




Marine Biological Excursion

Calvi 2021








Marine Biological Excursion

STARESO Station, Calvi, Corsica

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Participants

Trainers

Bert Hobmayer

Reinhold Hanel

Sandra Bračun

Students

Alexander Vorleuter

Marie-Luise Contala

Matthias Achrainer

Michael Sodja

Moritz Nowakowski

Nicolas Bioly

Pamela Nenning

Veronika Peer

universität innsbruck

C A U Christian-Albrechts-Universität zu Kiel

UNI GRAZ

Contents

Daily reports

Boulder Field 6

Identification of Algae in the Mediterranean Sea 12

Posidonia oceanica - its enormous ecological importance and functional unit of diversity conservation 22

Plankton 32

Fauna of the sandy beach Plage de Revellata/ l'Alga* 36

Coralligène 44

Girolata & Fango 50

Project reports

Sea urchin development

Environmental pollutants and how they interfere with the development of sea urchin embryos (*Arbacia lixula*) 57

Inhibition of sea urchin development by the invasive alga *Chrysopheaum taylorii* 69

Fish surveys

Fish Diversity and Abundance – Transect 81

Fish diversity in dependence of human presence on the north-east coast of Corsica 96

Gut content analysis and age determination of the peacock wrasse (*Symphodus tinca*) and the brown wrasse (*Labrus merula*) 110

Daily reports

Boulder Field

PAMELA NENNING

INTRODUCTION

A rocky shore is an intertidal area that consists of solid rocks and is defined as a coast with no continuous transition from the continent to the sea. Only around 15% of the worldwide coasts consist of rocky shores, whereas in comparison more than 50% of the Mediterranean sea coast does (Hofrichter, 2003). It is a biologically rich environment with high nutrient supply and can include many different habitat types that can either be open rock structures like steep rocky cliffs, platforms or boulder fields or protected rock structures like crevices, caves or rock pools. Further it is influenced by both terrestrial nutrient sources and by coastal aquatic phytoplankton production (Levinton, 2018). So the intertidal zone is alternately a marine and a terrestrial habitat but the predominant fauna consists of marine animals. Therefore the boulder field around STARESO station as a representative area for rocky shores was chosen as a habitat of interest during the excursion.

Because of the constant action of the tides rocky shores are characterized by erosional features. These lead to continuous abrasion of the lower part of the coast, forming a cliff notch. Above this layer an overhang starts to form until it breaks off due to its own weight. The broken material accumulates along the cliff and gets continuously excavated through the wave activity (Hofrichter, 2003). Because of hydrodynamic forces this material can get fragmented and boulder fields arise whereat the big rocks are located near the surface and with direction to the sea the rocks get smaller. This leads to different levels of diversity depending on the size of the rocks. Small boulders contain only early successional communities, large boulders also usually have fewer species, except in the spring, when defoliation of the algal canopy during the previous winter has opened space for colonization. In comparison intermediate-sized boulders contain der highest level of diversity (Sousa, 1979). Further also protected microhabitats like cavities or sheltered rock pools are known to show a high diversity (Le Hir and Hily, 2005). This leads to a vertical zonation which is defined by the occurrence of specific dominant species in distinct horizontal bands or zones on the rocks (Levinton, 2018). The intertidal zone lies between the highest and lowest extent of the tides and can be divided in the following four zones: The Spray zone, the supralittoral zone or high tide zone, the eulittoral zone or mid-tide zone and the sublittoral zone or low tide zone (Schärer, 2007). For the investigations during the excursion mainly the eulittoral zone and the sublittoral zone were observed. The eulittoral zone on the one hand is a turbulent zone and not always covered with water. It is a barnacle-dominated zone either overlapping with a mussel-dominated zone or with mussels below. The sublittoral zone on the other hand is usually covered with water and is dominated variously, but usually by sponges, sea stars, mussels, sea urchins, shrimps, snails and crabs (Schärer, 2007).

But the distribution of the dominant species per zone is not always following the same pattern, it also depends on other factors like wave exposure, stress resistance and nutrient content. For example in quiet-water habitats the mid-intertidal zone is dominated by seaweeds, but with more wave exposure, barnacles and mussels come to dominate, especially at moderate to high nutrient contents. The upper intertidal is dominated by herbivorous snails in all cases with a band of lichens toward the top. In strongly wave-swept habitats, the vertical extent of organisms is expanded (Levinton, 2018).

Material and Methods

To assess typical and abundant species associated with the boulder field habitats around STARESO, the group randomly searched the seafloor in two 1.5 hour snorkeling surveys. On average the boulder fields around STARESO station were around 1 to 8 meters deep. So together with searching the shoreline, swimmers had to apnoe-dive to search the sediments top layer and turn around movable stones to investigate their ground surface, always trying to have the least impact to the environment. The animals were collected by hand or caught using commercially available scoops. Intermediately after catch, they were transported in water-filled plastic bags and later stored in containers filled with sea water. These boxes were placed in the shadow to prevent the individuals from increasing temperature and sun radiation. The collected animals were documented by picture and identified according to the available literature (Riedl, 1983; WoRMS, 2021) using a stereo microscope if necessary. Determination was performed to species level whenever possible. Species diversity per phylum as well as per class was calculated and depicted.

Results

The task for this assessment was to specifically focus on macrobenthic animals easy to recognize and catch. Therefore no fish, algae or very small individuals were collected. Table 1 shows the final list of all species observed on that day with accepted names according to "Fauna und Flora des Mittelmeeres" (Riedl, 1983), and if not included there, according to the World Register of Marine Species (WoRMS, 2021).

Table 1: List of collected species at the boulder fields around STARESO (Revellata bay). Scientific names refer to the World Register of Marine Species (WoRMS, 2021). Names in brackets are currently unaccepted names according to WoRMS but refer to literature used during the course (Riedl, 1983).

Phylum	Class	Species	German trivial name
Cnidaria	Scyphozoa	*Pelagia noctiluca*	Leuchtqualle
Cnidaria	Anthozoa	*Actinia equina*	Pferdeaktinie, Purpurrose
Cnidaria	Anthozoa	*Anemonia sulcata*	Wachsrose
Plathelminthes	Rhabditophora	*Thysanozoon brocchii*	Teppichplattwurm
Mollusca	Polyplacophora	*Acanthochitona fascicularis, (Acanthochitona communis)*	Käferschnecke
Mollusca	Polyplacophora	*Rhyssoplax olivacea, (Chiton olivaceus)*	Käferschnecke
Mollusca	Gastropoda	*Bittium reticulatum*	Nadelschnecke
Mollusca	Gastropoda	*Cerithium vulgatum*	Gemeine Nadelschnecke

Mollusca	Gastropoda	*Clanculus corallinus*	Kreiselschnecke
Phylum	**Class**	**Species**	**German trivial name**
Mollusca	Gastropoda	*Columbella rustica*	Schlichte Täubchenschnecke
Mollusca	Gastropoda	*Conus ventricosus,* *(Conus mediterraneus)*	Mittelmeer-Kegelschnecke
Mollusca	Gastropoda	*Haliotis tuberculata,* *(Haliotis lamellosa)*	Grünes oder Gemeines Seeohr
Mollusca	Gastropoda	*Melarhaphe neritoides,* *(Littorina neritoides)*	Zwergstrandschnecke
Mollusca	Gastropoda	*Luria lurida*	Kaurischnecke
Mollusca	Gastropoda	*Patella caerulea*	gewöhnliche Napfschnecke
Mollusca	Gastropoda	*Phorcus turbinatus,* *(Monodonta turbinata)*	Würfelturbanschnecke
Mollusca	Gastropoda	*Pisania striata*	Kleine Wellhornschnecke
Mollusca	Gastropoda	*Stramonita haemastoma,* *(Thais haemastoma)*	Rotmund-Leistenschnecke
Mollusca	Gastropoda	*Steromphala rarilineata,* *(Gibbula c.f. (rarilineata))*	
Mollusca	Bivalvia	*Barbatia barbata*	Bärtige Archenmuschel
Mollusca	Bivalvia	*Mimachlamys varia* *(Chlamys varia)*	Kammmuschel
Mollusca	Bivalvia	*Pecten jacobaeus*	Mittelmeer-Pilgermuschel
Mollusca	Cephalopoda	*Octopus vulgaris*	Gewöhnlicher Krake
Annelida	Polychaeta	*Eupolymnia nebulosa*	Erdbeerwurm
Annelida	Polychaeta	*Harmothoe extenuata,* *(Lagisca extenuata)*	
Arthropoda	Crustacea	*Calcinus tubularis*	Röhreneinsiedler
Arthropoda	Crustacea	*Maja crispata*	Kleine Seespinne
Arthropoda	Crustacea	*Pachygrapsus marmoratus*	Felsenkrabbe
Arthropoda	Crustacea	*Palaemon elegans*	kleine Felsengarnele
Arthropoda	Crustacea	*Xantho poressa*	Jaguar Rundkrabbe
Echinodermata	Holothuroidea	*Holothuria (Roweothuria) poli*	Weißspitzen-Seegurke
Echinodermata	Holothuroidea	*Holothuria (Platyperona)*	Röhrenholothurie

		sanctori	
Phylum	**Class**	**Species**	**German trivial name**
Echinodermata	Holothuroidea	*Holothuria tubulosa*	Röhrenseegurke
Echinodermata	Echinoidea	*Arbacia lixula*	Schwarzer Seeigel
Echinodermata	Echinoidea	*Paracentrotus lividus*	Steinseeigel
Echinodermata	Echinoidea	*Sphaerechinus granularis*	Violetter Seeigel
Echinodermata	Asteroidea	*Asterina gibbosa*	Fünfeck-Seestern, Kissenseestern
Echinodermata	Asteroidea	*Astropecten spinulosus*	Kletterkammseestern
Echinodermata	Asteroidea	*Echinaster sepositus*	Purpurstern
Echinodermata	Ophiuroidea	*Ophioderma longicaudum*	Brauner oder glatter Schlangenstern
Echinodermata	Ophiuroidea	*Ophiothrix fragilis*	Zerbrechlicher Schlangenstern
Chordata	Ascidiacea	*Ascidia mentula*	Rosa Seescheide

In about 3 hours collection time 42 different species were collected which belong to 14 different classes (Figure 2) and 7 different phyla (Figure 1). Therefore Figure 1 shows the 7 phyla with the number of collected species within the respective phyla. These results show a bias to slow-moving organisms like Mollusca or Echinodermata. To find as many different animals as possible no counting per species was performed, hence the results only show a qualitative observation, not a quantitative.

