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Ecological study of nudibranchs in the Armona Biodiversity Hotspot

UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS E TECNOLOGIA

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Master in Marine and Coastal Sciences

Work performed under the supervision of:

Prof. Doutores Duarte Duarte and Sofia Gamito

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam na listagem de referências incluídas

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Sandra Hoett

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Abstract

Since the 1980s, scientific interest in nudibranch species has grown due to their potential in biotechnology and neurobiology. But only a limited number of ecological studies have been undertaken specifically on this mollusk group in a lagunary environment.

This MSc thesis aims at assessing the nudibranch ecology of the Armona biodiversity hotspot in the Ria Formosa lagoon and at collecting information helpful to its conservation. The scope includes investigating the abundance of each species, which environment it prefers, its behaviour, biometrics and role in the trophic web as well as their relationship with the other benthos. In situ data acquisition during scientific diving forms the basis of the study. The diving survey took place during May 2018, a time of the year for which previous studies had typically observed higher densities of nudibranchs.

Data gathered from 10 dives was dissimilar but consistent. *Flabellina affinis* was the most abundant species identified in the Armona hotspot during the survey. Whilst previous surveys pointed out to other species of nudibranchs in the Ria Formosa area, these findings could not be confirmed in May 2018. This leads to questions about migration and general behaviour patterns of the nudibranch.

Determining the density of invertebrates other than nudibranchs, observing their behavior and recording their biometrics were secondary goals of this thesis. These tasks were performed through random quadrat sampling and should contribute to the knowledge of benthos inside the surveyed biodiversity patches.

The Armona biodiversity hotspot or patch consists of sea urchins accumulating in the sandy bottom of the Ria Formosa and generating livable niches for a great number of organisms. This results in the creation of a specific community with unique features.

Keywords: Sea slugs, Nudibranchs, sea-urchins hot spots, Biodiversity patches, Ria Formosa lagoon system macrobenthos

Resumo

Desde a década de 1980 que a comunidade científica tem vindo a desenvolver trabalhos de investigação centrados nas múltiplas espécies de Nudibrânquios, dado o seu potencial na biotecnologia, na neurobiologia e como bioindicadores. Porém poucos têm sido os estudos desenvolvidos sobre estes moluscos em ambiente lagunar.

O principal objetivo desta tese de mestrado versa contribuir para o estudo ecológico dos nudibrânquios observados no *hotspot* de biodiversidade localizado na zona lagunar adjacente poente da barra da Armona, na Ria Formosa e registar informações que possam contribuir para a sua conservação. Este trabalho pretendeu estudar a abundância de nudibrânquios, identificar as eventuais preferências de cada espécie, o seu comportamento, obter informações biométricas, determinar qual o seu papel na rede trófica, bem como avaliar a sua relação com os outros bentos. Pretendeu-se igualmente determinar as densidades dos macroinvertebrados presentes nas manchas de biodiversidade, para além das dos nudibrânquios. Cada *hotspot* ou mancha de biodiversidade estudada na zona adjacente à barra da Armona é constituída por populações de ouriços-do-mar e ascídias assentes numa camada de valvas de bivalves acumuladas no fundo arenoso da Ria Formosa, gerando nichos habitáveis para um grande número de animais (esponjas, crustáceos, moluscos, pepinos-do-mar, estrelas-do-mar, ofiurídeos, poliquetas, cavalos marinhos e outros peixes, que compõem um ecossistema. Várias manchas de biodiversidade foram analisadas e a respetiva comunidade macrobentónica estudada. Na identificação de nudibrânquios usou-se uma chave dicotómica modificada, baseada numa chave existente de um estudo anterior realizado na Ria Formosa. Foi necessário alterar e adaptar essa chave às espécies nudibrânquios versadas nesta tese, de modo a facilitar a sua utilização durante a campanha de mergulho.

A aquisição de dados no ambiente lagunar *in situ* decorreu com recurso a várias técnicas que envolveram o mergulho científico. Foram utilizados métodos específicos de mergulho de forma a não interferir ou danificar as manchas de biodiversidade e ainda técnicas e metodologias científicas de aquisição de dados

dos habitats em estudo e dos vários parâmetros, tendo para o efeito sido dada formação específica à equipa de mergulhadores. Para a aquisição de dados *in situ* recorreu-se ao método dos transeptos combinados com quadrantes (0.50x0.50m) para uma melhor avaliação do macrobentos nas manchas de biodiversidade. A aquisição de dados iniciou-se em maio de 2018, numa época do ano em que estudos anteriores apontavam para densidades mais altas de nudibrânquios nas manchas de biodiversidade da área em estudo. Para o efeito, foram realizadas 10 campanhas de mergulho distintas, mas relacionadas entre si.

Na primeira campanha de mergulho foram estudadas seis *hotspots* de biodiversidade, que incluiu etiquetar, geolocalizar e caracterizar morfologicamente essas manchas. Embora se tenha observado variação no tamanho e forma, verificou-se que genericamente têm uma forma oblonga. A hipótese apontada para que essa forma oblonga ocorra é a hidrodinâmica da Ria Formosa. Durante as campanhas de aquisição de dados *in situ* seis espécies diferentes de nudibrânquios foram identificadas nas manchas de biodiversidade durante estes trabalhos*: Flabellina affinis*, *Cratena peregrina*, *Dendrodoris grandiflora*, *Doriopsilla areolata*, *Felimida purpurea* e *Pleurobranchaea meckeli*, uma lesma do mar pertencente ao género *Pleurobranchaea*. No que respeita aos resultados quantitativos, *D. areolata* e *F. purpúrea* não foram considerados por falta de dados. A espécie *Flabellina affinis* foi a mais abundante entre os nudibrânquios no *hotspot* da barra da Armona. Os resultados obtidos apontam no sentido de algumas espécies de nudibrânquios preferirem os habitas das manchas de biodiversidade como fonte de alimento, reprodução e abrigo (*Flabellina affinis*, *Cratena peregtina*), enquanto as espécies *Dendrodoris grandiflora* e *Doriospilla areolata* preferem o habitat arenoso envolvente. Durante este trabalho não foram encontradas as mesmas espécies que tinham sido observadas em campanhas de anos anteriores. As razões para esta diferença podem ser: variações sazonais; pela localização escolhida para estudo; concentração de alimento ou pelas correntes costeiras. As densidades de macroinvertebrados identificadas durante estes levantamentos nas manchas de biodiversidade da barra da Armona foram inferiores à observada noutros habitats na Ria Formosa, embora a metodologia de amostragem não seja diretamente comparável. Estas baixas densidades observadas poderão estar relacionadas com o elevado stress físico nesta zona da Ria Formosa, devido às fortes correntes de maré e ainda a diversas atividades antrópicas. Neste local é habitual a ancoragem de múltiplos veleiros e consequente destruição das manchas de biodiversidade pelas respetivas ancoras, a pesca continua na área em estudo presenciada pela equipa de investigação aquando das campanhas, os impactes resultantes da intensa passagem e paragem de embarcações de recreio e marítimo turísticas, entre outras.

Os resultados obtidos no âmbito deste trabalho podem assim contribuir para que sejam tomadas medidas para a melhoria da gestão, preservação e conservação desta área lagunar em estudo, bem como servir de base para trabalhos futuros. Seria da maior importância, em trabalhos futuros, proceder ao estudo ecológico dos nudibrânquios nestas manchas de biodiversidade, em diferentes estações do ano, estudar uma eventual variabilidade comportamental diurna, diferenças no âmbito de outras condições hidrodinâmicas às estudadas (designadamente a marés extremas, fases de maré intermédias às estofas e períodos de maior aporte de água doce ao sistema lagunar) assim como possíveis padrões de migração, períodos de acasalamento e posturas. Estes estudos da ecologia das espécies de nudibrânquios, realizados não só à escala local da barra da Armona, mas também à escala do sistema lagunar da Ria Formosa, permitiriam comparações a um nível mais abrangente com outros sistemas lagunares, de forma a utilizar estes organismos como bioindicadores das mudanças no ambiente (naturais e antrópicas) e das alterações climáticas.

O estudo destas espécies de gastrópodes em ambiente natural, baseado em observações *in situ*, sem interferir ou condicionar o seu habitat requerem muitos recursos humanos, técnicos, logísticos e de segurança, sendo muito difíceis e dispendiosos as respetivas campanhas, mas de primordial importância a informação ecológica obtida. Os resultados obtidos no âmbito deste estudo apontaram, para trabalhos futuros, formas de melhorar em alguns detalhes as metodologias usadas neste trabalho (por exemplo, no tipo de levantamento usado com scooters, nos métodos dos quadrantes, ...), e de melhorar também o planeamento dos mergulhos quanto à parte logística e na previsão das condições ambientais adversas.

Palavras-chave: Lesmas do mar, nudibrânquios, agregações de ouriços-do-mar em substrato móvel, biodiversidade, Ria Formosa, Macrobentos

1. Introduction

1.1 Theme justification

Nudibranch, which means “naked gills” are shell-less sea slugs and members of the class Gastropoda of the phylum Mollusca (Wägele *et al.*, 2005). The majority of research conducted on sea slugs involves either taxonomic research or biomedical research (Dean *et al*., 2017). Some discoveries have shown the potential role of sea slug aggregations in nutrient and bio-control of certain organisms and recent work in their production of rare or novel chemical compounds through dietary sequestration (Dean *et al*., 2017). Additionally, a number of sea slug species, especially some of the larger species have played an important role in neurobiological research due to the relative size of many neurons and neural ganglia, which is useful in both neurological studies and teaching purposes. The research around the ecology of sea slugs has yet to get more attention. Most of the present ecological knowledge on the species is derived from studies which only peripherally cover nudibranchs (Geange and Stier 2010; Rossi *et al.*, 2012). Some interesting ecological discoveries have appeared in the recent years including the potential use of monitoring sea slug ranges in mapping climate change impacts in the North American region (Dean *et al*., 2017). Regarding the low amount of literature concerning specific groups of nudibranchs there are still many nudibranchs to be properly studied and that is the goal of the present study: to contribute to the knowledge of nudibranchs’ ecology in the Ria Formosa.

As a secondary aspect of this thesis the benthic community apart from sea slugs are also investigated. The diversity of benthic invertebrates in the Ria Formosa is high when compared with other lagoonal systems or transitional waters, with the highest diversity found on seagrass beds (Gamito, 2008). Examining the benthic community is very important to assess the general environmental conditions. Macro invertebrates are limited in their mobility and integrate environmental condition easier than pelagic organism, therefore, they are the perfect group to use for monitoring anthropogenic impacts or the assessment of ecological patterns. For the second goal is therefore to get knowledge about the benthic community present in the Armona biodiversity hotspot.

1.2 Nudibranchs

There are more than 3,000 known species of nudibranch (Wägele *et al*., 2005) and although they can be found throughout the world's oceans at various depths as well as in tidal pools and coral reefs, they are most common and diverse in shallow tropical waters (Gofas., 2014). Their scientific value lies in their suitability for evolutionary studies and their use in pharmaceutical studies (Cimino *et al*., 2004).

The evolutionary and ecological adaptations of nudibranch such as modifying their bodies for defense and feeding were driven by the loss of their shell (Faulkner *et al.*, 1983). They possess a very effective chemical defense mechanism which is obtained from biochemical accumulation or biochemical transformation of molecules present in their prey (Cimino *et al*., 2004). Many nudibranch biosynthesize compounds similar to the metabolites of their preys (Spinella *et al*., 1993). The ability to produce own defenses through the sequestration of dietary chemicals found in prey is an exceptional evolutionary advancement (Cimino *et al*., 2004).

