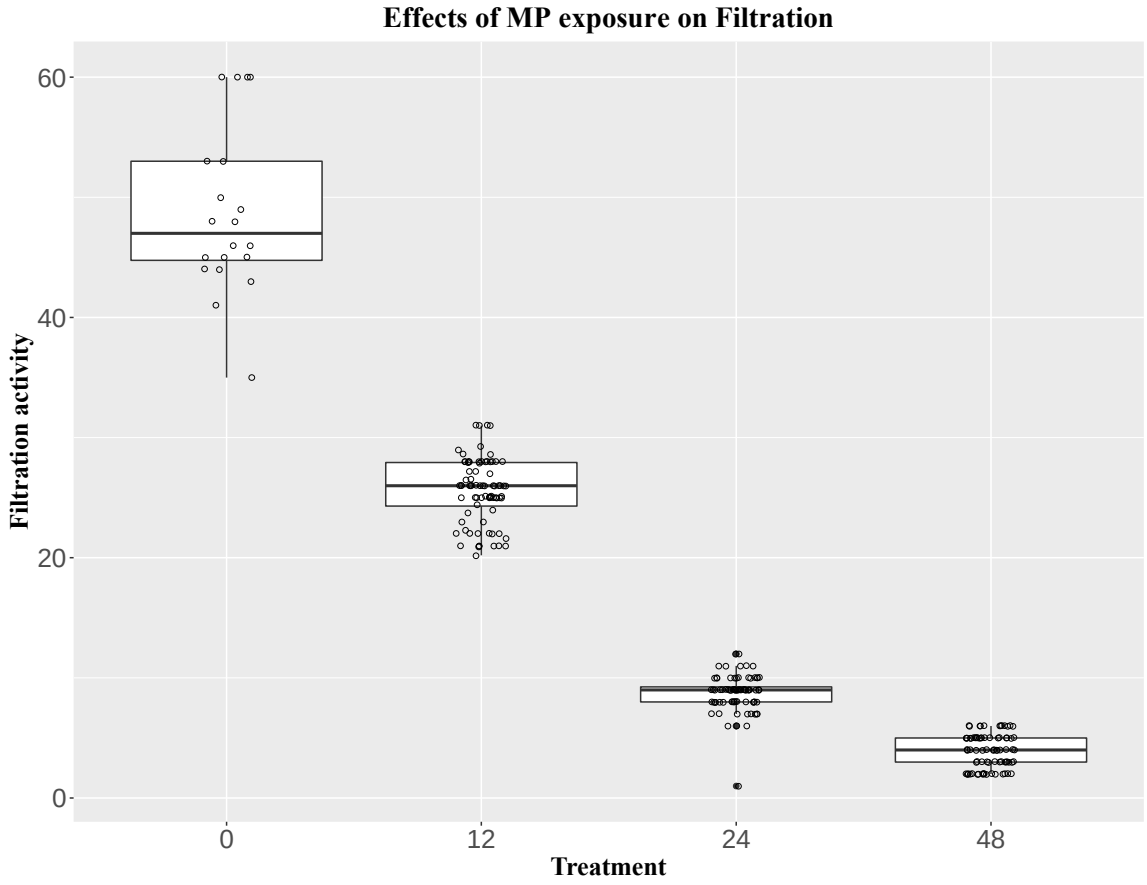

Figure 82. Number of times the oral siphons opened of the ascidians after the Microplastic exposure opened during low turbidity (micro-algae cells in the water).