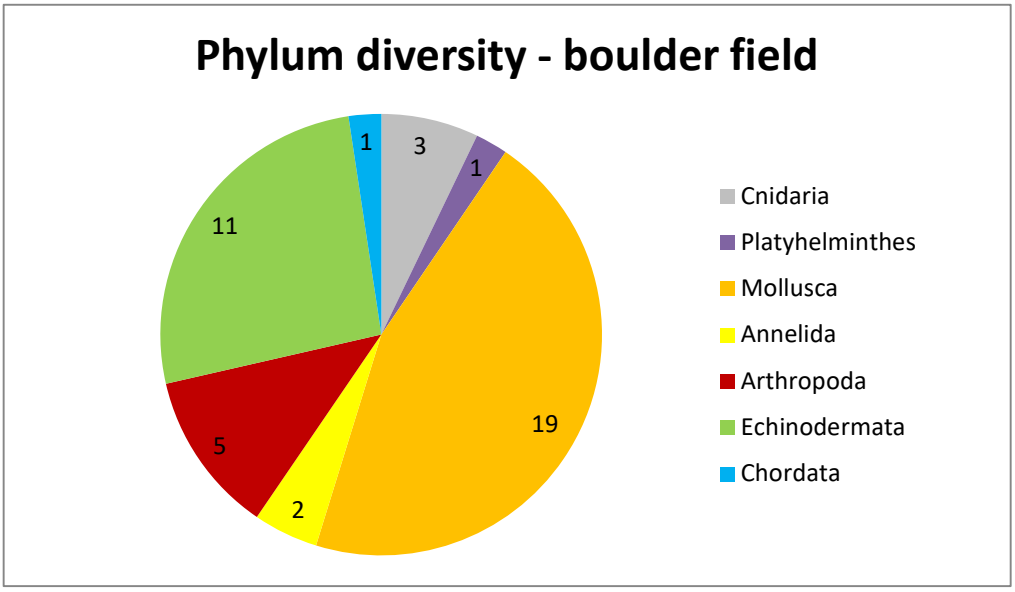

Figure 1: Phylum diversity and distribution in the boulder field around STARESO station at Revellata bay.

Further Figure 2 compares the species richness within these 14 classes using the same color code as shown in Figure 1. Whereas Echinoderms show an even distribution between the four captured classes, the Molluscs show a bias towards Gastropods.

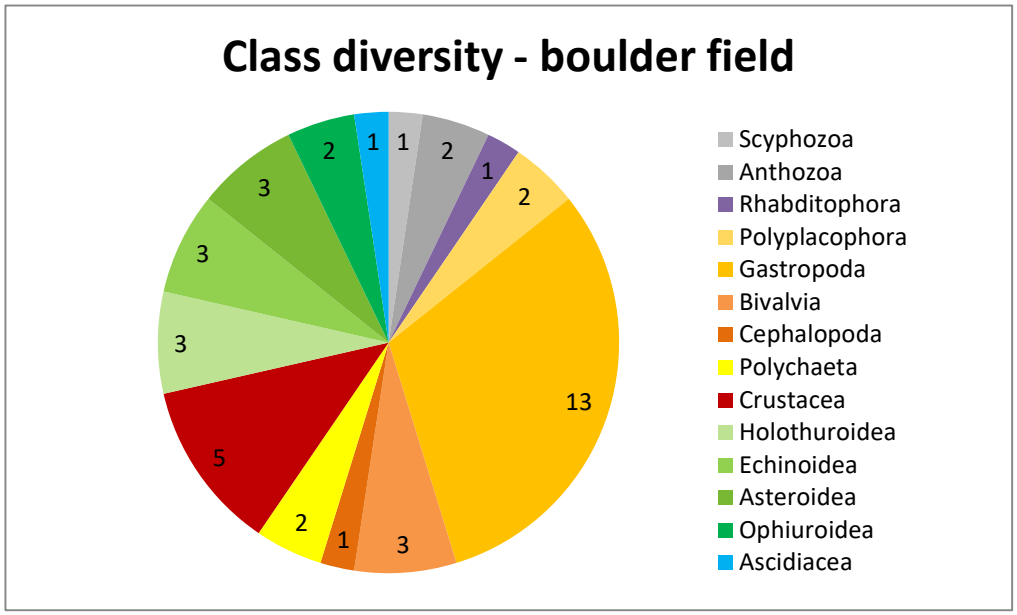

Figure 2: Class diversity and distribution in the boulder field around STARESO station at Revellata bay.

DISCUSSION

Compared to the previous courses of 2016 and 2018 the 2021 assessment showed similar results. Predominantly benthic species were collected, mainly belonging to the phylum Mollusca followed by the Echinodermata and Arthropoda. In general the discovered phyla were largely the same with exception of the Nemertini, which was not found in 2021. Instead members of the phyla Cnidaria and Chordata were added, which were not part of the results in 2018. Overall, the assessed diversity was slightly higher with 42 different species compared to 32 species in 2018.

The Molluscs, and here specifically the Gastropods, represented the phylum with the highest species richness. Due to their high abundance on mid-sized to big rocks (Sousa, 1979) they were easy to recognize and catch for the students. Furthermore gastropod species were even counted and identified, if only empty shells or shells inhabited by hermit crabs were found. However, Gastropods are a diverse group in boulder field habitats, occupying different ecological niches not only as grazers but also as predators with their shells leading to improved resistance against desiccation, extreme temperatures or wave exposure. Since mid-intertidal zone habitats in the Mediterranean are usually dominated by seaweeds or barnacles and mussels, depending on wave exposure, Gastropods find suitable ecological niches either as grazers or predators (Levinton, 2018). A further explanation for the high abundance of Mollusca within the assessed area are unfavorable conditions for their main predators. Sea stars for example can only hunt within the water. This limits their territory to the lower part of the shore, leading to some kind of a refuge area for Molluscs within the eulittoral zone and sublittoral zone.

However the results of this observation should not be considered as a representative assessment of the local species richness. As already mentioned the collection was random and largely dependent on the snorkeling skills of the group members, of which some were just beginners. Hence, very small and/or fast moving species have not been added to the species list (Table 1). Algae and crust-building animals like sponges and Bryozoa were disregarded and will be discussed separately in this report.

LIST OF FIGURES AND TABLES

Figure 1: Phylum diversity and distribution in the boulder field around STARESO station at Revellata bay. 9

Figure 2: Class diversity and distribution in the boulder field around STARESO station at Revellata bay. 10

Table 1: List of collected species at the boulder fields around STARESO (Revellata bay). Scientific names refer to the World Register of Marine Species (WoRMS, 2021). Names in brackets are currently unaccepted names according to WoRMS but refer to literature used during the course (Riedl, 1983). 7

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WoRMS (2021) WoRMS Editorial Board - World Register of Marine Species.

Identification of Algae in the Mediterranean Sea

NICOLAS BIOLY

ABSTRACT

Several algae samples were taken and determined around the STARESO research station in Calvi. The relationship between brown, green and red algae was evaluated with these samples and compared with the results of previous courses. This made it possible to demonstrate that the ratio of brown, green and red algae species in the port of the STARESO is relatively stable. In addition, the distribution of the algae in terms of depth and whether they grew in shadowed or light areas was observed. There was no clear pattern found. However, it could be shown, that a lot of brown and red algae were already growing at a depth of 0-5 meters. Finally, the invasive algae Caulerpa cylindracea and Chrysophaeum taylorii were identified.

Key Words: Algae in Mediterranean Sea, algae composition STARESO, Invasive algae, Caulerpa cylindracea, Chrysophaeum taylorii

INTRODUCTION

Algae species are incredibly rich in shape. There are multicellular as well as unicellular species, that colonize a wide variety of habitats. Their ways of life are just as different as the ecosystems in which they live. For example, some species have a phototrophic, heterotrophic or mixotrophic way of life, but there are also parasitic algae species. Many ecosystems dependent on them, as they provide food and habitat for many different animal species. They are essential for almost all organisms on earth, too, as many types of them photosynthesize and thus generate oxygen (Sahoo and Seckbach, 2015). But what exactly are algae? This is difficult to classify precisely because algae are not a taxon in the biological sense, which is also the reason why there are so many different types of classifications (Sahoo and Seckbach, 2015) (Sengbusch, 2004). The simplest classification is that of macroalgae and microalgae, although initially only the size is classified here (Bux, 2013). Much more precise and also more accepted, however, is the proposed classification by F. E. Fritsch (1935), which also suggests the well-known classification of red algae, brown algae and green algae. Several characteristics, above all the different pigments, were taken into account for this classification (Sahoo and Seckbach, 2015). These pigments allow algae to absorb different wavelengths and so the algae can survive at different depths (Larkum *et al.,* 2020). This is the case for green, brown and red algae, whereas the green algae are more likely to be found in shallow water, followed by the brown algae being more dominant in deeper water. The red algae can survive at greatest depths. In some cases, they can still be found up to around 150 meters (Sengbusch, 2004). However, there are now far more classifications for the different types of algae than this classical one, based e.g. on the nature of the chloroplast membrane (Sahoo and Seckbach, 2015). As a consequence the classification of algae has changed again and again over the past few years (Lewin and Andersen, 2021).

The aim of the study was to investigate, whether a specific green, brown and red algae distribution can already be recognized in depth between 0 to 5 meters. It was also taken into account, which algae predominate in shadow and light areas. This is particularly interesting with regard to the differ-

ent pigments of the groups. It has also been hypothesized, that algae closer to the water surface are more robust to be better able to deal with mechanical stress caused by wave action. In this work it was also examined how strongly the algae biodiversity of green, brown and red algae has developed over the years from the algae found in the Innsbruck marine biology course.

MATERIAL AND METHODS

Sampling took place on July 27th, 2021 over a period of eight hours. The samples were taken from the harbour of the STARESO research station near Calvi, western Mediterranean Sea (see Figure 1), by the help of four snorkelers. During the sampling, the depth the samples were taken and whether the algae grew in light or shadowed areas, were noted (see Table 1). The algae were then identified on land. For this purpose the identification literature Riedel 1983 was used. However, to avoid misclassifications, species assignments to higher taxonomic categories (tribe, class, order and family) was crosschecked with the "Algebase" database (see Table 1) (Guiry, 2021). The present assessment only considers macroalgae from the rocky shores in the port of STARESO (see Figure 1). After the determination, the algae were returned to the sea.