The analysis of nudibranch enzymes for biogenetic processes may also provide key ingredients for drug development and in time lead to a new class of antibiotics (Cimino *et al*., 2004). The knowledge of basic natural phenomena is crucial for future ecological, pharmaceutical and biotechnological advances (Leal *et al*., 2012).

Nudibranchs are important components of benthic marine ecosystems and can commonly be found grazing on corals, sponges and macroalgae or crawling over rocks or on any other substrate (Behrens and Valdés, 2004). Despite their presence in many diverse habitats, nudibranch have rarely been the subject of dedicated ecological studies.

1.2.1 Taxonomic features

The classification of nudibranchs is still evolving due to a number of taxonomic revisions in the past (Dean *et al*., 2017). The prevailing view today, as reflected in the WORMS database, considers nudibranch to be members of the sub-class of Heterobranchia, a highly diversified and successful group of marine gastropods. They split into two sub-orders: Doridina and Cladobranchia (Bouchet, 2017). Their vivid colors and patterns are used to identify the nudibranch species but variation in color and pattern can appear within a species, occasionally causing taxonomical confusion (Behrens and Valdés, 2004). In cases where individuals have analogue morphology, but different colors and/or patterns, external and internal anatomy and larval stages are important characteristics to define whether the individuals can be considered to be a single or two distinct species (Hirose *et al*., 2014).

1.2.2 External morphology

Generally oblong in shape, nudibranch can be thick or flattened, long or short. Their colour can be vivid to warn their predators or dull to match their surroundings (Faulkner *et al*., 1983). The surface of the mantle may bear tubercles which vary in size, shape and number and are often a character used to identify nudibranch sub-groups (Calado and Silva, 2012). In aeolid nudibranchs the mantle is extended into long finger-like projections called cerata (Picton, 2006).

To identify prey, nudibranchs have a pair of highly sensitive tentacles, called rhinophores, located on top of their heads (Wägele and Willan, 2000). These structures are primarily chemosensory (smell, taste) in function (Cummins *et al*., 2009).

The most important respiratory organ in nudibranchs are likely to be the gills, however gaseous exchange also occurs over the entire body surface (Wägele and Willan, 2000).

In some species the cerata covering their back also serves as gills. It performs multiple functions such as breathing, digestion, and defense (Aguado and Marin, 2006). The ceratal epidermis is thin enough to enable dissolved oxygen from the surrounding water to diffuse in and carbon dioxide to diffuse out (Wägele and Willan, 2000). The cerata can be branched or tuberculated, which increases the surface area available for gas exchange (Aguado and Marin, 2007).

As nudibranch have an underdeveloped eyesight, the front head region of some species is extended on either side of the mouth to form an oral and/or propodial tentacle (Goodheart, 2016; Rudman, W.B., 1999). These structures act as feelers, allowing the nudibranch to touch the environment around itself and to determine the direction (Rudman, W.B., 1999). They are likely to also allow the nudibranch to identify food by touch (Goodheart, 2016).

All of the nudibranchs have in common a tail and a foot. The latter is a flat and broad muscle which clings to rocks, corals, sponges or other surfaces and which allows them to move (Rudman, W.B., 1999). Some of them even have the ability to swim short distances in the water column by flexing their muscles (Lawrence and Watson, 2002).

1.2.3 Reproductive strategy and life cycle

Nudibranchs have both male and female sex organs, making them hermaphrodites (Todd, 1978). Although hermaphrodite, self-fertilization does not occur (Kristof *et al*,. 2010). After fertilization they lay masses of spiral-shaped or coiled eggs in rocks or other substrates (Claverie and Kamenos, 2008). The eggs hatch into free-swimming larvae which eventually settle onto the ocean bottom as adults (Perron and Turner, 1977).

The life cycle of nudibranchs varies with different species: those developing more than one generation per year have a short lifespan (weekly or monthly) while those spawning once a year tend to have a longer lifespan (annual) (Rudman, W.B., 2004). The latter species are normally bigger than the first ones, showing synchronous sexual development and spawning behaviour and total mortality after reproduction (semelparous) (Aerts, 1994).

Opinions differ regarding the optimal spawning and settlement dates. Todd (1985) mentions that the timing is mainly due to environmental conditions such as photoperiodicity and temperature leading to individual maturity and spawning variations inside a species. Grant and Williamson (1985), however, suggests optimal spawning dates are not dependent on environmental factors but on the settlement condition of the prey.

Those factors are particularly relevant whenever ecological studies of nudibranchs are carried out in the intertidal zone. Moreover, the mobility of groups of organisms needs to be considered when studying community ecology based on parameters such as abundance, biodiversity and habitat preferences.

1.2.4 Relationship between nudibranchs and other species

The responses to brief encounters between nudibranchs vary, however, they are generally peaceful (Lambert, 1993). Lambert (1993) suggests that due to the absence of aggressive interactions among individuals and in spite of the apparent lack of a limiting resource, competition is unimportant in this community. Due to their limited mobility, nudibranchs' whole life cycle is associated to their trophic source and they are strongly related to their natural habitats and the benthic ecosystem where they feed for life on a broad range of different substrates (Aerts, 1994). The distribution and the population size of nudibranchs depend greatly on their dietary species and nudibranch predation doesn't seem to affect community structure (Aerts, 1994).

1.2.5 Horizontal movements (migrations)

The sudden appearance and disappearance of a large number of some nudibranch species in certain intertidal and subtidal locations are seasonally observed events (Aerts, 1994; Knowlton and Highsmith, 2000). Several theories are trying to explain this behaviour: the most accepted one is the migration theory. Migration as defined by Gibson (2003) is a seasonal movement of large numbers of animals over great distances. Nudibranch would migrate inshore to copulate and lay their eggs (Pelseneer, 1922; Nybakken, 1978; Claverie and Kamenos, 2008). Other theories for inshore migration include the influence of tidal action, currents or waves (Costello, 1938; Wyeth and Willows, 2006a), food foraging (Wyeth and Willows, 2006a), and geomagnetic field influence (Willows, 1999) all these being essential stressors in determining their life cycle.

Those different and abundant theories and justifications highlight the lack of information on the life cycle and general ecology of nudibranchs as well as on the diverse strategies of life that they present (Crane, 1972). A low number of ecological studies have been undertaken on this mollusk group and no broad study of the biodiversity of nudibranchs in the Ria Formosa exists up to now.

1.2.6 Feeding ecology

Nudibranchs feed on species such as hydroids, anemones, corals, sponges, tunicates, other marine invertebrates and even on other nudibranchs (Cimino *et al*., 2004; Crane, 1972; Robinson, 2004). Each species or family of nudibranchs may specialize in specific prey which it feeds on (Ruppert and Barnes, 1994; Purchon, 1977). Some are able to feed on different types of organisms, while others limit themselves to a single type of food (Todd *et al*., 2001; McDonald and Nybakken, 1997). The diet can easily be identified by analyzing DNA barcodes of nudibranchs' gut content (Robinson, 2004).

1.2.7 Defense

Between the different species of nudibranchs, one can observe a huge morphological and chemical diversity as well as unique adaptations caused by various evolutionary pressures (Cimino and Ghiselin, 1999). As nudibranchs have evolved towards a complete loss of an external or internal shell, they lack any mechanical defense (Faulkner *et al*., 1983). One of the curious evolutionary adaptations is their capacity to retain some specific chemical compounds obtained from their diet to use them as a chemical defense mechanism against their predators (fish, sea stars, sea spiders, crabs, birds and even other sea slugs) (McClintock *et al*., 1994).

When aeolid nudibranchs eat prey with nematocysts, the nematocysts are eaten but not discharged, instead they are stored in special sacs in the nudibranch cerata where they can be used to catch food or to sting predators. If feeling threatened, the nudibranch is capable of discharging these stinging cells through a terminal pore in the ceras which is an effective deterrent to predatory fish (Aguado and Marin, 2007). Dorid nudibranchs make their own toxins or absorb toxins from their prey creating an unsavory or toxic taste to their predators (Karuso, 1987). Nudibranch *Melibe leonina* can emit chemical odors to deter predators (Barsby *et al*., 2002).

The different patterns of nudibranch and especially the bright colours they obtain trough their prey serve as a warning signal to predators of their chemical defenses (Aguado and Marin, 2006). Alternatively, the patterns and the coloration of some nudibranchs could also be a camouflage mechanism, allowing them to blend in with their surroundings (Becerro *et al*., 2006).

1.3 Ria Formosa and Armona biodiversity hotspot

The unique Ria Formosa coastal lagoon is located in the South of Portugal and consists of a barrier islands system (two peninsulas and five barrier islands) (Pikey *et al*,.1989). It is a protected area since 1978 and classified as a natural park 1987 extending over 180 km^2 (CDL 373/87; ICNF,2015). It is a very dynamic and constantly evolving system due to the continuous movement of winds, currents and tides. It is considered as a shallow mesotidal lagunary system with a wet area of 100 km^2 (Falcão and Vale, 1990).

The lagoon has several channels and a large intertidal area which corresponds to roughly 50% of the total area, mostly covered by sand, muddy sand-flats and salt marshes (Falcão *et al*., 2003). In addition to the nature conservation areas there are several conflicting uses, such as fisheries, aquaculture and tourism (Duarte *et al*., 2008). Freshwater input to the lagoon is negligible and salinity remains close to 36, except during sporadic and short periods of winter runoff (Falcão *et al*., 2003). The tidal amplitude varies from a maximum of 0.5 meters at neap tide to 3.5 meters at spring tide and the mean water depth is 3.5 meters (Falcão and Vale, 1990). There is an intense exchange of 50 – 75% of water mass during each tide (Sprung, 1994). During spring tide, the total area covered by water varies between 14.1 and 63.1 km^2 (Aguas, 1986).

Estuaries and coastal lagoons are characteristically highly productive and important nursery grounds for fish and crustaceans, including various commercially valued species (De Wit, 2011; Kjerfve, 1994). Many of these species are depending on these areas for survival (Allen, 1982; Bennett, 1989). Four different types of habitat have been identified in previous studies in the Ria Formosa: sand, mud, seagrass and seagrass patches (Gamito *et al.,* 2008, 2012). The substrate of the Ria Formosa is either covered by vegetation (seagrasses and macroalgae) or sediment with grain size varying from muddy in the inner channels to coarse sand near the inlets (Falcao and Vale, 1990). Due to the various habitats in the Ria Formosa, the coastal lagoon sustains a large number of species (Almeida *et al.*, 2008).

Existing literature on the Armona biodiversity hotspot has not covered patches consisting of sea urchins trapping algae. Animals living in the sand or inside seagrass/algae meadows have been analysed (Allain, 1975) but this represents an exception. Additionally, in the Armona hotspot case, the sea urchins are using their spins to trap the algae transported by the tides from the ocean (pers.obs.)

In regions such as Arcachon, France, *P. lividus* lives in coarse sand. This species is smaller than the open sea one (Allain, 1975). *P. lividus* also settles in zoostera/seagrass meadows inside the sand (Allain, 1975). However, this kind of habitat is only partially suited for the animal (Cuenot, 1912). Bonnet (1925) stated that those individuals living in the sand or in the meadows are “belittle”. The sea urchins living inside those seagrass meadows find shelter and food and they can expand but this process is significantly slower than in the open sea.

1.4 Objectives

The main goal of this study is to contribute to the understanding of nudibranchs ecology in the Armona biodiversity hotspot. In order to reach this main goal, two major field campaigns and a series of laboratory procedures were accomplished.

The physiography of the biodiversity patch and their general characteristics such as size, composition, shape and location were studied. The diversity, species richness, abundance and biometrics of nudibranchs as well as of macrobenthos from different habitats and substrate were compared. We also validated the existing dichotomous key of nudibranchs for Ria Formosa.