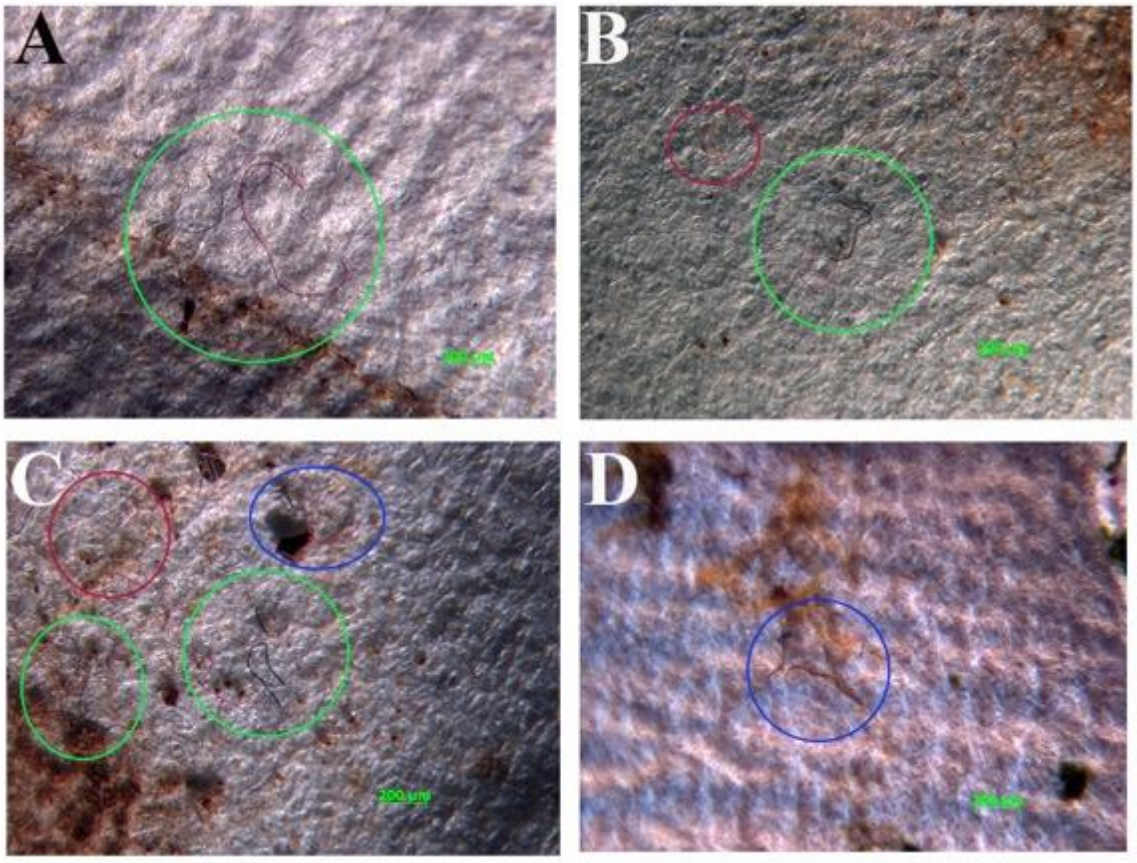

Figure 9. Type of polymer found each organ and faeces in one tunicate sample after 12-hour exposure, green: Polyethylene terephthalate (Polyester), blue: Polyvinyl alcohol and red: Polypropylene. A. Intestine, B. Stomach, C. Pharynx, D. Faeces.

Experiment 2: Effects of MP on the clearance rate and absorption efficiency

Clearance rate

The number of particles cleared after each exposure time was reduced proportionally to the length of the treatment. The linear model showed an effect of the duration of exposure ($n= 60$; $p<0.05$) and the ascidians' clearance rate response in all the exposure treatments suggesting that the microplastics interrupted the normal filtration behaviour in *P.captiosa* (Figure 10).

Absorption efficiency

The absorption efficiency decreased after each exposure time but was significantly less in the 24 and 48-hour treatments. The generalized linear model showed an effect of the length of experimentation (n= 60; $p<0.05$) on the absorption efficiency of *P. captiosa* (Figure 11).