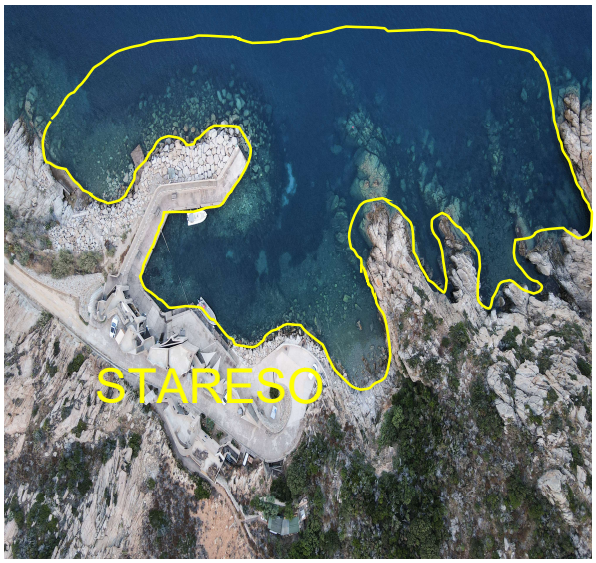

Figure 1: Shows the Stareso research station and the sampling region. See area framed in yellow.

RESULTS

A total of 24 different algae species were identified (see Table 1). The algae found from Table 1 are shown in Figure 5. The numbering can be traced back to Table 1. The algae from Table 1 were determined according to phylum, class order, family and species. Based on this data, it was possible to determine the species richness of green, brown and red algae in relation to one another (see Figure 2). Since this experiment was carried out every two subsequent years from 2006 - with the exception of 2018 to 2021 - the ratio of species found could be observed and represented more precisely over a longer period of time. See Figure 3. This shows that from 2006 to 2010 there was an increase in sampled red algae. However, this increase fell continuously over the years up to 2021. Furthermore, slight fluctuations in green and brown algae can also be seen over the years. Table 1 also shows in which depth regions the algae were found and whether they were found in bright light or shadowed places. This was also evaluated graphically (Figure 4). It becomes apparent, that green algae were preferably located in depths of 3-5 meters, as well as in shadowed areas. In contrast, more brown algae were sampled in light areas. A preferred depth for brown algae could not be determined, because they were relatively evenly distributed over the various depths. Red algae had roughly the same distribution in both light conditions, whereby the red algae that grew in the light preferred a proximity to the surface and the red algae that grew in shadowed areas were more located in deeper regions.

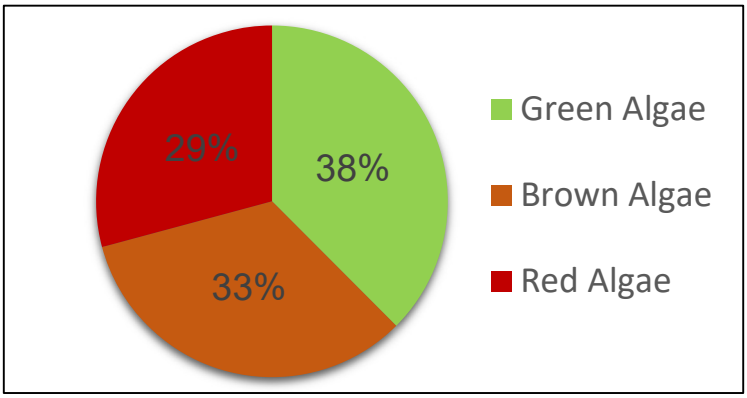

Figure 2: Indicates the ratio of green, brown and red algae which were found. The distribution is given in percent. The data for the chart were taken from Table 1.

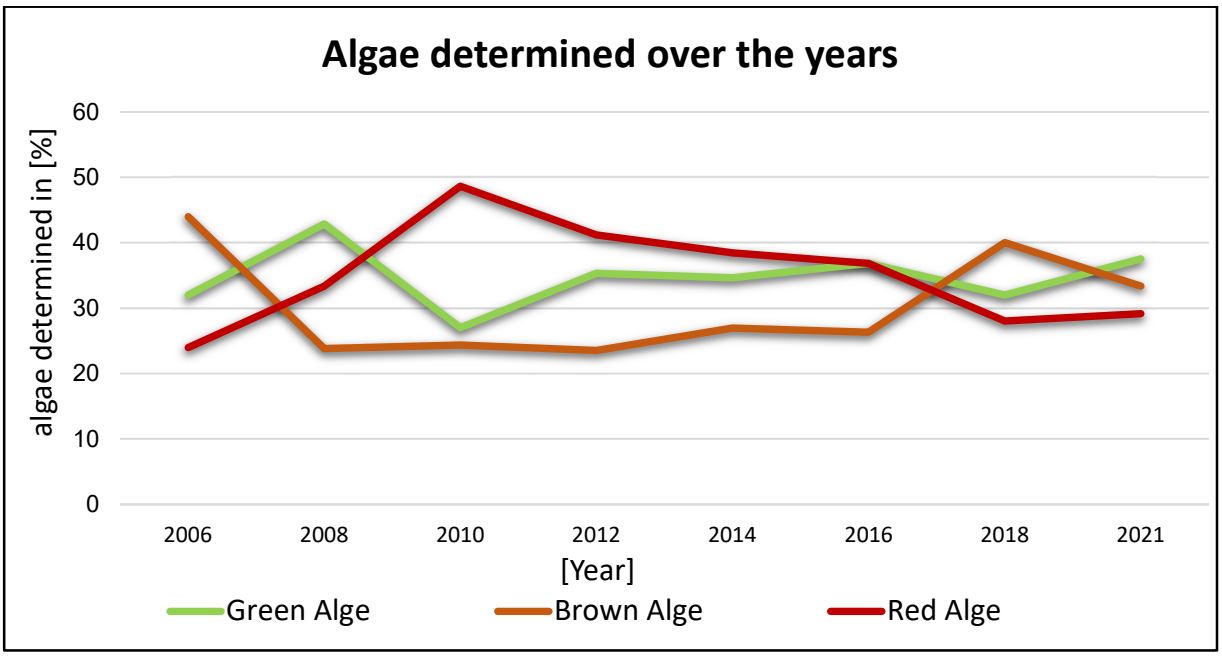

Figure 3: Shows the concentration of the three types of algae (green, brown and red algae) from 2006 to 2021.

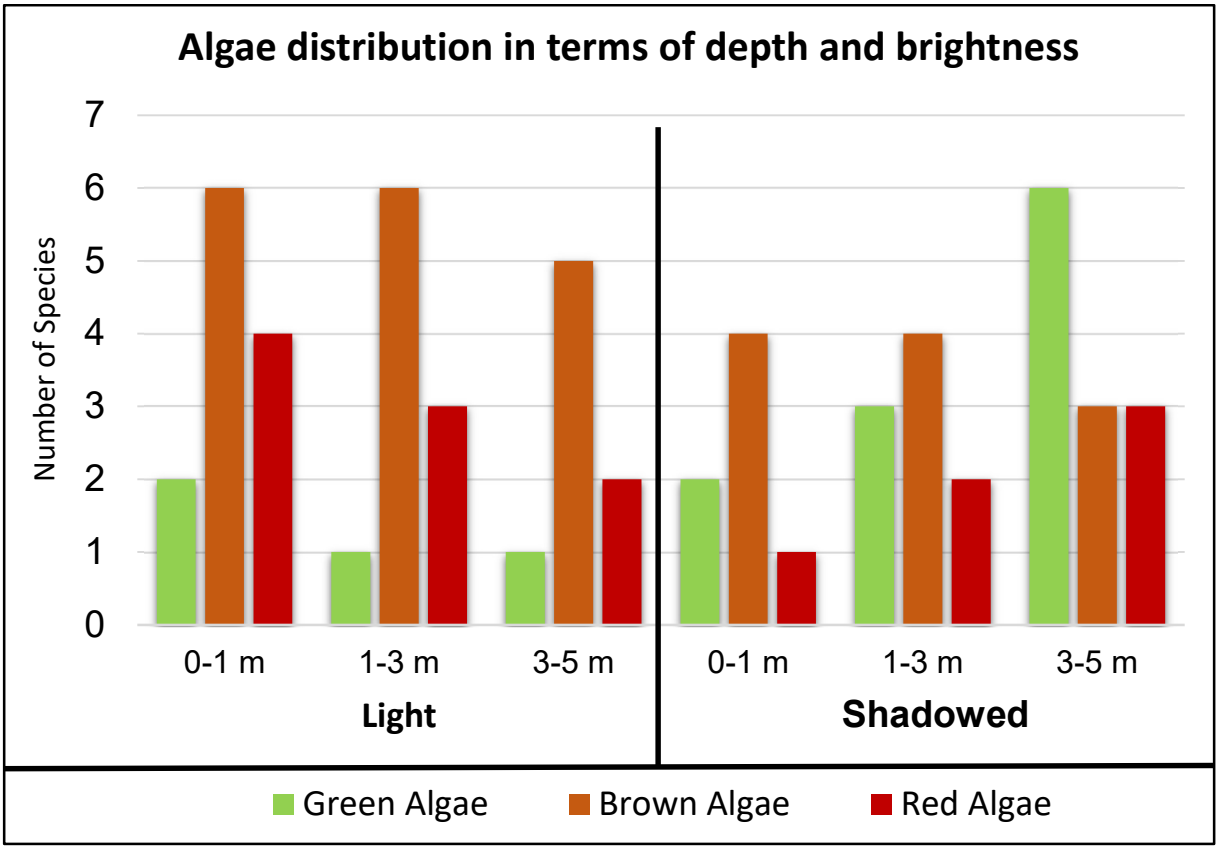

Figure 4: Indicates the number of algae species found at the various dephts. This is indicated for light as well as for shadowed locations. The data fort he chart were taken from Table 1.