We addressed the following questions:

- Which are the dominant nudibranch species in the Armona Hotspot?
- According to their behaviour, what are the nudibranchs using the Armona Hotspot for?
- Do nudibranchs have a preferred habitat, namely substrate?
- Which are the other dominant invertebrates in the Armona Hotspot?
- Do hydrodynamics such as tidal variation or coastal currents influence the nudibranchs density and species richness?

2. Methods and materials

2.1 Study area

For this thesis project, the focus area is in proximity of the Armona Inlet (Figure 2.1.1) where biodiversity patches have formed. This area represents one of the highest energy sectors of the Southern Portuguese coast as it is exposed to dominant wave conditions and storminess (Freitas *et al.*, 2011). The Armona biodiversity patches are mainly composed of sea urchins. They use empty shells to protect themselves (Allain, 1975) and to create a harder bottom (pers. observation) which they prefer to sandy ones. Macroalgae such as *Ulva* sp. are trapped by the sea urchin colonies, creating liveable niches for a large number of different organisms.

Figure 2.1.1 Study area: South of Portugal, Ria Formosa (Google Earth, 2018)

During the winter months, the patches are surrounded by empty shells. These are leftovers from the summer patches, which are larger and exhibit no or almost no shell boundaries (pers.obs.).

Over the study period the environmental conditions are usually varying between 18-20 °C (IPMA reports). Depending on prevalent wind and rainfall conditions, the water visibility can be reduced or good. The depth varies from less than two meters to a maximum of five meters depending on the tides.

2.2 Diving

The diving team was composed by Duarte Duarte, Benjamin Schmid, Filipe Parreira and Sandra Hoett.

Before pool training and the actual survey, the details of the diving techniques and the working methods were discussed in the classroom. The divers received theoretical training on how to achieve a perfect trim position (Figure 2.2.1) and learnt how to use the compass, the wet notes, the scooters and how to correctly perform transects. They also study the various nudibranch species and associated recognition patterns.

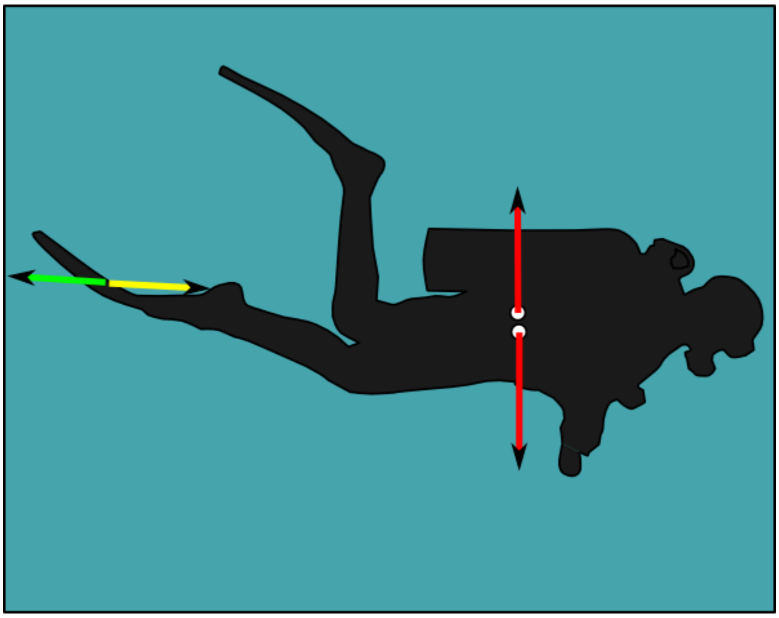


Figure 2.2.1 Horizontal Trim Position

A controlled horizontal trim position allows the diver to minimise swimming effort by reducing the front area of the diver passing through the water and minimizing disturbance of sediments on the bottom. The risk of the diver's fins interfering with delicate benthic organisms living close to the sea bed is also reduced (source: Peter Southwood https://creativecommons.org/licenses/by-sa/3.0, from Wikimedia Commons) (Figure 2.2.1).

During pool training sessions, the theoretical part was turned into practice. At the beginning, the divers learnt to master the trim position (Figure 2.2.1). In a second step they had to learn how to work underwater without disturbing the environment. Finally, they trained how to use the scooters and to complete a transect survey.

In this type of environment, to improve the quality of the photographs and to facilitate the orientation of the divers, it is advisable to employ as many complementary aids as possible such as flashlights and spotlights. Taking photos with ID tag helps to locate the site position relative to the seabed features. If photography or video footage of the site is not feasible due to adverse weather conditions, a detailed map can be drawn, illustrating distance and direction of the study site from other conspicuous features. Having any kind of visual help simplifies the task of identifying and recognising the study area. Before diving, watching a video footage of the location and pathway can also prove effective in aiding divers to form a mental map of the area and to recognize features of the site (Eleftheriou, 2013). For the safety measurements see Annex A.

2.3 Physiography and general characteristics of the patches

The first underwater survey was realized with underwater scooters. The divers had done transects in a previously defined study area (Figure 2.3.1).

Figure 2.3.1 Planned transect in the study area (Google Earth, 2018)

As soon as a biodiversity patch was encountered (on average bigger than 1x1 meter), the divers marked it with an ID tag attached to a stick (Figure 2.3.2) and with a buoy in order to take its GPS coordinates.

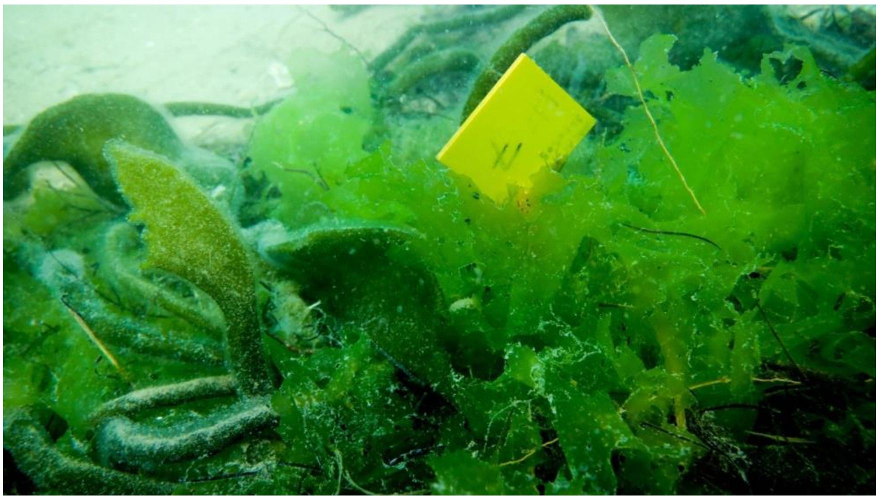


Figure 2.3.2 ID Tag (Photo: B. Schmid,2018)

As this method alone was not efficient enough, surface transects were performed from the boat during low tide and times of good visibility. Patches that are located close to each other were checked by scuba divers with the circular search method (Figure 2.3.3).

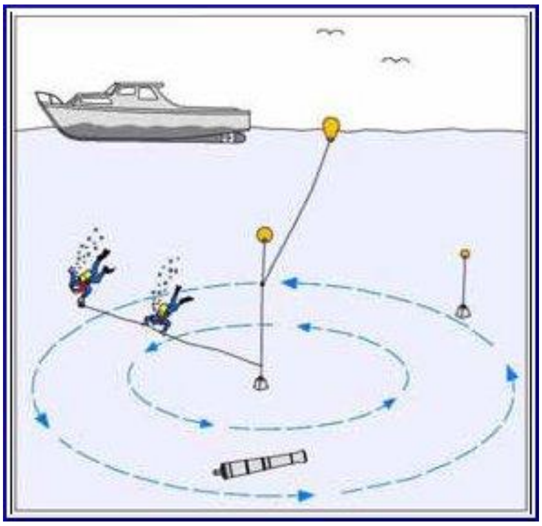


Figure 2.3.3 Divers conducting a circular search (source: cismas.org.uk)

A general distribution of the patches and the dimensions (length and width) are noted during this first underwater survey. Derived from this knowledge, six to ten patches were randomly selected for the following detailed study.

In each selected patch an underwater cartography was carried out, creating a map noting the geometry, orientation and biodiversity. A stick placed in the border of the patch at 0 degrees North of the center stick and a metric tape is tied to both. A third stick is then moved to each new location around the patch and again the angle, depth, distance (radius from center) and observations are recorded into the wet notes. The boundaries of the patch were determined visually based on the presence of algae and sea urchins.

To calculate the coordinates of the biodiversity patch the following formulas were used based on the parametric equation of a circle ($X = d \times cos(\alpha); Y= d \times sin(\alpha)$).

Only minor adjustments had to be made such as adding X_{HS1} and Y_{HS1} as the center was not at the origin. This is just translating the circle from the origin to its proper location (Page, 2018)

$$X_1 = X_{HSI} + [d1 \times \cos(\alpha_1 + 90°)] \quad (1)$$

$$Y_1 = Y_{HSI} + [d1 \times \sin(\alpha_1 + 90°)] \quad (2)$$

X_1 and Y_1 represent the actual coordinates, d_1 represents the distance in meters, α the angle, X_{HS1} and Y_{HS1} have the values of 6052294 m and -4095902 m, respectively.

2.4 Distribution and abundance of nudibranchs in and around the biodiversity patches

There are several underwater methodologies that allow us to define the species richness, diversity, the distribution and behaviour of the different organisms (Krebs, 1989). The transect search is the most suitable method for the study area (rough water and low visibility). For each biodiversity patch three parallel transects were installed: one going through the centre, one to the left of it and one to the right. The transects covered the three habitats (sand, shell and patch) (Figure 2.4.1).

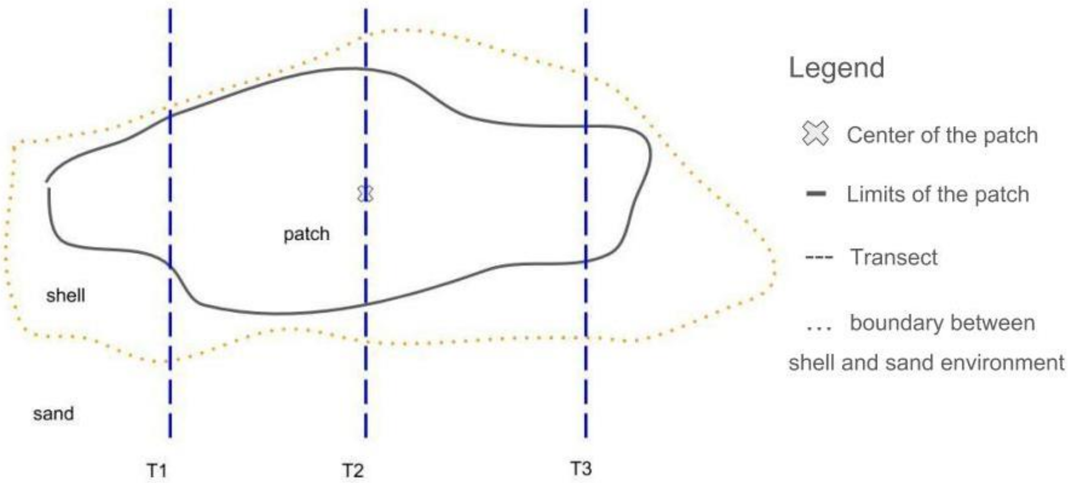


Figure 2.4.1 Schema of the transect methodology

As a next step, one diver calibrated the camera then did a video transect and took photos of the environment and the animals encountered. Divers were placed left and right along the transect line and covered one meter each side of the line. Then the divers started the survey swimming in a slow and constant speed to cover the areas each side of the line (Heine, 1999). When an organism of interest was found, a series of signals were displayed to tell the other divers to stop and to approach the animal. Each diver carried out a set of specific tasks (observations, species identification, biometric measurements, photo and videogrametric transect for benthic acquisition according to Heine (1999). Flash illumination is indispensable for videos and photos to bring out the contrast of colors as the sunlight is gradually diminishing with depth (Calado and Silva 2012). Observation time was limited to 30 seconds to avoid any unnatural behaviour as nudibranchs can feel threatened by the researchers´ presence. During the whole survey no nudibranch or any other organism was sampled as the goal was to protect and to conserve this special habitat.