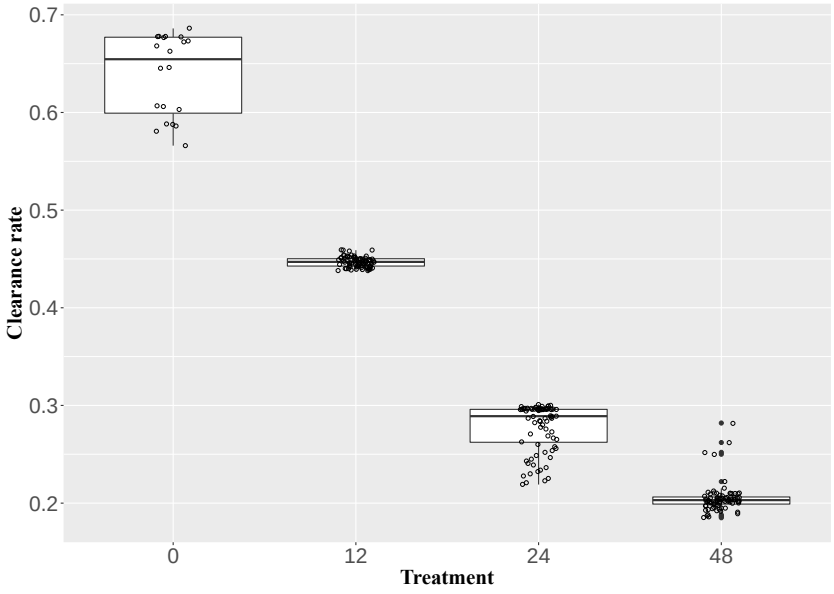

Figure 10. Clearance rate after each microplastic fibre exposure experiment.

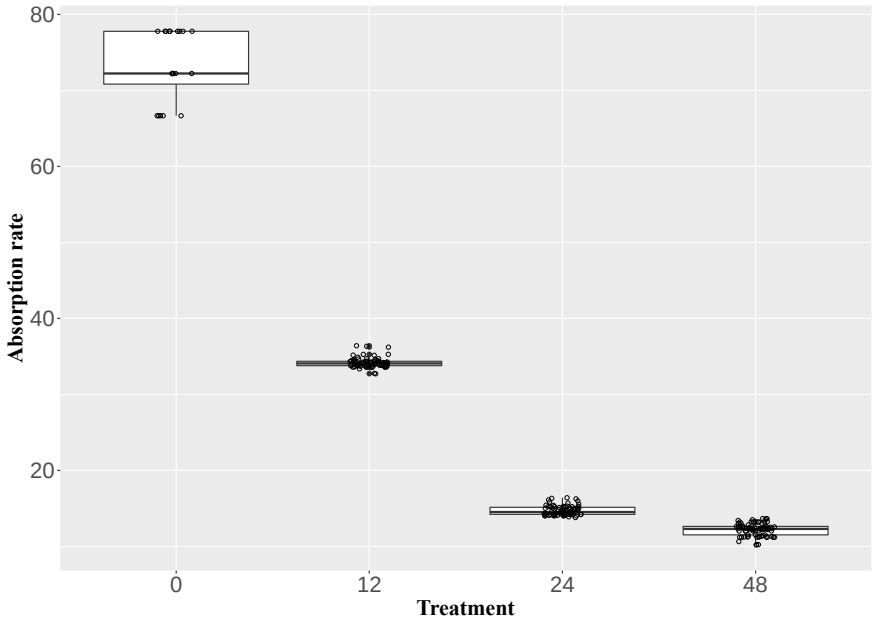

Figure 11. Absorption efficiency (%) after each microplastic fibre exposure.

Biological effects of MP fibres exposure

After all experiments, 70% of ascidians that were exposed to 12-hour exposure and all ascidians exposed to 24 and 48-hour treatments performed total gut ejection. However, after examination of each pharynx a significant damage was observed in comparison to previous evisceration after environmental disturbances (chapter 5 and 6). Tissue was considerably disintegrating and yellow coloured; when dyed in haematoxylin solution 10% almost no coloration was obtained, probably due to a reduction in irrigating blood in the pharynx revealing a severe gastrointestinal deficiency. In addition, from the tunicates that ejected their guts, only 10% regenerated them within the following 20 days post-treatment. The rest of ascidians did not recover their guts and subsequently deceased. Post-mortem dissections were made to those specimens and a massive decomposition of the musculature and gonads was observed inside the tunic (Figure 12).