Table 1: List of identified algae found at different depths and light conditions

Nr.	Phylum	Class	Order	Family	Species	Light condition	Sea depth		
							0-1 m	1-3 m	3-5 m
1	Chlorophyta	Ulvophyceae	Bryopsidales	Halimedaceae	*Halimeda tuna*	Shadowed			x
2				Udoteaceae	*Udotea petiolata*	Shadowed		x	x
3				Caulerpaceae	*Caulerpa cylindracea*	Shadowed	x		
4				Codiaceae	*Codium adhaerens*	Shadowed			x
5					*Codium bursa*	Shadowed		x	x
6					*Codium effusum*	Shadowed			x
7			Cladophorales	Cladophoraceae	*Cladophora sp.*	Both	x		x
8				Anadyomenaceae	*Anadyomene stellata*	Both		x	
9			Dasycladales	Polyphysaceae	*Acetabularia acetabulum*	Light	x		
10	Ochrophyta	Phaeophyceae	Dictyotales	Dictyotaceae	*Padina Pavonica*	Both	x	x	x
11					*Dictyota linearis*	Both	x	x	x
12					*Dictyopteris membranacea*	Light	x		
13					*Dictyota dichotoma*	Both	x	x	
14			Fucales	Sargassaceae	*Cystoseira Fimbriata*	Both		x	x
15					*Cystoseira barbata*	Both	x		

16			Sphacelariales	Stypocaulaceae	*Halopteris sco-paria*	Light	x	x	x
17			Pelagomonadales	Pelagomonadaceae	*Chrysophaeum taylorii*	Light		x	x
18	Rhodo-phyta	Florideophyceae	Nemaliales	Liagoraceae	*Liagora Viscida*	Both	x	x	
19			Peyssonneliales	Peyssonneliaceae	*Peyssonnelia squamaria*	Both		x	x
20			Halymeniales	Halymeniaceae	*Cryptonemia lomation*	Shadowed			x
21			Corallinales	Lithophyllaceae	*Amphiroa rubra*	Shadowed			x
22				Corallinaceae	*Corallina elon-gata*	Light	x		
23					*Jania adhaerens*	Light	x	x	x
24					*Corallina medi-terranea*	Light	x		

Figure 5: Shows images of the different species from table 1 that were found in the marine biology course. In addition, images from literature sources were shown for non-recorded species. These are provided with references.

DISCUSSION

As shown in Figure 2, green, brown and red algae were present in similar species numbers in the sampling area, with only one more green algae found compared to brown algae species. This suggests, that the three macroalgae groups are relatively evenly present around STARESO. Nevertheless, these findings need to be critically interpreted, since they are based a single assessment by inexperienced divers only. However, by looking more closely at the dataset gathered during the past marine biological excursions of the Universities of Innsbruck and Kiel (Figure 3), it becomes clear, that the species richness ratio the three algal groups didn't differ much over the years, despite some obvious fluctuations, such as a strong increase in red algae in 2010. But this is little surprising, since short-term assessments are often influenced by various factors. In 2010 for example, much more algae were collected than in the other years, which may be caused by an increased sampling effort in deeper zones. This would explain the peak of red algae. Of course, one could also speculate that in the years from 2008-2010 a few more red algae species might have settled in the study area. However, this is rather unlikely, since this peak of red algae subsequently continuously decreased again. So in conclusion, the 3 macroalgae groups seem to be similarily diverse in the port of STARESO. This is particularly interesting given that the three different algal groups for the most part have very different habitat needs. It is known that green algae are usually found in the shallow areas, followed by brown algae in slightly deeper regions and by red algae in even deeper areas. This pattern caused by different photosynthetic pigments and therefore absorbtion optima for different wavelengths (Larkum *et al.,* 2020). Of course, it is questionable, whether such a depth distribution already exists in the study area, reaching from 0-5 meters only. Figure 4 provides an answer to this question. On closer inspection, the first noticeable thing is, that the green algae were increasing at depths of 3-5 meters. They also preferred shadowed areas. In contrast, brown algae preferred bright areas. However, the data show, that they do not have a preferred depth. Furthermore the behavior of the red algae showed no clear trend. Some grew at a depth of 0-1 meters when it was bright and, on the other side, at a depth of 3-5 meters when it was shadowed. Finally, the results show, that at a depth of 0-5 meters no typical distribution of green, brown and red algae is present. This is not too surprising, since all light wavelengths still reach down to a depth of 5 meters. It is therefore not surprising that green algae occur at depths of 3-5 meters. Now, one might think, that the light in this depth and in our study area is not a major influencing factor and that the algae are more dependent on other factors, such as wave action. However, this assumption is refuted by the work of Fields and Hubach 2014. In this study, algal composition and diversity was determined over a range of wave loads. It was revealed that there was no significant influence of the calculated wave activity on the diversity and composition of the algal communities around the STARESO station. The study also shows, that light has a great influence, not on the depth distribution, but on the number of species represented. These two factors could explain why so many brown and red algae species can already be found in a range of 0-5 meters. The reason is, the conditions seem to be very favorable, so that brown and red algae do not have to retreat to deeper areas. A high light intensity would also explain why so many green algae can grow in shadowed areas. Another argument in favor of a good habitat is, that STARESO port is located in a protected area (Katz *et al.,* 2021). As a result, the harbour is not heavily burdened by human disruptive factors. This could be another reason, why so many different algal species were found.

Furthermore, the invasive species *Caulerpa cylindracea* was found in this study, which is a variety of *Caulerpa racemosa*. Together with *Caulerpa taxifolia*, this species poses a massive problem, as it displaces the seagrass *Posidonia oceanica* and spreads massively (Molenaar *et al.,* 2009) (Pierucci *et al.,* 2019). In recent years this species has massively expanded its distribution in the Mediterranean area (Piazzi *et al.,* 2005). It already has been proven that *Caulerpa racemosa* var. *cylindracea* influences the behavior of other organsimes (Vázquez-Luis *et al.,* 2013). In the end, fortunately, this species was not found to be too widespread in the study area. Nevertheless, the spread of this invasive species should be closely monitored over the next few years at the Stareso station and other locations in the Mediterranean sea, as it could have catastrophic consequences for the native fauna.

Much more problematic at the moment is the algae, that appeared in the port around 2016. The alga that was found at that time is yellowish and produces large amounts of slime (see Figure 5. Nr.17). This year this species was identified as *Chrysophaeum taylorii*. This microalgae, which prefers to settle on hard substrate, is now quite common in the studied area. Even so far, that it is now one of the five main algae species, that can be found in the harbour of the Stareso station (To get a closer look at the distribution of this type of algae in the studied area see chapter: Line transect: Results, Discussion). This is particularly problematic, because it has already been proven that the mucus has a strong influence on various marine communities (Caronni *et al.,* 2015). This influence could also be observed in this work on sea urchin development. (See chapter: Influence of algae treatment on sea urchin development: Results, Discussion) Ultimately, the spread of *Chrysophaeum taylorii* should be closely observed over the next few years. Further studies must be carried out on the influence of this species on the marine ecosystem in order to be able to assess any consequences and to prevent negative effects for the ecosystem.

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Posidonia oceanica - its enormous ecological importance and functional unit of diversity conservation

MARIE-LUISE CONTALA

INTRODUCTION

Seagrass, the only marine angiosperm growing completely submerged is a monocotyledonous plant and has in contrast to algae fruits, blossoms, and roots. Seagrass species appear monotypic, which means that seagrass meadows consist of one species. In the Mediterranean, this would be predominantly the type of *Posidonia oceanica* which belongs to the family Posidoniaceae. This species is an endemic species within the Mediterranean Sea and can be found mainly on sedimentary substrate (e.g., coarse sand) in the depth between 3-40 meters (max. 50m; Luminy, 2006).

Posidonia oceanica is a photosynthetic-autotrophic plant exchanging oxygen and carbon dioxide throughout the plant leaves – vegetating by "Conveyor-belt growth"(Hook *et al.*, 1988). Based on this growth form, the leaves of this seagrass take on a wide variety of maturation stages (from bottom to top). Roots which are linked by rhizomes are important for stabilization with the sediment as well as nutrient transfer of the plant and can form extended root systems, especially within large seagrass meadows. These functional parts (maturing leaves and roots) of seagrass meadows form a special habitat for a wide variety of sessile and vagile species.

In this student course we investigate the species diversity within seagrass (meadows) and also include the importance of seagrass within the marine ecological system.

MATERIAL AND METHODS

To investigate on species diversity of *Posidonia oceanica*, a few seagrass pieces were collected from an edge-growing meadow section (5-6m depth) within the harbor of STARESO station (Calvi, Corsica; Figure 1). This collection was carried out by snorkelers cutting the seagrass at the lowest possible point of the plant (around the roots) using a diving knife. The plants were collected into a plastic bag which was carefully put over before cutting (Figure 2). Also, the collection of the surrounding water within the plastic bag was important to catch as much as possible of the organisms inhabiting the seagrass meadows. Right after, the plastic bag was closed under water to ensure no loss of the sampling while surfacing. For this course we were sampling only one time at one location of the seagrass meadow to keep the invasive intervention as small as possible, and thereby to not strain this protected species unnecessarily. This sampling was previously agreed with the STATION, as they also have a permission of collecting this species for research purposes.

Back onshore the samples were placed into buckets and later brought to the laboratory, where we searched for vagile and sessile organisms on five different sections of the plant. These sections were classified based on the growth of the plant and thus by the maturation of the leaves: young leave-basal segment (YI), young leave-apical segment (YII), old leave- basal segment (OI), and old leave-

apical segment (OII; Figure 3: left side). The classification was thereby based on Bračun et al. (2020) which is demonstrated in Figure S1. Additionally, the rhizome (R) was analyzed as the 5th part of the plant. The plant parts as well as the collected surrounding water were then searched for organisms using a microscope. The species of the surrounding water were assigned to the plant parts old leave-basal segment (OI) and old leave-apical segment (OII), because many of these species can be assigned to these plant parts. These were most likely removed by shaking out and washing off the old leaves, which from experience are richly colonized.

Detected sessile or vagile species were taxonomically classified on phylum, class and species level using the determination key of Riedl (1983). If possible the different species were photographed through the oculars of the microscope (Figure 3: right side) and cross-checked with the sources of Flanders Marine Institute (2021).