After the dives, the data was organized and analysed. Through the pictures and videos, the in-situ identifications, measurements and observations were verified with the help of books and the dichotomous key. Simple statistic calculations were performed to better represent and understand the data.

2.5 Distribution and abundance of benthos excluding nudibranchs in and around the biodiversity patches

Besides the transect search, quadrat sampling was another proven tool, best suited for coastal areas where access to a habitat is easy (Krebs, 1989). Along each transect line one quadrat of 50x50 cm is placed in the different habitats (sand, shell and patch habitat) (Figure 2.5.1).

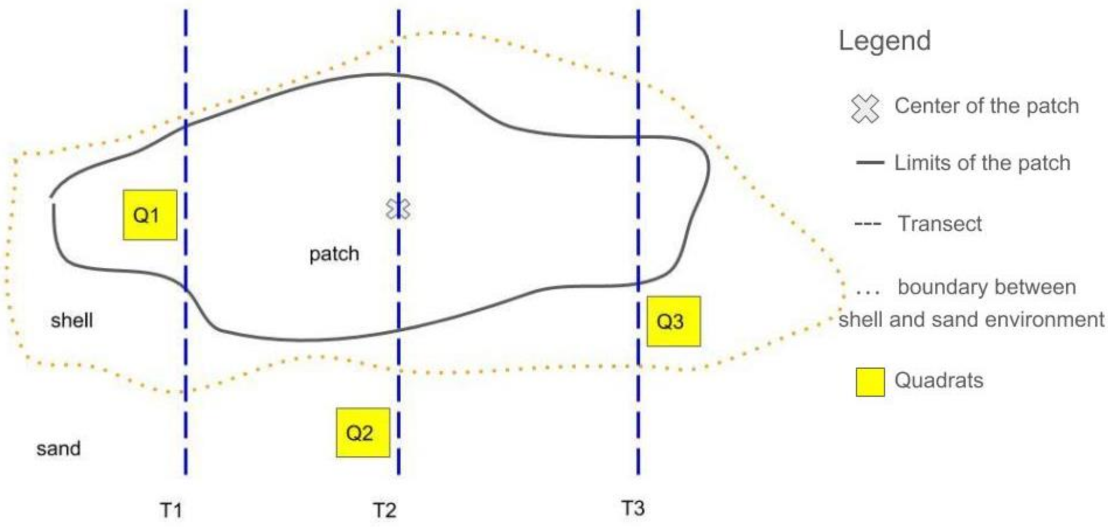


Figure 2.5.1 Schema of the quadrat methodology

The quadrat was subdivided in 10x10 cm smaller quadrats. Three small quadrats were randomly chosen and the species inside those small quadrats were identified and recorded. The content of the small quadrats was put on a support with a scale which simplified the postliminary work as through photo and video analysis the recorded species have to be identified, measured and their abundance calculated.

With the quadrat method the densities of organisms found at the study site could be established using the number found per quadrat and the size of the quadrat area. Subsequently, sites can be compared to determine whether for example, the abundance or diversity of organisms varies at different locations.

3. Results

3.1 Physiography and the general characteristics of the patches

During three diving days a total number of 44 potential patches were identified by scooter survey and from the surface by boat as the underwater scooter survey alone was not efficient enough. Heavy boat traffic in the Ria Formosa lagoon combined with a strict safety protocol meant that covering large areas by underwater scooter without interruption was not possible. Searching for biodiversity patches from a boat proved easier and more efficient. The relatively clear and shallow water made biodiversity patches easy to be seen and the larger distance covered made it possible to spot clusters of patches. Following the initial surface survey, diving sessions were undertaken at the identified sites in order to categorise them and to mark them with buoys for the gathering of GPS coordinates.

From the 44 identified patches, eleven were randomly retained as suitable biodiversity patches. Out of these eleven, six representative patches underwent a full in-depth survey (Figure 3.1.1, Table 3.1.1.).

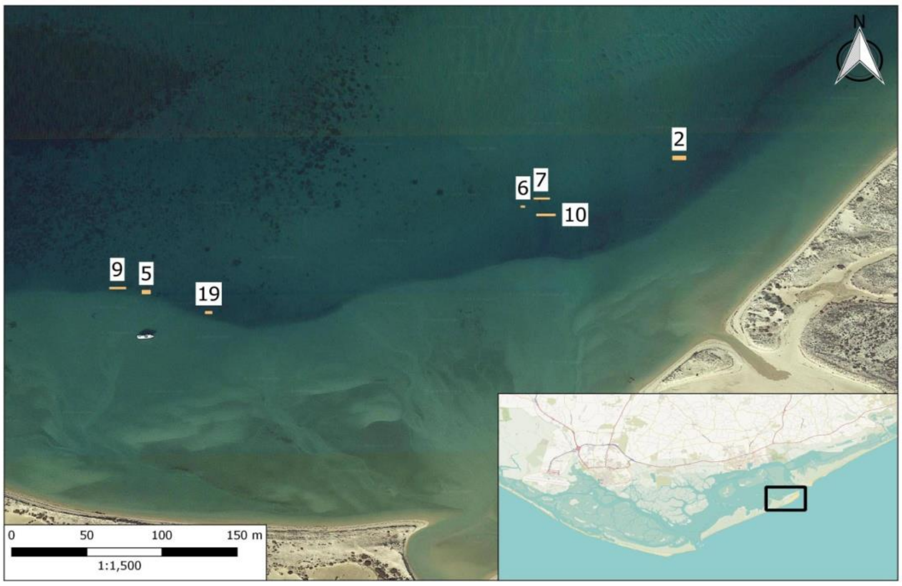


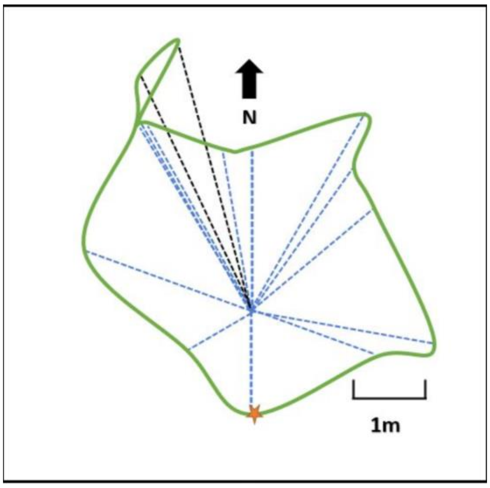
Figure 3.1.1 Map of the location of the biodiversity patches with their correspondent number and dimension (yellow rectangles). Map created from satellite imagery provided by Planet (2018) and OpenStreetMap (2018).

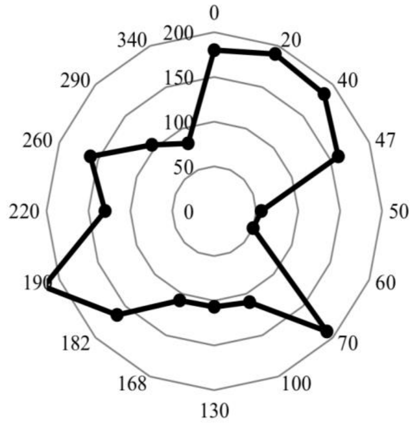
Due to time limitations and a very shallow location, patch 2 was located and measured but it did not undergo a full survey (no transect or quadrat sampling carried out).

Table 3.1.1 Relative dimensions of the patches fully surveyed

Patch	Length (m)	Width (m)	estimated 31rea (m^2)
2	8.6	3.2	28
5	5	3	15
6	2.5	1.1	2.8
7	9.6	1.1	10.6
9	9	1.5	13.5
10	12	1.5	18
19	4.1	2	8.2

The characteristics of the patches varied significantly, both in shape and size (Table 3.1.1 and Figure 3.1.2). Generally, the patches tended to be more oblong shaped. This is likely to be caused by the hydrodynamics present in Ria Formosa.

<table>
<tr>
<td>

Morphology of Patch 5
5x3 meters</td>
<td>

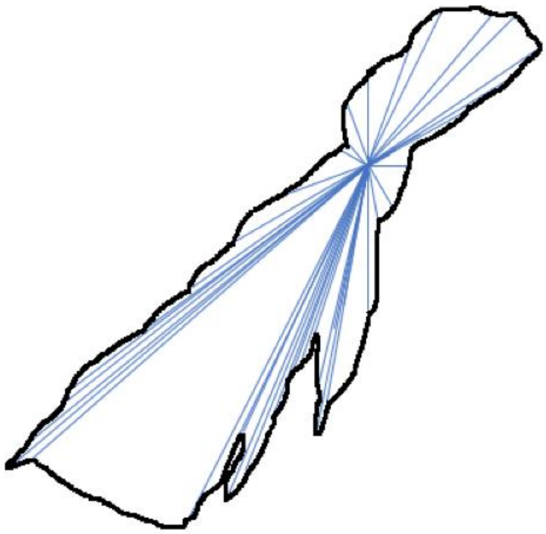
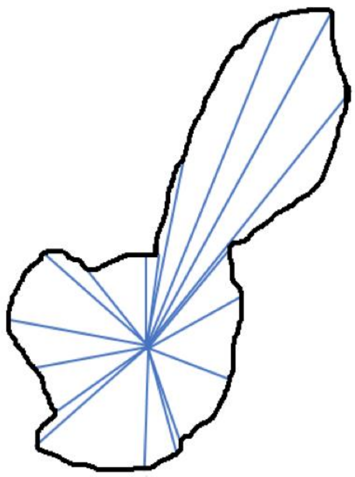
Morphology of patch 6
2.5x1.1 meters</td>
</tr>
<tr>
<td>
Morphology of patch 10
12x1.5 meters</td>
<td>
Morphology of patch 7
9x1.5 meters</td>
</tr>
</table>

Figure 3.1.2 General shape of some of the studied patches (made by MaCS Master students)

These results were obtained with the help of the first year MaCS Master students who produced the cartography of some of the elected patches for a course.

3.2 Investigation of nudibranchs in and around the biodiversity patches

3.2.1 Methods for identifying species

The already existing nudibranch dichotomous key (Annex B) was validated during this survey and changed with minor adjustments. The key facilitated underwater nudibranch identification and a comparison of photos taken during the dives with relevant literature later confirmed the results. The key is an indispensable tool for on the spot identification but requires periodic modifications and updates to ensure that it remains up to date. The literature comparison after the dive serves to validate the initial findings and helps to train the diver's ability to identify species.

3.2.2 Species of nudibranchs found

During the diving campaigns in May 2018, 66 individuals of different species (see Annex C) and sizes were found along the transect lines. The most common species was *Flabellina affinis*, followed by *Cratena peregrina*. Other nudibranch species such as *Dendrodoris grandiflora*, *Doriopsilla areolata*, *Felimida purpurea* and *Pleurobranchaea meckeli* which is a sea slug belonging to the genus of *Pleurobranchaea* were also observed. For the quantitative results, *D. areolata* and *F. purpurea* were discarded from the analysis because specimens were not found on the transect lines.