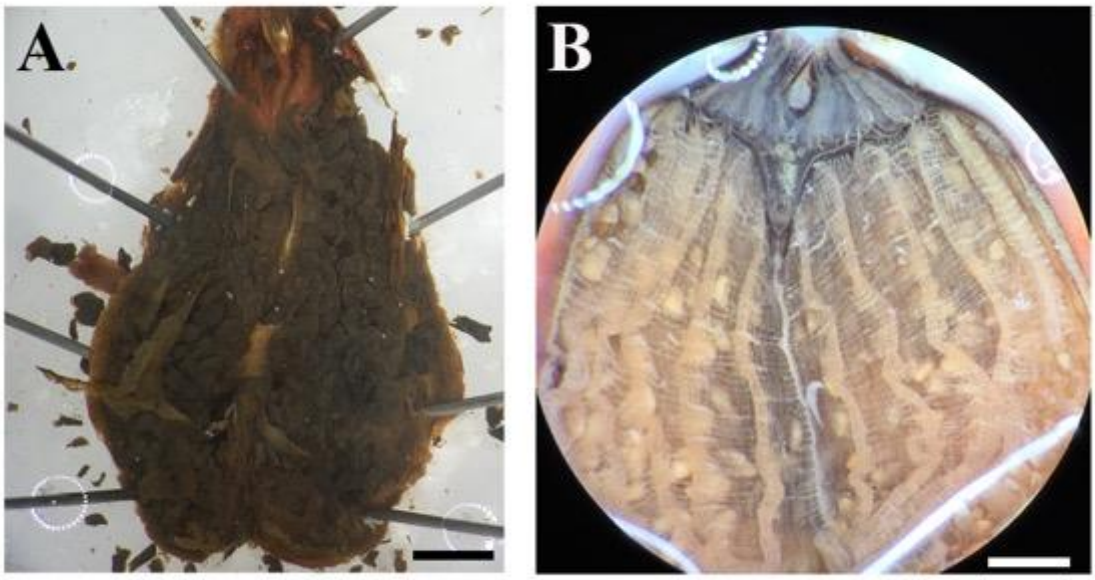

Figure 12. Butterfly dissection of Polycarpa captiosa. A) Post-mortem after deceased non-gut recovery, decomposed gonads and rest of the previous pharynx observed, scale bar: 0.5 cm. B) Fresh dissected material, healthy pharynx and gonads observed, scale bar: 0.5 cm.

DISCUSSION

The microplastics exposure treatments showed that the filtration behaviour of ascidians was severely affected by the ingested plastic fibres negatively affecting the animal's overall filtration capacity, clearance rate and absorption efficiency. Following exposure ascidians struggled to achieve their optimal clearance rate. Individuals exposed to the 12-hour treatment reduced their filtration activity expressed in the number of times they opened and closed their oral siphons and the amount of faeces produced after the treatment, significantly different from the control. After the longer microplastic exposure (24-hours and 48-hours), ascidians dramatically decreased their filtration behaviour, clearance rate and absorption efficiency. This behaviour could be explained by a reduction of their metabolic rate after the microplastic shock resulting in an inability to feed on the available suspended organic matter.

The amount of ingested fibres found in the animals exponentially decreased with exposure time as evidenced by the fibres found in faeces and retained in the digestive tract. During the experimental time, ascidians could not absorb organic matter effectively once their digestive system was blocked by the fibres. In the 48-hour exposure treatment, ascidians retained more fibres in their pharynx. Two weeks after micro-plastic exposure, 80% of ascidians who eviscerated their guts did not properly recover and died compared to a usual recovery rate of *P. captiosa* under other environmental stresses such as high turbidity and ocean acidification (Chapter 5 and 6).

There is evidence from many other studies of the impacts of microplastic intake on a range of organisms, such as copepods, mussels and shrimps in laboratory conditions, and these studies also found bioaccumulation of plastics in their guts (Browne et al. 2008; Devriese et al. 2015). Cole et al. (2015) also found that copepods feeding rates reduced after microplastic exposure, which had the same effect on their digestive system as the ascidians treated in this research.

Marine animals exposed to microplastics may only retain the plastics in the digestive tract or expelled in faeces, although Browne et al. (2008) found translocation from the gut of a mussel to the circulatory system. Dewar-Fowler (2019) conducted the

first study on microplastic intake on *Ciona intestinalis* ascidians using plastic beads (10 um) and fibres made of polyethylene, to test the ingestion of particles and accumulation in the essential tissue. The study found that animals ingested more beads than fibres when exposed to the same concentrations and that some translocation in the ascidian's tunic, was possible after long exposure times, we could not find evidence translocation of microplastic fibres in this study. The transfer of plastics to predators has been previously reported in crabs, (Farrell & Nelson, 2013), shrimps (Setala et al., 2014) and lobsters (Murray & Cowie, 2011).