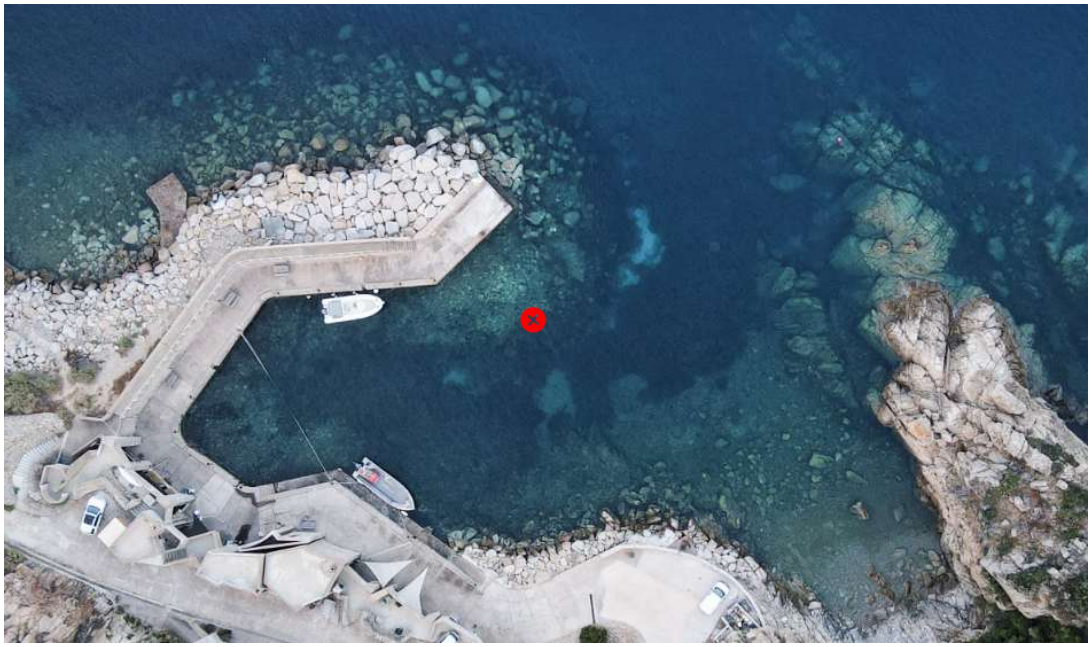

Figure 1: Sampling location of Posidonia oceanica plants within the harbor of STARESO station (Calvi, Corsica).

Figure 2: Documentation of the sampling of Posidonia oceanica by snorkelers using the plastic bag to collect the cut plants.

RESULTS

Within the five plant subdivisions of *Posidonia oceanica* (based on Figure 3), a species list was created, which represents all the different species of each location. The number of same species found was not considered. These species list includes in total 40 different species, whereby 23 of them are sessile species and 17 species live vagile (Figure 4, Table 1). Thereby the rhizome includes the highest number of vagile species whereby the old leaves include the most sessile species in comparison between the three general plant sections (young leave, old leave and rhizome; Figure 4). In comparison to the student courses held the years before similar number of species could be found whereby no distinction was made here between sessile and vagile species (2014: 45 species; 2016: 35 species; 2018: 29 species).

With more exact determination of the different species and their systematic affiliation we could count altogether nine phyla (Table 1). A large share of species had mainly the classification of bryozoans and arthropods followed by annelids (Figure 5: bottom graph). The distribution of the phyla within the three main sections of the plant looks different to the whole plant. Here, young leaves mainly include the phylum of sessile living bryozoans (45%; Figure 5: top graph) while the phyla of Cnidaria, Echinodermata and Annelida are missing. Bryozoans are also dominating old leaves with 31% whereas retarians include 23% of the species (Figure 5: left circle diagram).

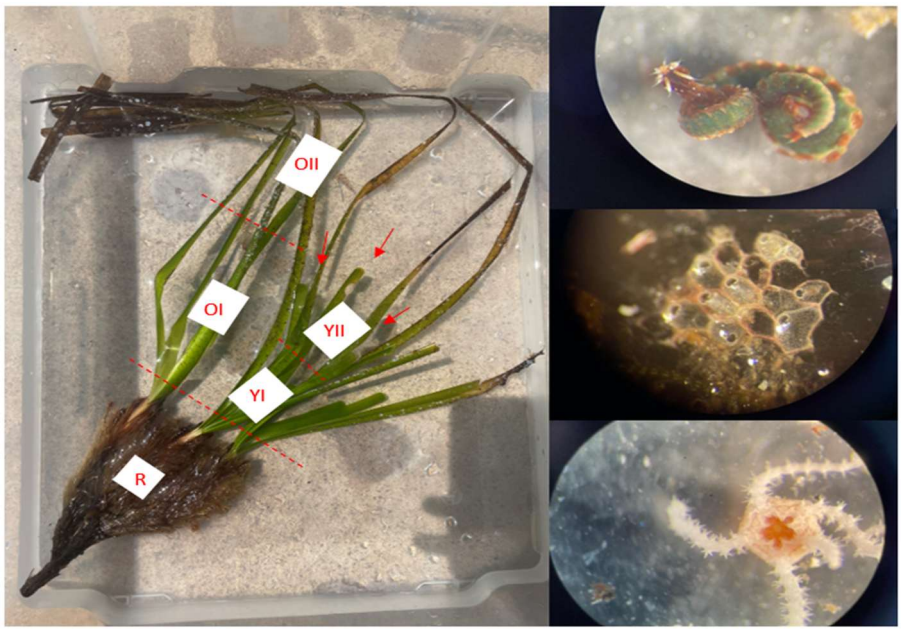

Figure 3: Classification of the five sections (YI, YII, OI, OI and R) and description of Posidonia oceanica (left side of figure) and exemplary photographs of Eulalia cf. macrocerus , Schizobrachiella cf. sanguinea and Ophiopsila aranea (right side of the figure; top to bottom) through the oculars of the microscope.

Table 2: Species list (indicated with stem, class, and family and if available German synonym based on Flanders Marine Institute, 2021) subdivided into vagile and sessile species based on the subdivision of the plant and thus the exact localization at Posidonia oceanica.

sessil	vagil	Phylum	Class	Family	*Species*	German
Old leaf						
• ***Apical & basal segment (OII & OI)***						
x		Cnidaria	Hydrozoa	Anthoathecata		
x		Cnidaria	Hydrozoa	Bougainvilliidae	*Bimeria vestita*	
x		Bryozoa	Stenolaemata	Lichenophoridae	*Lichenopora sp.*	
x		Retaria	Foraminifera			„Kammerlinge“
	x	Arthropoda	Malacostraca	Gnathiidae		
x		Bryozoa	Gymnolaemata	Electridae	*Electra posidoniae*	
x		Bryozoa	Cheilostomata	Phidoloporidae	*Sertella beaniana*	„Neptunschleier“
	x	Mollusca	Gastropoda	Discodoridiidae	*Baptodoris cf. cinnabarina*	„Sternschnecke“
	x	Echinodermata	Ophiuroidea	Ophiopsilidae	*Ophiopsila aranea*	„Schlangenstern“
x		Retaria	Foraminifera	Rosalinidae	*Tretomphaloides cf. coucinnus*	„Kammerlinge“
	x	Arthropoda	Crustacea	Malacostraca	*Phtisica nativa*	„Gespensterkrebs“
x		Retaria	Foraminifera	Peneroplidae	*Peneroplis planatus*	
x		Bryozoa	Cheilostomata	Beaniidae	*Beania sp.*	
Young leaf						
• ***Apical segment (YII)***						
	x	Nematoda				
x		Bryozoa	Cheilostomata	Aeteidae	*Aetea truncata*	
	x	Arthropoda	Crustacea			
x		Bryozoa	Cyclostomata	Tubuliporidae	*Tubulipora flabellaris*	
x		Bryozoa	Cheilostomata	Aeteidea	*Aetea sp.*	
x		Bryozoa	Cyclostomata	Tubuliporidae	*Tubulipora sp.*	
x		Mollusca	Polyplacophora	Leptochitonidae	*Chiton cf. phaselinus*	
• ***Basal segment (YI)***						
x		Retaria	Foraminifera	Rosalinidae	*Tretomphaloides cancinna*	„Kammerlinge“
x		Rhodophyta	Florideophyceae	Corallinaceae	*Fosiella sp.*	„Kalkrotalge“
Rhizome						

x		Annelida	Polychaeta			
x		Bryozoa	Gymnolaemata	Schizoporellidae	*Schizobrachiella cf. sanguinea*	
x		Annelida	Polycheata			
x		Rhodophyta	Florideophyceae	Corallinaceae	*Fosiella sp.*	„Kalkrotalge“
x		Bryozoa				
x		Annelida	Polychaeta	Serpulidae	*Janua heterostropha*	
x		Rhodophyta	Florideophyceae	Ceramiaceae	*Ceramium sp.*	
	x	Annelida	Polychaeta	Polynoidae	*Lepidonotus clava*	
	x	Nematoida	Nematoda			
	x	Annelida	Polychaeta	Phyllodocidae	*Eulalia cf. macrocerus*	[„Drachenpolychet“]
	x	Arthropoda	Malacostraca	Idoteidae		
	x	Annelida	Polychaeta	Serpulidae		
	x	Arthropoda	Malacostraca	Leucothoidae	*Leucothoe spinicarpa*	
	x	Mollusca	Gastropoda	Cerithiidae	*Bittium reticulatum*	
	x	Arthropoda	Malacostraca	Inachidae	*Achaecus cranchii*	„Seespinnenartige“
	x	Echinodermata	Ophiuroidea	Amphiuridae	*Amphipholis squamata*	„Schuppiger Schlangenstern“
	x	Arthropoda	Malacostraca	Lysianassidae		
	x	Arthropoda	Malacostraca	Leucosiidae		
MORE species seen by snorkeling						
	x	Chordata	Actinopterygii	Scorpaenidae	*Scorpaena porcus*	„brauner Drachenkopf“
	x	Chordata	Actinopterygii	Sparidae	*Sarpa salpa*	„Goldstrieme“
	x	Chordata	Actinopterygii	Labridae	*Centrolabrus melanocercus*	„Putzerlippfisch“
x		Cnidaria	Anthozoa	Caryophyliidae	*Cladocera caespitosa*	„Rasenkoralle“
	x	Chordata	Actinopterygii	Muraenidae	*Murena helena*	„Mittelmeermoräne“