3.2.3 Density of nudibranchs

The density of nudibranchs per patch is shown in Figure 3.2.1 and Figure 3.2.2

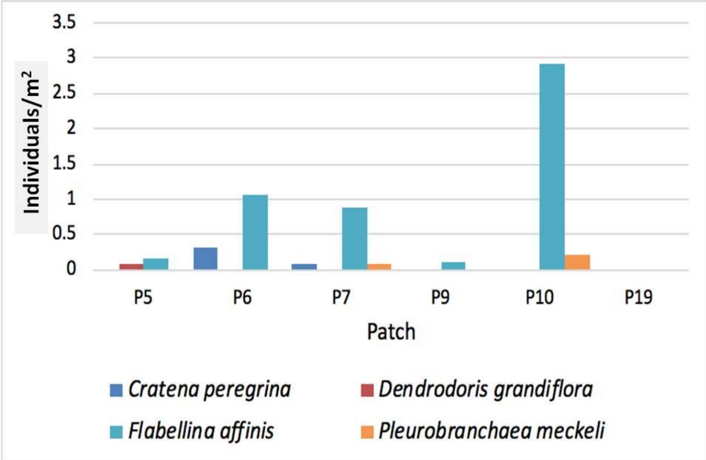


Figure 3.2.1 Density of nudibranchs species per patch

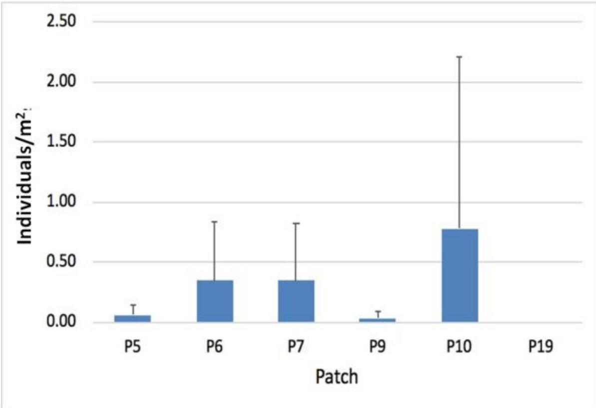


Figure 3.2.2 Nudibranch density with standard deviation

Patch 10 presents the highest abundance of nudibranchs with an average (±SD) of 0.8±1.4 individuals/m^2, patches 6 and 7 showed similar densities with an average (±SD) of 0.3±0.5 individuals/m^2 whereas both patches 5 and 9 exhibited lower densities with average (±SD) of 0.06±0.08 individuals/m^2 and 0.03±0.06 individuals/m^2 respectively. No nudibranch was found in patch 19.

Figure 3.2.3 plots the density of nudibranchs found during the transects in a patch against that patch's size. For simplification purposes, the latter is calculated using the formula for a rectangular area (length x width).

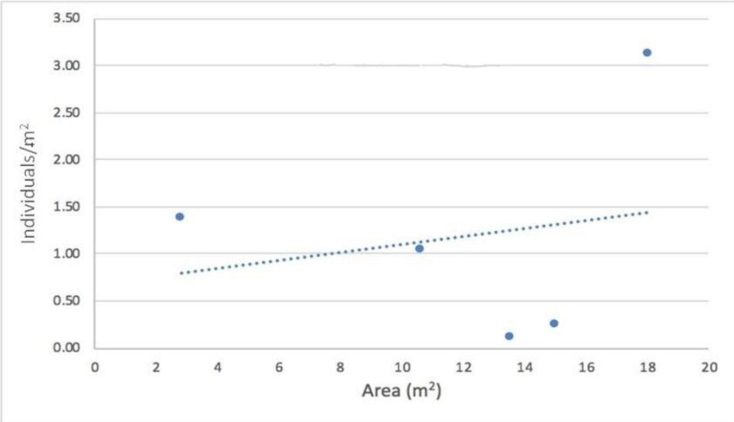


Figure 3.2.3 Density vs. Area

The graph shows no correlation between patch size and nudibranch density and the low R^2= 0.0147 confirms this lack of linear relation between the two variables. Although patch size is not a suitable explanation for a high nudibranch density, other variables need to be considered when evaluating the attractiveness of a biodiversity patch to nudibranchs. These factors could include patch location, composition (e.g. more *Ulva* sp. or more *Codium*), presence of sea urchins or availability of hiding spots. Another factor worth considering is tidal activity. Observations made during previous years indicate that the abundance of nudibranchs varies during neap and spring tides. This could be explained by a tendency of nudibranchs to be more active during neap tide when currents are weaker and movement is easier.

However, Figure 3.2.4 which compares the number of nudibranchs observed during neap tides (32) and spring tides (34) shows no major discrepancy between the two.

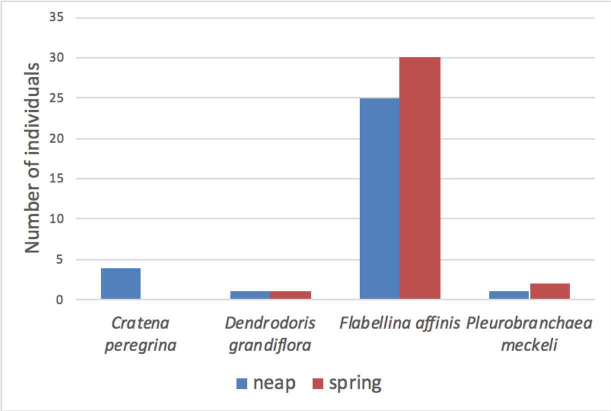


Figure 3.2.4 Number of nudibranchs species per tide type

The variation is even smaller in aggregate across all species (Figure 3.2.4 and Figure 3.2.5).

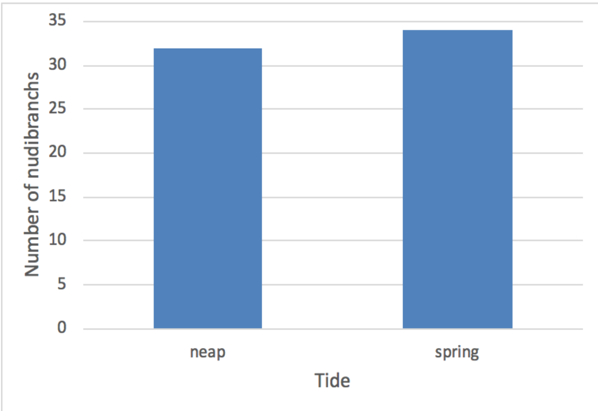


Figure 3.2.5 Total number of nudibranchs per tide type

3.2.4 Predominant species of nudibranchs

The dominant species during the survey was *Flabellina affinis* with an average (±SD) of 0.86±1.1 individuals/m^2, followed by *Cratena peregrina* (0.06±0.14 individuals/m^2), *Pleurobranchaea meckeli* (0.05±0.1 individuals/m^2) and *Dendrodoris grandiflora* (0.02±0.04 individuals m^2) (Figure 3.2.6).

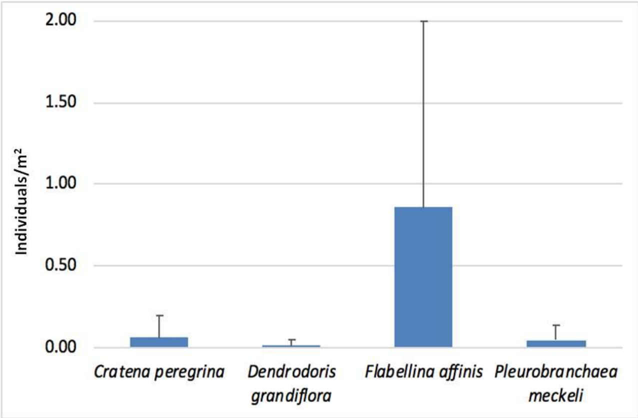


Figure 3.2.6 Average density of nudibranch species and standard deviation

The median size of *Flabellina affinis* was 1-1.5cm long with a minimum and maximum length in the sample of less than 0.5cm and 3cm and the graph follows a normal distribution (Figure 3.2.7).

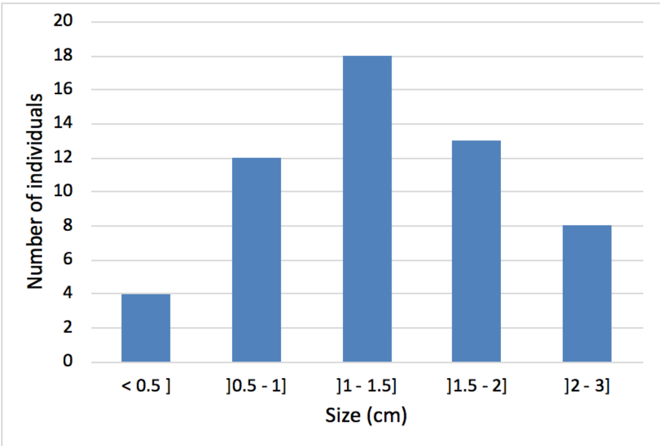


Figure 3.2.7 Size distribution of Flabellina affinis

3.2.5 Habitat of the different species

With the exception of *Dendrodoris grandiflora* (0.08 individuals/m^2) which was only observed in the sandy environment (Sa), all other species were predominantly found inside the patch (P) (Figure 3.2.8). Some *Flabellina affinis* (individuals/m^2) were observed in the shell habitat (Sh) and in the sandy environment. The density of *F. affinis* (0.9 individuals/m^2) was lower in those habitats compared to inside the patch (3.1 individuals/m^2). *Pleurobranchaea meckeli* had a density of 0.1

individuals/m^2 in the shell habitat while its density inside the patch was 0.17 individuals/m^2. *Cratena peregrina* was seen more frequently in the sand habitat (0.23 individuals/m^2) but also inside the patch (0.15 individuals/m^2) (Figure 3.2.8). *Flabellina affinis* (21 ind.) as well as *Cratena peregrina* (1 ind.) preferred to be on algae and seagrass (26 ind; 3 ind) inside the biodiversity patch (Figure 3.2.9) A small number of *F. affinis* were found in the sand environment (7 ind) and on ascidias (1 ind) (Figure 3.2.9). *Pleurobranchaea meckeli* was seen in the sand (1 ind), under and on algae (1 ind) and seagrass (1 ind) (Figure 3.2.9).

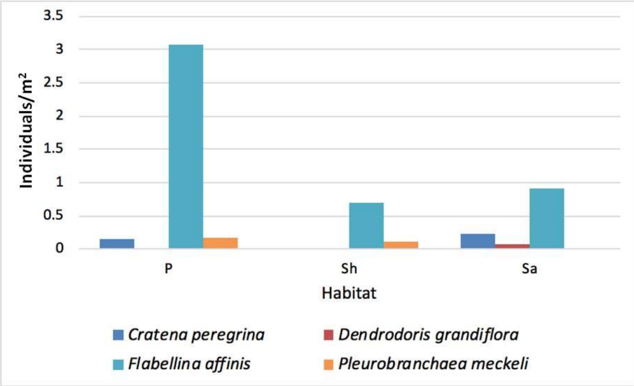


Figure 3.2.8 Density of nudibranch per habitat, patch (p), shell (sh), sand (Sa)

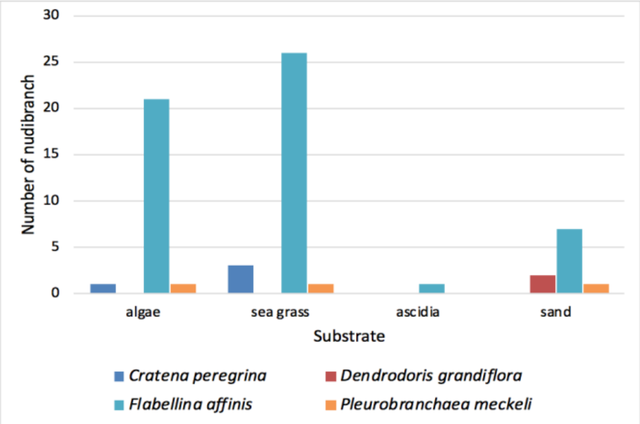


Figure 3.2.9 Distribution of nudibranch by substrate

3.2.6 Activities of the nudibranchs

It is difficult to quantify the activities of nudibranchs. Determining whether a stationary nudibranch is feeding, sleeping, or resting is impossible and can therefore not be classified. To simplify the assessment, we only considered three different activities: 1) static which includes sleeping, resting, holding on to seagrass/algae and feeding; 2) in motion; and 3) reproducing (nudibranchs together with pinkish flower-like eggs close by).