Ascidians are part of the gastronomic culture in several countries like Chile and Korea (Lambert et al .2016), and the transfer of microplastics ingested by these organisms to humans is a concerning issue that must be studied in depth to determine whether or not this could be a plastic transferring pathway.

Despite increasing concern of microplastic pollution in the marine environment, few studies have analysed the impacts of those plastics on the biological roles the animals play within their ecosystems. The impacts on the ingestion and retention of microplastics by marine invertebrates, and especially filter feeders like ascidians can severely affect their ecosystem services and even the homeostasis of the ecosystems and biota they coexist with in many ways (Wright et al. 2013).
Ascidians have received little attention when it comes to microplastic pollution; this study shows how the growing plastic fibres exposure affects important sessile organisms at low trophic levels. The results of this experiment demonstrate how easily microplastics fibres are ingested over a short period of time.
This ingestion caused 90% of the ascidians to eviscerate their guts and later to die. In chapter 5 and 6 we demonstrated this gut ejection behaviour when ascidians were exposed to high turbidity and ocean acidification and warming scenarios but 80% of the ascidians recovered their guts within two weeks after evisceration. In this study ascidians were not able to recover. *P.captiosa* ejected their gut voluntarily after microplastic exposure stress. During the experimental time it was recorded that some ascidians regenerated and regrew their guts within two weeks, however, the damage to the digestive systems was severe and most of them could not regenerate and died.

The plastics fibres caused blockages in the stomach and intestines of the treated ascidians and these could not filter or ingest any organic particles when exposed to low concentration of microalgae. The animals used in this study were collected in shallow water in Singapore shores where some studies have found microplastic particles in water and sediment samples from coastal areas (Ng & Obbard, 2006). Following the results presented in this research is possible to presume there is a high possibility *Polycarpa captiosa* could be ingesting plastic debris from their natural niche of occurrence increasing the need to implement conservation strategies in that area to reduce negative impacts driven by plastic pollution.

CONCLUSIONS

Microplastics have a direct effect on the ecosystem services of ascidians, reducing their filtration capacity and their clearance rate which will directly affect other organisms that depend on clear waters to survive. As the majority of plastic fibres were ingested, there was a significant amount of bioaccumulation of plastics within the animal's digestive systems, leading to detrimental biological effects. When plastics were not consumed, they precipitate or remained attached to the animals' skin. Upon examination the digestive tissue was severely damaged which eventually led to the death of the ascidians, after only a short exposure time. If the accumulation of plastics is in their natural habitat, the ascidians and other organisms which will presumably lead to high mortality rates.

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Chapter 8: General discussion

Coastal marine ecosystems are important from many perspectives, firstly, most marine species seasonally approach the coasts to fertilize or lay their eggs, because the coastline systems provide protection for larvae and juveniles (Halpern et al. 2008). Secondly, most human activities at the coast depend on the services obtained from coastal marine ecosystems, such as coastline protection, fisheries, scenery and leisure (Lopes & Videira, 2013).

The imminent impacts of climate change on the coastal ecosystem are of great concern; ocean warming, and greenhouse gases have elevated mean sea-surface temperature and in shallower areas it has modified the thermocline curve (IPCC, 2007). This stressor triggers a series of subsequent affects including the melting of polar ice which increases the sea level (Andersson et al., 2008; Rahmstorf et al. 2007), increases tropical storms and even modifies upwelling patterns (Debiolles et al., 2016; Knutson et al., 2010). In addition to those environmental stressors, the continuous uptake of anthropogenic CO_2 in the ocean is provoking a reduction in the water pH and causing serious stress for marine fauna, especially marine invertebrates with calcified skeletons such as bryozoans (Swezey et al., 2017; Crook et al., 2013). The major threat of this stressor is that it is expected to continue increasing through the end of the century, affecting most marine and human life (Caldeira & Wickett 2003; Dickson, 1990).