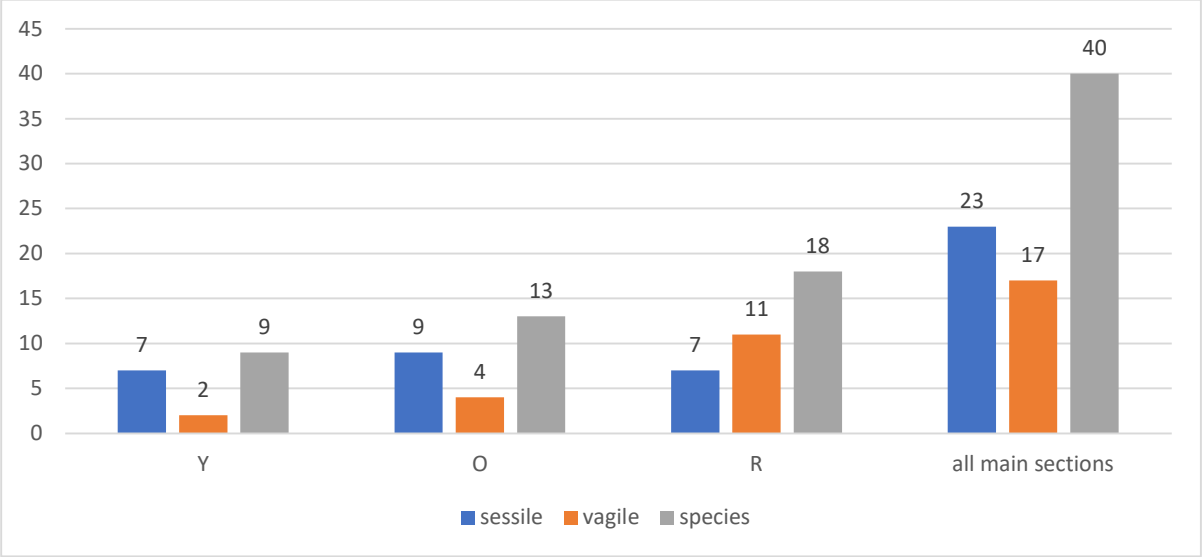

Figure 4: Number of living species on Posidonia oceanica for all three main plant sections (Y = young leaves, O = old leaves, R = Rhizome) as well as the summary of these sections- also including the two different life forms: sessile and vagile.

Within this distribution the phyla Nematoida, Rhodophyta und Annelida are missing. Annelids and arthropods are dominating the habitat of the *Posidonia oceanica* rhizome with 33% and 28 % (Figure 5: right circle diagram).Only two Phyla, Retaria and Cnidaria, are not represented .

In comparison to previous held student courses on Corsica, the main part of phyla were arthropods and mollusk's (course report 2014, 2016 and 2018). Within this course also vagile living arthropods were frequently found whereby more sessile living bryozoans were described (Table 1 & Figure 5).

DISCUSSION

In this year's course we looked for sessile and vagile living organisms on *Posidonia oceanica*, to get an impression of the diversity of species within this habitat. Sessile living bryozoans and vagile living arthropods as well as annelids were dominating the species list within this year, relatively similar to the years before (Figure 5). Therefore, sessile living organisms had a higher proportion as vagile species, mostly found on the old *Posidonia* leaves, whereby vagile species were mostly found at rhizome structures (Figure 4, Table 1).

Within this excursion, the focus was mainly on the rhizome, which could explain the high number of vagile species – also in comparison to previous courses. In addition, vagile species are mobile, but because of this as well as including their size, they are usually easier to recognize which also explains the richness within the species list. This is also true for annelids, which are the next most common group of vagile animals in our species list (Figure 5).

Seagrass in this perspective is an important habitat for vagile – especially for small invertebrates like crustaceans, small fish, and juvenile larger fish – and *Posidonia oceanica* is also known as "nursery

habitat" (Cheminée *et al.*, 2021). Sessile living organisms like bryozoans, invertebrates, bacteria as well as Rhodophyta (Table 1) can be found as epiphytes or directly on the living seagrass. For this purpose, all five plants section are suitable, because they are a stable substrate for plants and animals.

The accumulation of smaller organisms amongst and on the seagrass blades as well as the seagrass as a plant, attracts bigger animals whereby some of them are permanent resident (like "more species seen by snorkeling" in Table 1) while others are temporary visitors (Kalogirou *et al.*, 2010). The life form or type of inhabitants may change over time as the plant changes over time due to by "Conveyor-belt growth"(Hook *et al.*, 1988) – rejuvenating the leaves from the bottom to the top. This leads to an ever-increasing algae colonization overgrowing sessile species due to favorable photosynthetic conditions based on good light conditions at the top of the plant. The high algae overgrowth results in dead seagrass leaves as light can no longer reach the leaves of the seagrass for photosynthesis. All these changes within *Posidonia oceanica* as a habitat is leading to changes within species composition as well as on the foundation of coastal food webs.

Concerning future studies, we would recommend better zoning of *Posidonia oceanica* and compliance with zoning to understand better the differences of the specification of each habitat-zone in which species live in. However, due to the schedule, there was only half a day, so it was finally a little more generalized than originally thought (results only for the three general zones instead of the five zones). A clear classification of species within the complexity of the seagrass habitat as well as the ecological importance could be better treated within a whole day.

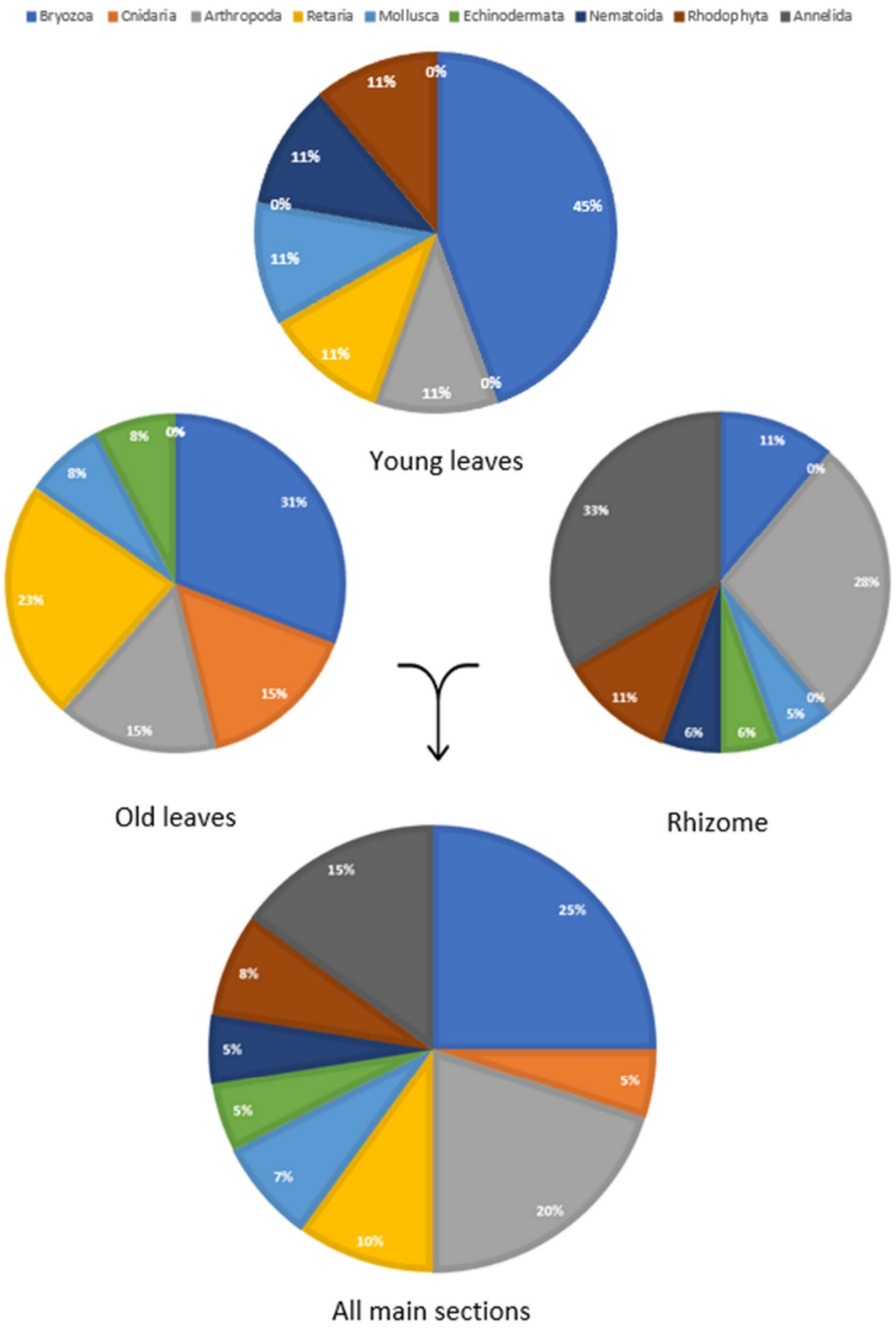

Figure 5: Phylum distribution of the three main plant sections (a subdivision into all 5 sections is not useful here) as well as the distribution taken all species of all sections together.

Another point that could be taken up further in the next course are the threads and conservation. Posidonia oceanica is showing a decline in the last decades which is mainly due to human reckless behavior. Therefore, we act as a marine stress factor by trawling, anchoring and shift the ecological function of the sea by eutrophication (Boudouresque et al., 2006; Boudouresque et al., 2012). However, restauration initiative (transplantation of seagrass and it's seedings) has excelled cutting of the fragmentation of this valuable habitat whereby some recovery is already being observed (Boudouresque et al., 2012, 2021; Paulo et al., 2019). Additionally, dead leaves of Posidonia oceanica are naturally used as protection wall on the coasts and is thereby important to fight against erosion. Because of this, our qualitative monitoring of Posidonia- based community species richness as well as future course investigations will point out the shift within species list as well as the local change of the habitat with all its subsequent consequences.