Most nudibranch observed (40 ind.) were immobile, while some were in motion (12 ind.) mostly whilst on sand. Three individuals were observed reproducing or attending eggs (Figure 3.2.10).

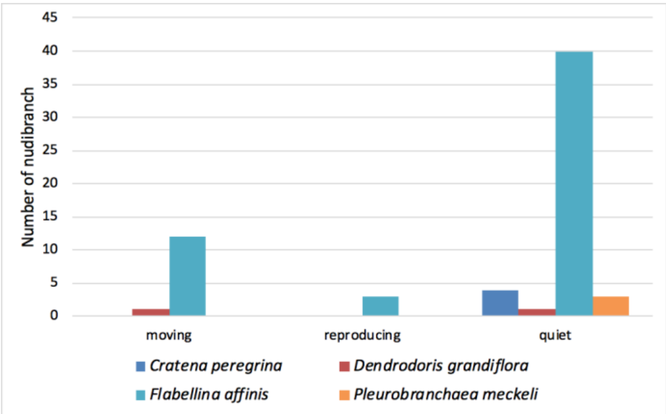


Figure 3.2.10 Activity distribution of nudibranch

3.3 Distribution and abundance of benthos excluding nudibranchs in and around the biodiversity patches

3.3.1 Benthic species identification

The invertebrates found during the quadrat sampling were identified with the help of the pictures and videos taken during the survey. The low quality of the material resulting from the poor visibility made this a challenging task (e.g. Figure 3.3.1)

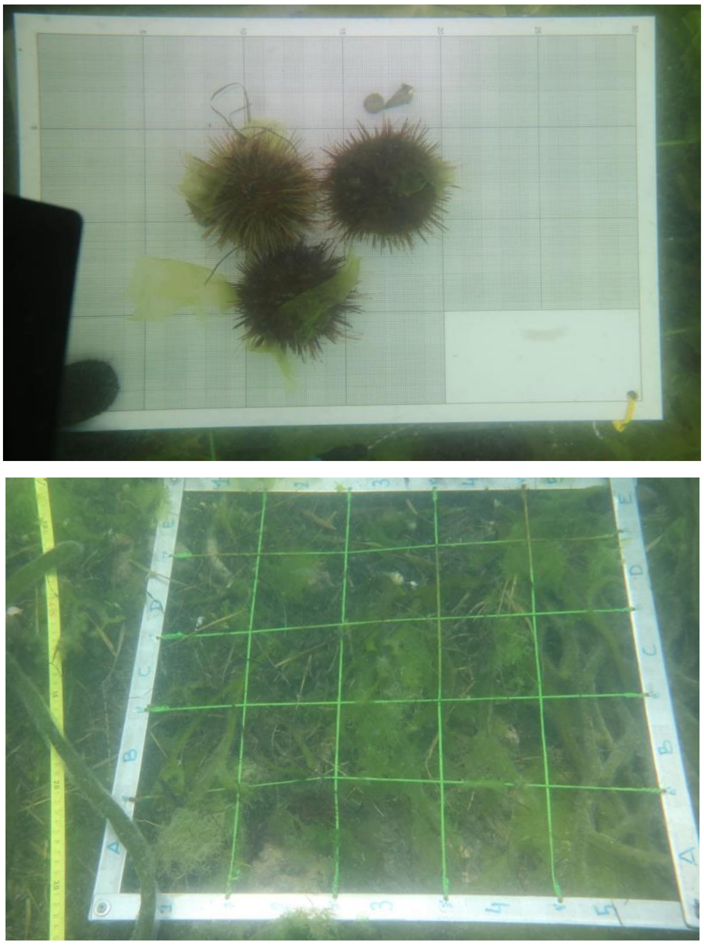


Figure 3.3.1 Taking measurements of the sea urchin and quadrat work (Photo: B. Schmid,2018)

3.3.2 Species of invertebrates found

The dominant species recorded on the patches were green macroalgae such as *Codium fragile, Ulva* sp. and *Caulerpa prolifera*, seagrass (*Zostera marina; Cymodocea nodosa*), brown algae (e.g *Dictyota sp.*), and some encrusting calcareous algae (e.g *Corallina sp.),* echinoderms (*Paracentrotus lividus*), nudibranchs, other mollusks (*Cerithium vulgatum; Gibbula umbilicalis*; *Nassarius* sp.), hermits crabs (e.g *Calcinus tubularis*), spider crabs, cuttlefish, polychaetes, bryozoa, some fish species like *Gobius buchichi*, *Symphodus cinereu*s or scorpion fish and seahorses (*Hippocampus hippocampus*). Also, a great abundance of ascidia has been observed. Other animals encountered during the dives included cuttlefish (*Sepia* sp.), jellyfish, sea stars (*Astropecten irregularis*; *Coscinasterias tenuispina),* ophiuroids (*Ophiura ophiura)*, sea cucumber (*Holothuria mammata)* and a pipefish (*Syngnathoides* sp.).

During the quadrat sampling, a variety of species was observed, such as *Calcinus tubularis* (hermit crab), *Cerithium vulgatum* (gastropod), *Coscinasterias tenuispina* (seastar), *Gibbula umbilicalis* (gastropod), *Nassarius* sp. (gastropod), *Ophiura ophiura* (seastar) and *Paracentrotus lividus* (sea urchin) (annex D). In total, eight invertebrate taxa were identified during quadrat sampling (Table 3.3.1).

Table 3.3.1 Size of the invertebrates encountered

Invertebrate	Average length or diameter (cm)
Calcinus tubularis	2
Cerithium vulgatum	2.5-3
Coscinasterias tenuispina	20
Gastropod	0,5
Gibbula umbilicalis	0.5-1
Nassarius sp.	3
Ophiura ophiura	0,5
Paracentrotus lividus	5-7

3.3.3 Local density of invertebrates

The patches had a varying invertebrate density, ranging from none to over 25 individuals per m^2. Patch 9 had an average (±SD) of 26.4±27.2 individuals/m^2 and held the most invertebrates in numbers but also in species whereas patch 7 did not hold any invertebrates (Figure 3.3.2 and Figure 3.3.3). Patch 5 had an average (±SD) of 6±12 individuals m^2 and only two species of invertebrates. Patch 6 had an average (±SD) of 7±12 individuals/m^2 and three species of invertebrates. Finally, patch 10 had an average (±SD) of 4.2±11.8 individuals/m^{2}and only one species of invertebrates.

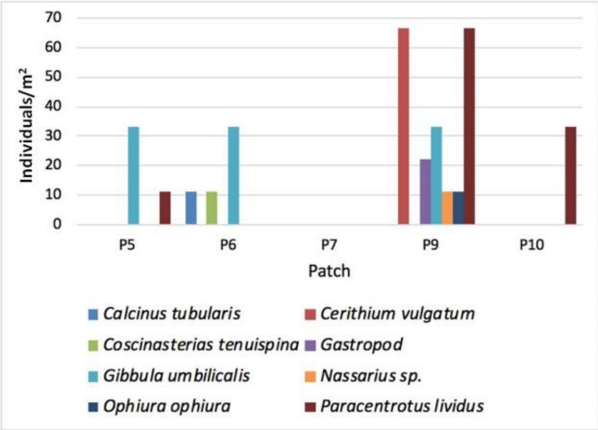


Figure 3.3.2 Density of invertebrate taxa per patch

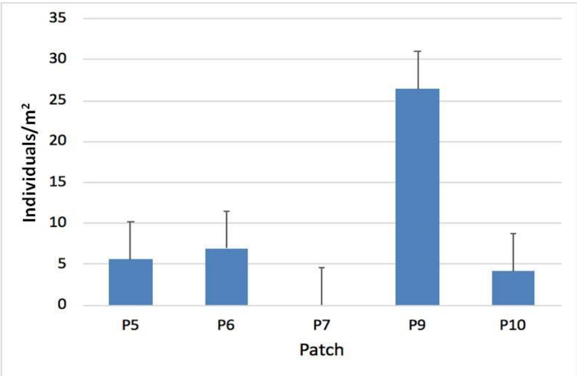


Figure 3.3.3 Density of invertebrates per patch and standard deviation

3.3.4 Habitat of the different species

The invertebrates were found in different habitats such as inside the biodiversity patch (P), inside the limits of the patch and the sand called shell (Sh) and in the sand (Sa) (Figure 3.3.5).

Most species were found inside the biodiversity patch (23±24.7 individuals/m^2) while some were observed in the shell habitat (3±5 individuals/m^2) and only very few individuals were found in the sand (1±2.4 individuals/m^2).

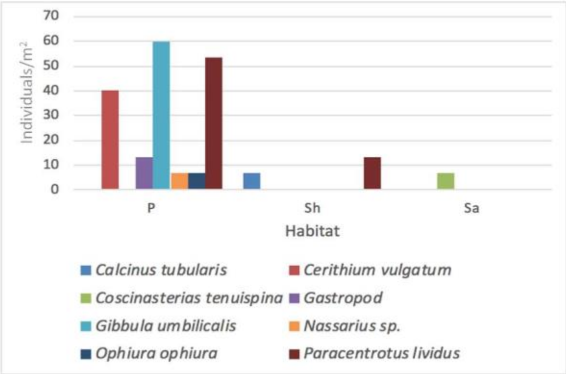


Figure 3.3.4 Density of invertebrates per habitat

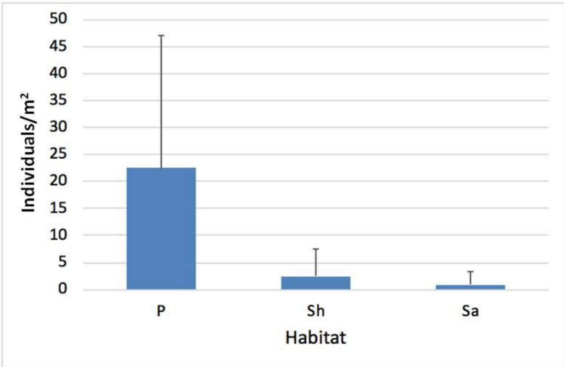


Figure 3.3.5 Density of invertebrates per habitat with standard deviation

Patches 9 and 7 has the maximum and minimum patch densities confirming the earlier observation. The preferred habitat of invertebrates seems to be inside the patch followed by shelly habitat and eventually sandy habitat. Figure 3.3.7 presents the preferred substrate of the invertebrates by species (Figure 3.3.6).