All these environmental variables affect all life stages in the marine ecosystems, provoking a series of behavioural and physiological responses from cells to systems and to ecosystem health. Understanding the effects of climate change on the organism is important in order to evaluate the degree to which adaptation or resilience to those stressors might occur. We interpreted ecological resilience as the ability of an organism to adapt and maintain biological functions under long-term environmental stress (Folke et al., 2004).

Based on the premise that biodiversity potentially increases the adaptative responses to climate change disturbances and the degree of stress compensations (Bernhardt & Leslie, 2013), the first chapter of this thesis (Chapter 2) involved the

description of the bryozoan deep-sea diversity in the Colombian Caribbean. The purpose of this was to complement the previous knowledge of these invertebrates in shallower areas of the Caribbean, and to determine potential hotspots of diversity. This information could be used as a baseline for the further understanding of population and ecosystem connectivity that would allow the “bryo-fauna” to adapt to climate change stressors (Hageman & Todd, 2014).

As part of this taxonomic revision, I found 94 species from which nine are new species for science, four correspond to new records for the Great Caribbean basin and 12 are new records for Colombia. This represents approximately a 30% increase in the previous knowledge of bryozoans in the country in regard to the update of bathymetric and geographical information. Finally, I concluded that bryozoans can generate habitat for the protection and establishment of smaller organism or even other bryozoans at the muddy bottoms of the Colombian deep-sea. I decided to evaluate the Colombian fauna due to the scarce knowledge about this phylum and due to previous knowledge and access to biological records and associated information. Diversity studies are crucial for our understanding of environmental ecology as meta-populations of the same species may better resist environmental disturbance than single isolated patches because the meta-population may increase the number and level of responses to stressors and potentially compensate overall population decline (Hughes, 2004).

Chapter 3 looked at the environmental conditions that determine the extent of occurrence of bryozoans along the Colombian Caribbean. I found three main growth forms (encrusting, erect and free living) (O’Dea & Okamura, 1999; O’Dea et al., 2007). I analysed the total bryozoans examined and compared phenotypic trends with the type of sediment, location and depth. I concluded that that erect flexible forms dominate the shallower depth range 1-10m and colonies were mostly associated with hard substrates, while deeper ranges (50-200m) were dominated by erect rigid forms associated with bivalves shells, dead corals and live rocks, lastly, I found that the 1000-3888m depth range mainly contained delicate flexible-articulated forms attached to soil grains, probably as a consequence of the limiting factors at those depths such as food and reduced water flow (Schopf et al., 1980). I also compared the bathymetric and geographic distribution of some species with contrasting growth forms with environmental data obtained at the time of collection.

I found that diversity depends on the temperature and nutrient availability. The bryozoan fauna in the northern part of the Caribbean is the richest in number of species and is more abundant from 10 to 80m depth, but the south part has higher species richness and abundance from 150m depth onwards. After comparing this trend with the mineralogical information of the selected species I concluded that the range 50-450m depth, presented the greatest assemblages of calcite-composed species with larger colonies than in shallower areas and the range 1500-3888m depth has more calcite-Mg composed species. This chapter is the first report of environmental relationships with bryozoans from 1 to 3888m in the Colombian Caribbean. Species assemblages could potentially recover from disturbance events (Hooper et al., 2005) such as stationary high turbidity or seasonal upwelling as in the case of the North Colombian Caribbean (Andrade & Barton, 2005; Mckinney & Jackson, 1989).

In Chapter 4, I analysed the ability of *Bugula neritina* to switch between reproductive strategies in response to changes in the environment (Håkansson & Thomsen, 2001). I collected colonies at contrasting geographical areas of the Atlantic and Pacific Oceans from 1 to 5m depth and counted the reproductive structure densities developed under different climatic seasons, substrates, and other environmental factors. Results showed that *B. neritina* colonies inhabiting similar ecosystems with contrasting environmental factors differed in their reproductive strategies and morphometry. In addition, we suggested a possible trade-off between zooid features and ovicell production, when colonies invested in sexual structures, a reduction in zooid and colony size was observed. The degree of adaptation to environmental stressor is a means of ecological resilience (Hall & Hughes, 1996)