SUPPLEMENTARY

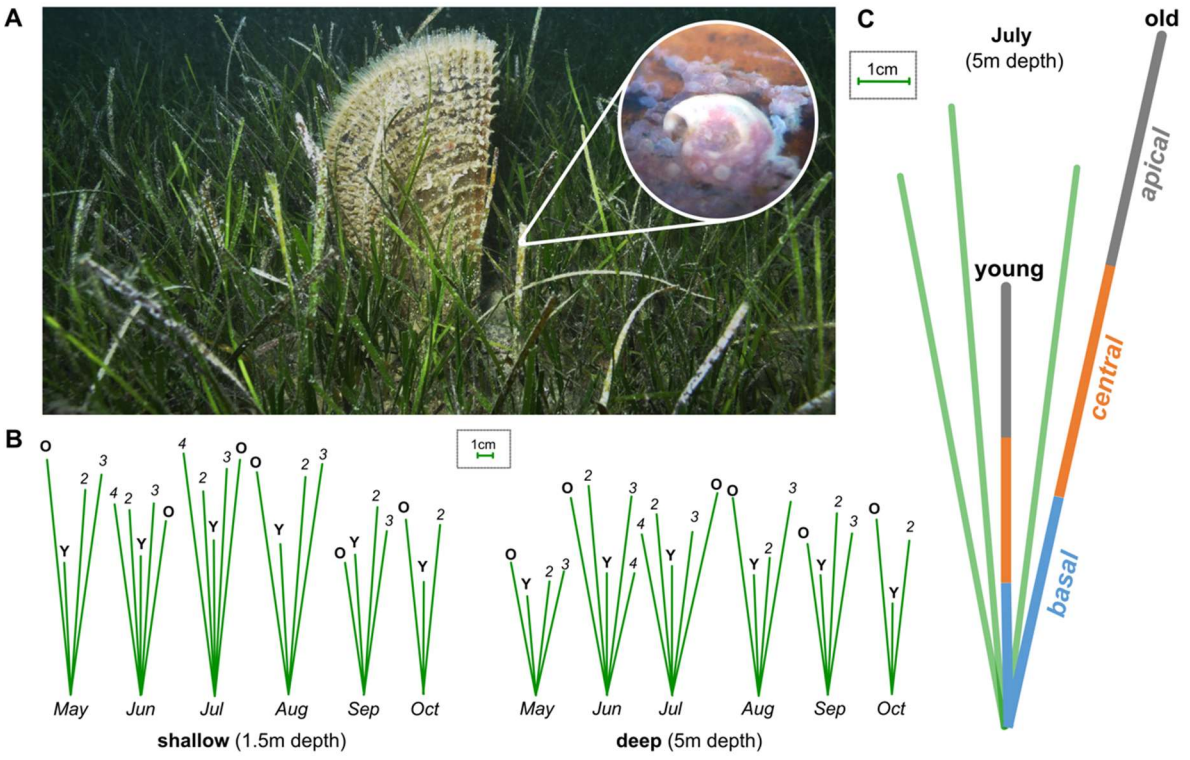

Figure S3: Classification of seagrass leaves of Bračun et al. (2020).

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Plankton

MATTHIAS ACHRAINER

INTRODUCTION

Plankton is defined as organisms floating with the current, but not actively swimming against the current (in opposite to the nekton). Plankton is the fundamental basis of the marine ecosystem and plays a key-role as primary nutrient source. Between 4000-9000 species belong to the plankton in the Mediterranean sea , from viruses to fishes, from small cyanobacteria to big cnidarians.

Plankton produces between 50-70% of the oxygen on our planet, and is thus a key factor for more or less all ecosystems in the ocean. Further, with 98%, plankton represents by far the largest part of the biomass in the ocean. In the group of plankton, diatoms represent with around 50% of the whole biomass the biggest subgroup. Diatoms can also be used to estimate the nutrient level of an environment, more diatoms normally indicate a higher nutrient level (known as tropic diatom index). Crustacea seems to be the most diverse group in the plankton and are also often the dominant species in the system.

As plankton is very diverse, it is difficult to classify it. Different methods where used to distinguish different plankton species, like size, lifecycle (holo- vs. meroplankton) or function (phyto- vs. zooplankton). The distribution and density of plankton follows in general the big ocean currents like the golf stream. Newer approaches and technologies try to use neuronal networks and deep learning to classify plankton (Lumini und Nanni 2019).

Like all other processes, climate change also influences the density and composition of plankton. In the year 2013, Beaugrand et al. reported an altered composition in spots with increased temperature. While the number of dinoflagellates decreased, the number of diatoms increased. The consequences are not clear yet.

In contrast, plankton parts can also act as invasive species and influence the local ecosystem and economy. *Mnemiopsis leidyi,* native in north and south America was found in the black and Caspian sea. Because of the higher temperatures and a lack of natural enemies, the Ctenophore population increased and lead to a 90% drop of the anchovy population.

MATERIAL AND METHODS

Sampling was done in front of STARESO station. We used a 4-5m long ring net with a mesh size of 500µm. We drove with the ship around 1km in front of the STARESO station, let the net sink till around 20m and decreased the speed of the boat to around 2-3km/h. After 20min (we sampled from 10:10-10:30), we dragged up the net and put it in a container filled with seawater. In the lab the seawater containing the net (and with that the plankton) were transferred into Petri dishes and analysed by binocular or the microscope.

RESULTS

Table 3: List of all determined plankton species

Phylum	Class	Order	Family	Species
Cnidaria	Hydrozoa			Hydroidomedusa sp.
Cnidaria	Hydrozoa	Siphonophorae	Diphyidae	Chelophyes appendiculata
Cnidaria	Hydrozoa	Siphonophorae	Diphyidae	Diphyidae sp.
Cnidaria	Hydrozoa	Siphonophorae	Diphyidae	Muggiaea sp. (juvenile)
Cnidaria	Hydrozoa	Anthomedusae		Anthomedusae sp.
Arthropoda	Malacostraca	Decapoda	Upogebiidae	Upogebia sp
Arthropoda	Malacostraca	Decapoda		Decapoda sp
Arthropoda	Malacostraca	Decapoda	Portunidae	Portunidae sp
Arthropoda	Malacostraca	Decapoda		Decapoda sp (lavae)
Arthropoda	Malacostraca	Decapoda	Processidae	Processa sp.
Arthropoda	Malacostraca	Mysida		Mysidaus sp.
Arthropoda	Hexanauplia	Calanoida	Centropagidae	Centopages typicus
Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia clausi
Arthropoda	Maxillopoda	Calanoida	Calocalanidae	Calocalanus sp
Radiozoa	Acantharea			Acantharea sp.
Radiozoa				Radiolaria sp.
Radiozoa	Acantharia	Arthracanthida		Acanthostaurus sp.
Dinoflagellatta	Dinophyceae	Gonyaulacales	Ceratiaceae	Ceratium sp.
Dinoflagellatta	Dinophyceae	Gonyaulacales	Ceratiaceae	Ceratium macroceros
Foraminifera	Globothalamea	Rotaliida		Globigerinia sp.
Formainifera				Foraminifera sp.
Chaetognatha	Sagittoidea	Aphragmophora	Sagittidae	Sagattia sp.
Mollusca	Gastropoda	Littorinimorpha	Atlantidae	Atlanta sp.
Mollusca	Gastropoda	Pteropoda	Creseidae	Creseis sp. (lava)
Mollusca	Gastropoda	Littorinimorpha	Littorinidae	Littorina sp.
Chlorophyta	Pyramimonadophyceae	Pyramimonadales	Pyramimonadaceae	Halosphaera sp.
Ochrophyta	Bacillariophyceae	Rhizosoleniales	Rhizosoleniaceae	Rhizosolenia styliformis
Ochrophyta	Bacillariophyceae	Chaetocerotanae incertae sedis	Chaetocerotaceae	Chaetoceros densus
Chordata	Actintopteri			Teleostei sp. (juvenil)
Annelida	Polychaeta			Polychaeta sp.

Figure 4: Examples of determined species. We determined Foraminifera sp. (A), Halosphera sp. (B), Sagattia sp. (C), a Teleostei juvenile animal (D), Polychaeta sp. and a Anthomedusae sp. (F)

We were able to identify 27 different organisms in the sample. In most cases, it was not possible to determine the specimen to the species level; only 3 samples were determined to the species level. The most represented group were the arthropods with 9 different specimens, followed by Cnidaria with 5 specimens (**Tab. 1**). The decapods were with 5 determined individuals the dominant order. In general we identified 11 different phyla in our sample, 2 larvae and 1 juvenile fish embryo.

Discussion

In general, we identified more than 20 different species in only one sample and with limited time. This shows the high diversity and density of plankton in the Mediterranean sea. As we had expected, the arthropods were the dominate species in the sample. This is also consistent with other data and older course protocols.

In comparison to older course reports from 2016 and 2018, we identified more or less the same number of species. If we look at the different phyla, we can consider that in our sample, the cnidari-

ans were overrepresent while some phyla were not observed at all in comparison to the old protocols. For example, we were not able to find or identify a platyhelminth or a tunicate species.

In most cases, we were not able to determine the specimens to the species level; the genus level was often the end. This is caused by the extreme diversity of characters, so that without molecular methods it is nearly impossible to determine between two closely related species.

In summary, our data are consistent with older course protocols, in the number of identified species as well as in the distribution of the different phyla, indicating a stable ecosystem.

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Fauna of the sandy beach Plage de Revellata/ l'Alga

MICHAEL SODJA

INTRODUCTION

Sandy shores are common habitats in the marine ecosystem. Following McLachlan's estimation, about 66% of the oceanic coastline is occupied by this biotope (coast lines covered in ice are not included). Therefore, sandy shores are globally the main border between land and sea. However, in the Mediterranean Sea the percentage of boulder field structures is way higher and Sfurlani (2018) claims that over half of the coastlines are rocky shores. Although no number was found this suggestion from Sfurlani could also be used on the Island of Corse, where the north and west coast are mainly rocky shores and sandy beaches most likely occur on the eastern side of the island (Sales et al.). Nevertheless, few sandy shores also appear on the northern coastline, such as the beach *La Revellata*.