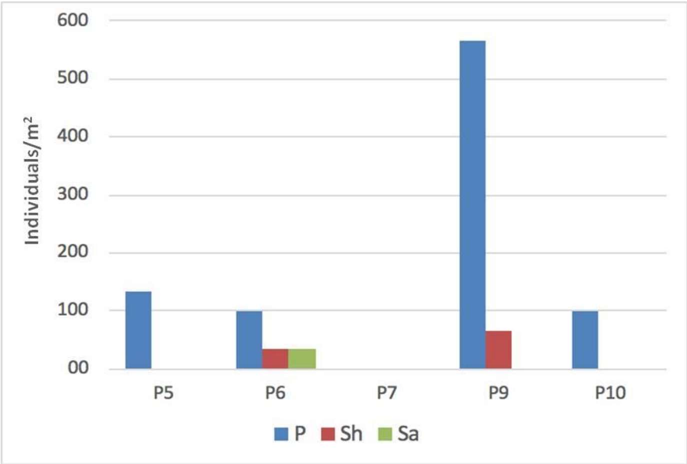


Figure 3.3.6 Density of invertebrates per patch and habitat

Sea urchins prefer to be on the ground in the sand, gastropods seem to mainly hide under the *Ulva* or between sea urchins. Sea stars can be found inside the patch on *Ulva* or outside in the sand (Figure 3.3.7).

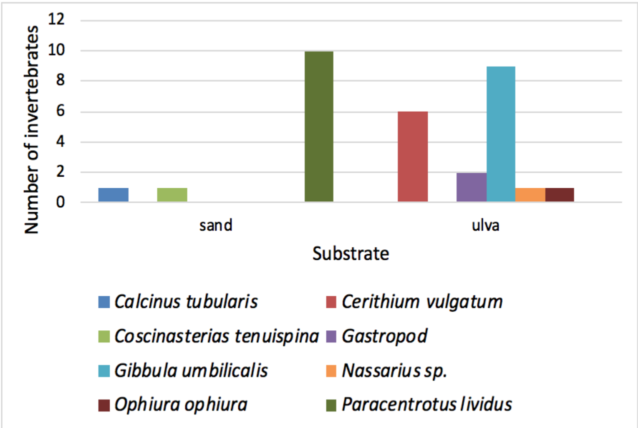


Figure 3.3.7 Distribution of invertebrates by substrate

4. Discussion

4.1 Environmental cartography

Environmental cartography plays a primary role both for basic ecosystem knowledge research and for territorial management purposes (Bartlett, 2000; Villa *et al*., 2002). But such ecological maps are much rarer for marine environments compared to those for land areas, largely due to the operational challenges and costs associated with underwater cartography. As a result, such studies tend to focus on single subjects to reduce the time and effort involved in producing them. The bionomic charts (combining an organism and its relation to its environment) are more complete, offering information of a given area. Benthic communities are the ideal study object for marine cartography due to their structural complexity, stability over time and space, and the ability to record environmental alterations (Bianchi & Zurlini, 1984). Méaille *et al*., (1988) showed that a comparison of bionomic charts produced at different times is a highly effective way of recognising the time evolution and the alterations in the ecosystems of a given region.

The quality of the cartographic data can be enhanced through specialist software which allows coordinates to be added and maps to be generated. However, for this thesis, Excel based radar charts were used as they were deemed to be sufficient for its purposes. The quality of the measurements strongly depends on the diver's accuracy. A methodical step by step approach combined with rigorous checks is necessary to avoid common pitfalls. For instance, choosing the wrong sampling points could result in a low resolution which does not correspond to reality. This risk can be mitigated by comparing the computed results to pictures of the patch. Or if underwater visibility was too poor, a sketch drawn during the dive can also be used for checks.

With the help of additional data points such as the slope of the substrate at each reference point, the map could be turned into a bionomic chart. Combined with the transects and quadrat sampling, this chart would serve as a record of the evolution and alterations in the ecosystems of the region over time (Bianchi *et al.,* 2004). Such an analysis would be of high scientific value as the seafloor and the shape of patches in lagoons such as the Ria Formosa are known to change significantly due to hydrodynamics.

4.2 Nudibranchs biology and ecology

In general, the difficulty of the applied methodology is to properly carry out the transects. Strong currents and low visibility render the task challenging, even more so as nudibranch can sometimes blend in perfectly with their environment. Additionally, individuals smaller than 0.5 cm in length are not identifiable with the naked eye. Six different species of nudibranch were identified with *Flabellina affinis* being the most abundant one.

Flabellina affinis and *Cratena peregrina* were observed together. It is unclear whether these two species can coexist permanently or whether they are only sharing the same food source peacefully as long as there is sufficient food available. *Dendrodoris grandiflora* was only observed in the sand in proximity of a patch sheltering under a shell. This hiding position could be a reason for the low number of individuals observed. They seem to prefer the area around the patch and bury themselves in the sand or hide under shells, making it difficult to spot them. Only one *Doriopsilla areolata* was found between patches; it was half buried in the sand and its yellow color gave it good cover and it was easy to oversee. However, the sample is too limited to offer a clear conclusion on this behavioral preference. *Pleurobranchaea meckeli* is not a nudibranch but belongs to the species of sea slugs. Other surveys have described it as feeding on other sea slugs including nudibranchs. During this survey P. *meckeli* was always observed inside a patch with a higher density of nudibranchs (*Figure 3.2.1*) but the nudibranch feeding thesis could not be observed. The data for *Cratena peregrina* and *Felimida purpurea* is too limited to draw any conclusions.

Although ecological studies have previously been conducted on the benthic macro fauna in the Ria Formosa (e.g Gamito, 2008 which is a compilation of several benthic surveys) it does not include any nudibranchs. However, scientific reports and literature mention the presence of nudibranchs at different spots of the Ria

Formosa (e.g Malaquias and Morenito, 2000). No species occurrence map has been established yet.

Many nudibranchs species previously reported have not been observed during this survey. The most likely explanations for this discrepancy lie in the seasonal variation and micro-locations of the observations. Other authors such as Clark (1975) describe seasonal differences in species in the Northwestern Atlantic as a consequence of temperature change sensitivity. Only very few species were found recurrently during the multi-annual study (Clark, 1975). Those irregular occurrences of nudibranchs can be due to concentration of food species in this region. Another possible factor could be coastal currents. Indeed, the Ria Formosa lagoon has a daily 50-75 % water exchange with the ocean. The incoming water would randomly deposit nudibranch eggs from a diverse range of species, frequently altering the population structure in the lagoon. Especially during the larvae state of the nudibranch (Clark, 1975). Mileikovsky (1960) reported that nudibranch larvae were transported over 500 miles from the hatching site.

The personal hypothesis that tidal variations influence nudibranch density could not be validated during this survey. It should however be noted that this survey is not ideally suited to the evaluation of such behaviour which effectively represents a migration pattern. A more suitable approach would be observing a particular patch on several occasions of neap and spring tide, ideally after having colour-coded the nudibranchs present on the patch. The isolation could be taken further by using a controlled aquarium environment with simulated tidal activity to determine the precise impact of such tidal variations on nudibranch behaviour.

Figure 3.2.8 and Figure 3.2.9 suggest that *Flabellina affinis* and *Cratene perigram* prefer the patch habitat which includes algae and seagrass as favored substrate. Nonetheless some were observed outside the patch in the shelly and sandy habitat and on sand and ascidia as substrate. Observation during the dives suggest that the individuals were caught and transported by the current to locations they would not have chosen themselves.

In the Ria Formosa, the presence of different biota due to both marine mixed and lagoon water, causes the appearance of a high number of species (Malaquias and

Morenito. 2000). Comparing the number of opisthobranches recorded in the Ria Formosa to the number of opisthobranches in other coastal lagoons in the Mediterranean according to Cattaneo-Viette and Chemello (1991), the Ria Formosa may present a high diversity for ophistbranch fauna (Malaquias and Morenito, 2000). During previous observations in the Ria Formosa *Doriosilla areolata* and *Dendrodoris graniflora* were observed on rocks (Malaquias and Morenito 2000). This may explain the rare encounters with these species during this survey as we were observing the sea urchin patches and not rocky bottoms.

Allmont and Sebens work (1988) highlight the immense impact a sea slug population can have on an existing ecological balance. He describes a total change in an ecosystem probably due to the sudden abundant appearance of a new nudibranch species. Lambert and Todd (1994) state that “the larval biology of nudibranch molluscs has been of increasing interest over the past two decades, but experimental data are presently available for too few species to permit generalizations about larval behavior within the order Nudibranchia” which confirms the little knowledge we have about them and how the presence of nudibranch can influence a whole ecosystem. In our case we could not relate ecological changes with the presence or absent of certain nudibranchs.

4.3 Benthic community

The diversity and density of invertebrates were expected to be higher, in-line with previous studies. This is especially true for the density of sea urchins. The density during the survey of 2014 (Fragkopoulou, 2014) was >50-100 individuals/m² (sample was only done one patch) whereas we have a density >25 individuals/m². Additionally, while there are three common species of sea urchins in the Ria Formosa (*Paracentrotus lividus, Sphaerechinus granularis, Echinus acutus*) (Fragkopoulou, 2014) only *Paracentrotus lividus* was observed during this survey. Seasonal changes, micro-location and water exchange are likely to be the main explanatory factors for the divergences. Some patches sheltered a large variety of species whilst others did not. The reason for this phenomenon is unclear but the methodology for data acquisition may have been unsuitable for this setting. The relatively low patch sample size could also have distorted results but the demanding diving conditions would have rendered the gathering of a larger sample highly challenging. It should also be noted that sampling was only carried out on the surface of the sand and not in the sand itself which would have yielded more complete results but would also have damaged the highly sensitive seabed environment.

Despite these shortcomings, the data clearly demonstrates the importance of the biodiversity patches; the highest abundance of species was found inside the patch (Figure 3.3.2 and 3.3.3).

The favored substrate depends on the species; while sea stars and sea urchins tended to be on the ground on top of the sandy/shelly bottom, most mollusk were hiding in the patch under *Ulva* sp., whereas hermit crabs were moving across all substrates.

In study areas such as the Ria Formosa, *P. lividus* can occupy sandy and even muddy substrates as living quarters (Boudouresque, 2001). The individual size of *P. lividus* inside a population remaining in lagunary systems is always smaller than for the ones living in the open sea (Boudouresque, 2001). They seem to be sensitive to long term low and/or high salinities exposure (Boudouresque, 2001). However, in the Ria Formosa, the variations in salinity are not significant enough or long termed to impact the sea urchin population. Numerous authors (Tortonese, 1965; Zavodnik, 1987; Delmas, 1992) suggest an insensitivity towards organic pollution. On the contrary it would even increase their development. Short term as well as long term changes in densities are normal (Boudouresque, 2001). Different parameters can be responsible for those fluctuations such as irregular spawning, larvae death, success or failure of recruitment, migrations, natural and unnatural changes of sea urchin predators, pollution, diseases and harvesting (Ebling *et al.*,1966; Byrne, 1990; Delmas 1992). Sea urchins like to eat macroalgae such as *Codium* sp. and seagrasses as main food source. Nonetheless they seem to be opportunistic generalists able to explore any kind of food resource. *P. lividus* has been observed to cover itself with stones, shells and debris (e.g., Zavodnik, 1987) and this was confirmed for the sea urchin found in the Ria Formosa. This covering behavior may protect the animal from light (Mortensen, 1927, Verling *et al.,* 2002).

Other literature suggest that this behavior is a predation strategy (Mortensen, 1927; Pastor, 1971) or cover-feeding behavior (DeRidder and Lawrence, 1982; Veraque and Nélélec, 1983b) allowing drifting algae and seagrass leaves to be caught and held for consumption. Another theory for this extraordinary behaviour of *P. lividus*, is that it protects their fragile vascular system from occluding with sand (Richner and Milinski, 2000). The authors state that this behavior appears to be a preventive mechanism and did not exclude any of the other reasons for this behavior. It could indeed be a combination of many reasons.