From Chapter five to Chapter seven, I focused on the understanding of the environmental stressors that modify the filtration behaviour of *Polycarpa captiosa* and that, in some cases, lead to evisceration and gut recovery. In Chapter 5, I looked at the effects of turbidity conditions on the behaviour of *P. captiosa* in controlled mesocosms to determine their optimal and limiting tolerance to this natural disturbance. I found that *P. captiosa* performs complete evisceration, 12-hours post high turbidity exposure and within 24-hours of medium turbidity exposure and found that animals regenerate their digestive system within 20-days post evisceration

when transferred to control conditions. However, when conditions remained unchanged, 100% mortality was observed. Finally, I concluded that animals can eject their gut more than once in response to stress disturbance, but their mortality rate increases with the number of eviscerations and their filtration activity declines. This is the first study reporting evisceration in Chordates as a consequence of environmental stress.

In Chapter 6, I evaluated the resilience of *P. captiosa* under ocean acidification and warming (OAW). The responses of ascidians were assessed by analysing their resilience (survival), and clearance, absorption and ingestion rates. I found that this disturbance significantly affects the resilience and performance of ascidians at the individual level. Animals reacted by skin shedding and gut ejection after all high temperature and elevated pCO2 treatments, and 20% mortality was observed.
I suggested that ascidians increased their metabolic rates under warm temperature treatments, as clearance and ingestion rates increased. In contrast, under higher pCO2 conditions, a significant reduction of the ingestion rates and clearance rate was registered. These findings could be considered important for the future of the coastal managements and conservation strategies of those important ecosystem improvers.

Finally, in chapter 7 I looked at the impacts of the ingestion of polyester, polyvinyl and polypropylene microfibres by *Polycarpa captiosa* on its biology and filtration activity (Fossi et al., 2014; Browne et al., 2008). I collected Microfibers from launderettes, to replicate a common contamination pathway. I found that 90% of the ascidians eviscerated very damaged guts around 15-hours post microplastic exposure, from which only 20% recovered. Dead specimens had decomposed organs and gonads after 15 days of exposure. In addition, I found significant differences in the microplastic accumulation in different digestive organs in relation to the exposure time. Ascidians exposed for longer, reduced their filtration activity, clearance rate and absorption efficiency and presented the biggest accumulation of fibres in the pharynx suggesting a reduction in their pumping activity. This study constitutes the first registering lethal impacts of microplastic fibres on chordates and suggesting direct impacts on ascidian's ecosystem functions (Messinetti et al., 2019).

Thesis conclusions and future directions

The discovery of new species for science is important for the understanding of the species richness in a specific area. The level of interaction the Colombian deep-sea might be having with adjacent areas of the Atlantic is evidenced by the discovery of species previously registered for other sides of the Atlantic. The number of species found surrounding deep-sea coral patches provide information about the level of interaction bryozoans can be having with other habitat forming organisms in those ecosystems.

Bryozoans do not have a static distribution, some species usually found at deep zones, have representatives in shallower areas indicating a possible level of connectivity between populations across a bathymetric range. Also, it was demonstrated here that the benthic assemblages do not necessarily decreases in species diversity from north to south as it has been reported. The Magdalena deep-sea fan is a geo-form produced by the strong action of the Magdalena river delta that creates a frontier between northern and southern species; however, the distribution is not decreasing north to south as previously described by other authors. A higher biodiversity has been identified at the south zone of the Colombian Caribbean at deeper zones than in La Guajira. In addition, environmental variables modify the growth form, distribution (bathymetric, geographic) and the reproduction in bryozoans. *Bugula neritina* is a species reported in most parts of the world as invasive, however, there is no record of this in the Caribbean. This species does not invest in energetically expensive ovicell production when inhabiting disturbed areas such as marinas or mangrove areas with big temperature and salinity fluctuations.

Polycarpa captiosa is a good model system for the understanding of the effects of climate change in coastal ecosystems. It ejects its guts as a stress response to environmental disturbances. This species has the ability to regenerate their digestive system several times, but its filtration activity decreased. The filtration performance declines with the number of guts ejected. Microplastics are very damaging for the ecosystem functions and cause a lethal intestinal damaged to *P. captiosa* after exposure.

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