5. Conclusion

None ecological studies has been done up to now in the Ria Formosa. Extensive ecological studies about nudibranchs are relatively rare (Dean et al., 2017)and recent reviews deal with nudibranch chemistry only. They are unspecific to nudibranchs or with a narrow scope of only eight examined species (Dean et al., 2017). Hence further research will be required to improve the understanding of nudibranchs. Recurring surveys across seasons would allow the monitoring of the biodiversity in the lagoon over time, thus highlighting population changes over time. These insights would be particularly valuable when determining human and climate change impact on the fragile lagoon eco-system.

The goals of this survey were accomplished: biodiversity patches were characterised, the nudibranchs richness and diversity were evaluated and the benthic community was studied. Six different species have been identified and *Flabellina affinis* was the most abundant species of nudibranchs in the Armona biodiversity hotspots during the survey. The nudibranchs seem to use the biodiversity patch and the near surrounding as food source and as a shelter. Comparing the benthic community data with other ecological studies is not straightforward. Previous studies found the macro benthic community in the Ria Formosa to be high compared to other lagoon systems and to have a generally good ecological status mainly due to the high water exchange rate (e.g. Gamito et al., 2012). In comparison, the densities of invertebrates identified during this survey are lower than expected from previous studies, however, this location has not been considered in other surveys and it is situated in a physically stressed area (close to the tidal channel, near one inlet entrance.

The results obtained in this study also showed that there is room to improve the methodologies (e.g. scooter survey, quadrats). The diving assessment approach still faces several limitations such as logistics and the adverse environmental conditions. Nevertheless, the survey yielded information which can form the starting point for further field work. The underwater cartography can be used to create bionomic maps and as a management tool for this type of area. Crisscrossing biota information with other methods such as remote satellite sensing techniques to access physical and chemical data would be ideal to obtain a global picture of the environment. In addition, future continuous observations of the biodiversity patches may reveal some interesting relations between the seasons, high and low tide, the hydrodynamics and the grow or regression of the patch and the biodiversity. An annual survey over a number of years could highlight a pattern of species change. Besides the nudibranch themselves, the whole benthic community and the patches with their characteristics (such as size, biodiversity) could be used as bioindicators and as reference for detecting changes in the environment. A biotic index could be established for future studies in order to show the quality of the environment by indicating the types of organisms present in it. The collected data is also contributing towards evolutionary science and medicine and helping against the fighting of pollution and contamination.

As a general conclusion, in spite of being in proximity to the inlet entrance which is associated with strong tidal currents (Freitas et al., 2011), these biodiversity patches are crucial to the environment as they present liveable habitats for a large variety of animals and should be protected and preserved. A more in-depth study of the patches and their uniqueness will help to better understand the ecosystem and how human activity influences the system. The latter could help to minimize the negative effects and reinforce the positive ones.

6. References

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Annexes

Annex A: Materials and safety measurements

Diving equipment: Scuba diving certification card; Identification card; Diving; Hood; Mask; Fins; Gloves; Boots; Lead belt with weights; Wing; Regulators set; Steel tanks 12l air 200 bar; flashlight; Whistle; Knife; Depth gauge; SMB buoy; Wet note.

Campaign material: Shot line; cable and auxiliary/safety buoy; Id tags; Iron sticks; 20m tape measure; Compasses; GoPro; Video HD+Photo camera systems; GPS; Alfa flag; 25l of gasoline. Quadrats;

Safety/Protection: signalizing buoy and nearby an assistance boat that is equipped with a first–aid kit, and oxygen bottle. The skipper needs to be aware of the marine traffic, meteorological conditions, currents, position of the divers and any other factor that can be a danger to the divers.

Emergency equipment: Emergency kit; O2 Kit; Telephone with full battery.

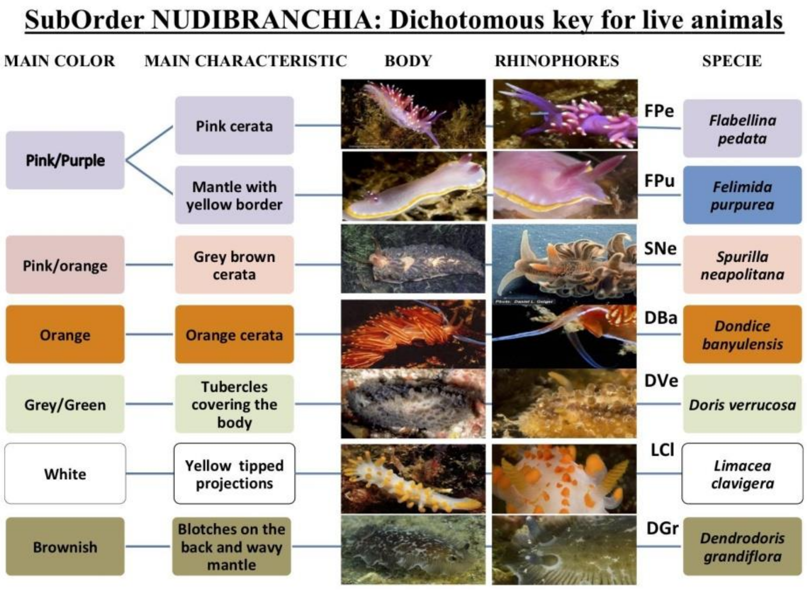
Risk assessment: caution with the currents– shorts slack waters; start and finish the dive on the shot line; organization of a united team (including gas, mental and SPG managements); individual/team/environment awareness (tides, currents, visibility); In case of low visibility flashlights on (less than 4m); personal SMB buoy emergency per diver for surface signalization; beware of sea urchin spines, floatability control might avoid being stung; safety and communication on the surface and underwater between divers; low visibility because of muddy, fine sandy sediment which can cause disorientation, fatigue and stress for the divers which also beget oxygen consumption; danger because the diving site is in a maritime traffic zone.

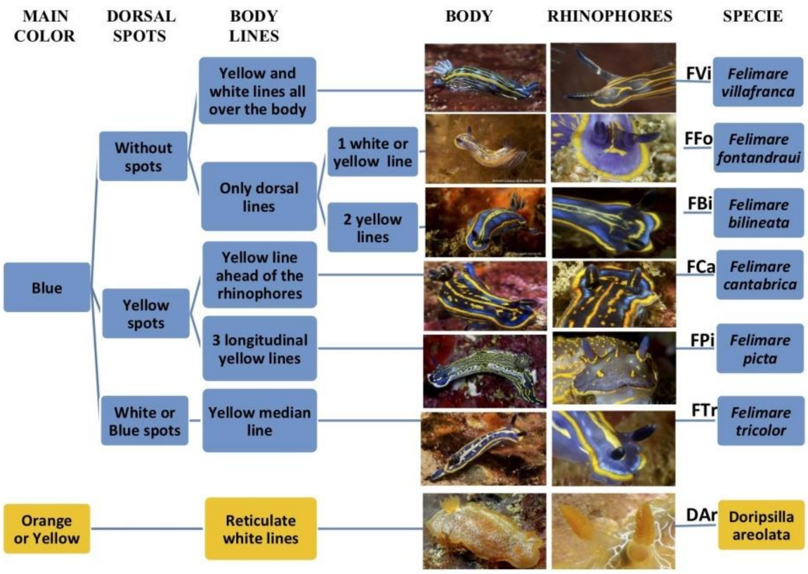
Emergency plan: Emergency phone numbers for illness, accident and intoxication: on board (CODU-MAR): 213303258 or 112; by radiotelegraphy: 500KHz; radiotelephony: 2182 Khz; VHF: 16 channel. On Land: 112.In case of accident call CODU or INEM and keep them online to monitor the clinical situation of the patient. Administer oxygen to the victim; Define a meeting point in land with INEM – Cais de Faro or Cais de Olhão; In case of hyperbaric accident contact Alvor Hyperbaric Chamber – 282420400 or 707282828. In situ procedures for victim's reanimation and evacuation.

Logistics: meeting at university or at Estaleiro Nave Pegos; assembling diving gear and loading the boat; verifying divers' cardiac information; **1h.** Boat trip; Hot spot point + dive preparation + 1st dive briefing scuba team; GPS point. 1st dive debriefing; before the tides changes again 2nd dive **1h.** boat trip back; Arriving to Estaleiro Nave Pegos; **1h.** Washing and packing up the gear. Each diver had to bring its own hot/cold fluids, food and proper clothing.

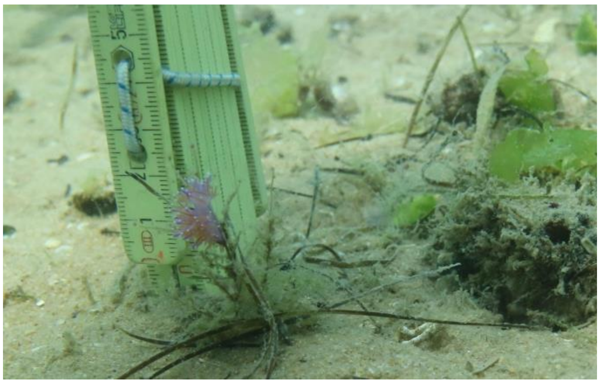
Annex B: Dichotomous key for nudibranchs

Based on Julia Solanas work



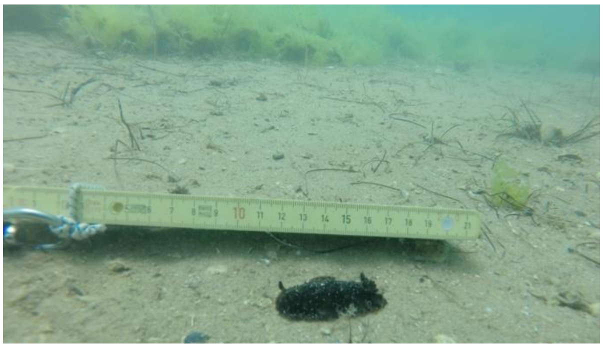


Annex C: Nudibranchs



Flabellina affinis

Cratena peregrina with Flabellina affinis / *Cratena peregrina*

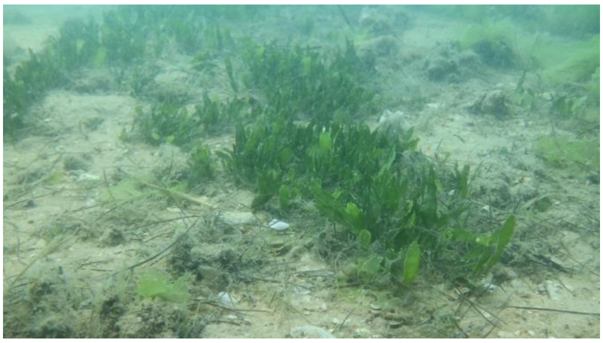


Dendrodoris grandiflora

	
Doriopsilla areolata	*Pleurobranchaea meckeli*

(Photos: B. Schmid, F. Parreira, D. Duart, S. Hoett, 2018)

Annex D: supplementary

	
Codium fragile	*Ulva sp.* and *Paracentrotus lividus*
	
Zostera marina	*Caulerpa prolifera*
	
Hippocampus hippocampus	Ascidia on hermit crab

	
Sepia sp.	*Astropecten irregularis*
	
Holothuria mammata	*Syngnathoides* sp.

(Photos: B. Schmid, F. Parreira, D. Duart, S. Hoett, 2018)