ZONATION

The sandy coastlines are generally sectioned into zones due to the tidal fluctuation, and wave mechanics. The description of the zones and the borders, however, can differ greatly between various literatures. An attempt to clarify the different zones was made by me using the figures from Beer (1997) and Webb (2019) and is shown in Figure 1. There you can see the 4 main areas of sandy beaches: the coast, the shore, the inshore and the offshore. The shore is determined by the high tide line on the upper end and the low tide line on the lower end. This zone again is also divided into the backshore and the foreshore, where the backshore is only covered with water when a storm expands the usual range of the water. Above the shore is the coastal zone, in which water abundance is nearly zero and only occasional drops from breaking waves wet the sand. On the lower end of the shore follows the inshore, which also consists of two different zones, the surf, and the breaking zone. The last area considered as part of sandy beaches is the offshore, where waves do not break usually, and the water movement is becoming less dynamic.

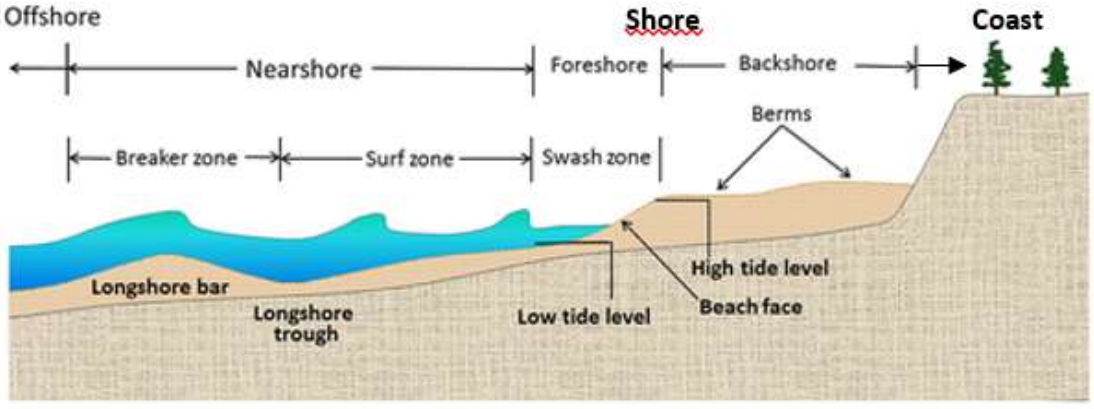

Figure 5. The figure shows the zonation of sandy beaches adapted from Beer and Webb

ENVIRONMENTAL FACTORS

Sandy beaches are highly dynamic areas, where the morphological properties of the beach and the resulting mechanical properties of the water play a key role in species richness. There are many characteristics that can be described when looking at a sandy shore such as grain size, salinity, pH, slope, wave actions, tide range, temperature, wind and so on. Barboza and Defeo (2015) selected 4 main characteristics to be relevant for the abundance of species, shown in Figure 2. These 4 variables have the largest impact on biodiversity and are either negatively or positively correlated with species richness. In the figure below you can see that taller grain of sand leads to a negative effect on species abundance, whereas a higher tide range supports the biodiversity.

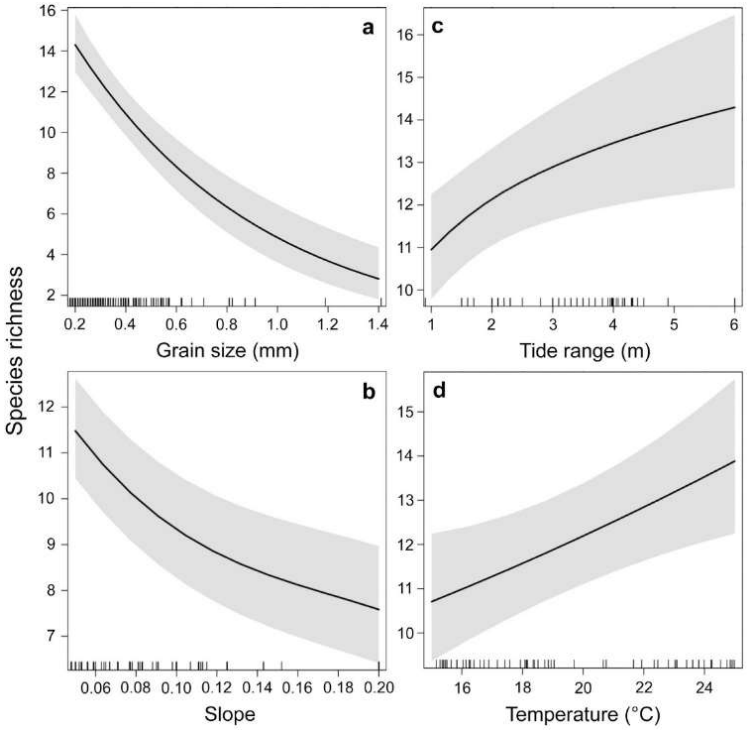

Figure 6. The most important characteristics and their effect on species richness. Species richness is always the y-axis and the different parameter are on the x-axis.

ADAPTATIONS

Due to the immense water movement, and the following substrate relocation, sandy beach species have gained some adaptations to cope with the environment. Every species living on a sandy shore has some sort of inner clock to determine whether its day or night or if the tide is rising or falling. This inner clock is subsequently more important to those species living in the substrate as to those swimming around in the water column. However, also the free-swimming animals must know when low tide comes, to avoid the danger of being captured in a saltwater pool with rising temperature and salt levels. Another feature that is important for our protocol is locomotion.

Locomotion is the main key of success when living on sandy shores. Through active movement the animals living in this habitat can either prevent from being relocated through burrowing, or regain

their location by swimming, crawling, or hopping. This is an essential characteristic of these animals, especially when living in sandy beaches with increased wave actions (McLachlan 2010).

However, the link between beach features and species richness as well as adaptations are researched mostly on the macrofauna. Our goal was to determine the megafauna (and mostly fishes) on this sandy beach and therefore the comparison between the found species on *La Revellata* and the total found species of the megafauna are considered in this protocol, as well as the species list from earlier protocols (earlier protocols are compared with the knowledge that the researchers also looked for macrofauna).

MATERIALS AND METHODS

SAMPLING SITE

The sandy shore where the data was collected was the beach *La Revellata* on the north-western end of the island of Corse. This beach is a hotspot for sailing and motor yachts which anchor on the sandy ground. Although it is described as a sandy beach, also rocky parts can be found adjacent. Another impact on the habitat has the seagrass *Posidonia oceanica* which is important for some species. In the figure below the different habitats are drawn into a bird view picture from the beach itself. The section B was a rocky area with small Posidonia meadows and sandy parts between rocks.

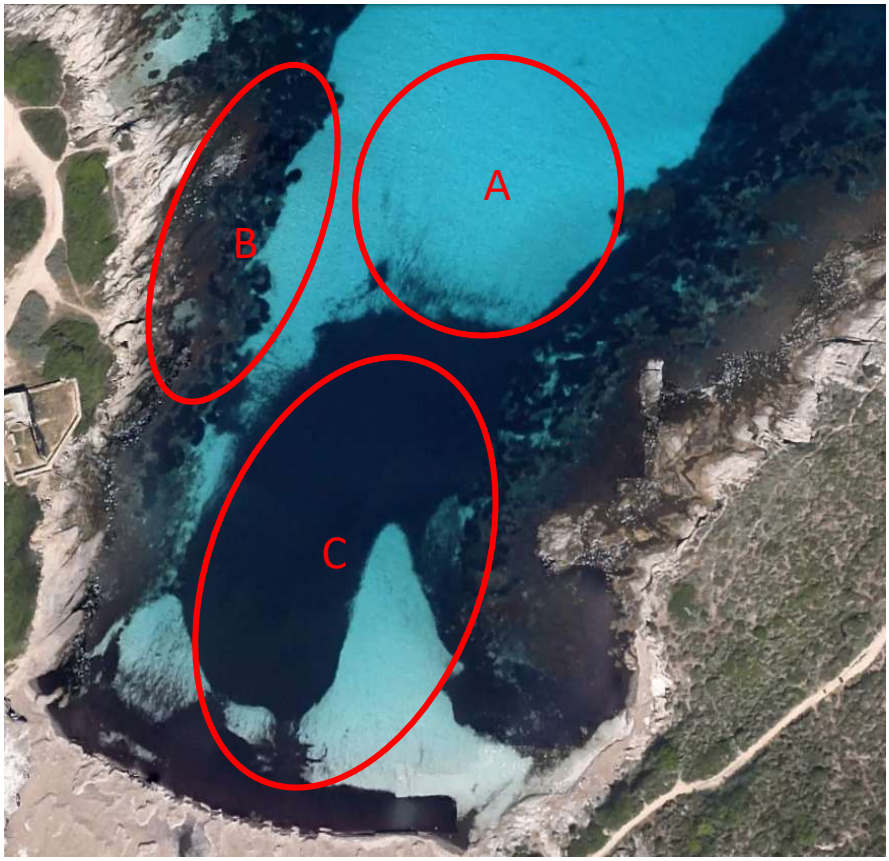

Figure 7. The beach La Revellata from above. The different habitats are (A) the sandy substrate, (B) the rocky grounds intermitted by sand with Posidonia meadows, and (C) the leftovers from Posidonia oceanica which accumulate in the bay of La Revellata. https://earth.google.com/web/@42.56205835,8.72782733,0.41646368a,279.07683837d,35y,0h,0t,0r

ASCERTAINMENT

The method that we used to determine how many species are living on the sandy beach on *La Revellata* was snorkelling. The students swam 2 times for about 30 minutes in the water and observed the different fish species. In certain cases, the species was added to the list in coordination with our fish expert Dr. Reinhold Hanel. The students were trained before in form of a lecture to identify the most common fish species in Corse.

In contrary to the last courses no meiofauna was investigated, and the main method used was the non-invasive snorkelling. Still, eventually animals were caught with the net to get a closer look. Afterwards all the animals were set back into the water column with no harm done (except the stress of catching). Also sampling by hand was used for some crustaceans, gastropods, or bivalves.

RESULTS

As a result of our sampling method, we found many different fish species, the most being in the family of breams followed by wrasses. Only few of the sampled animals did not belong to the taxa of Chordata, with Mollusca being the most represented. Also, some Arthropoda were found and despite active looking for some irregular sea urchins, only one Echinodermata was found: the black sea urchin *Arbacia lixula*. The total amount of species determined was 37, with 28 Chordata (1 Elasmobranchii and the rest Actinopteri), 4 Mollusca, 2 Crustacea, and 1 species of Cnidaria, Echinodermata, and Tracheophyta (Table 1).

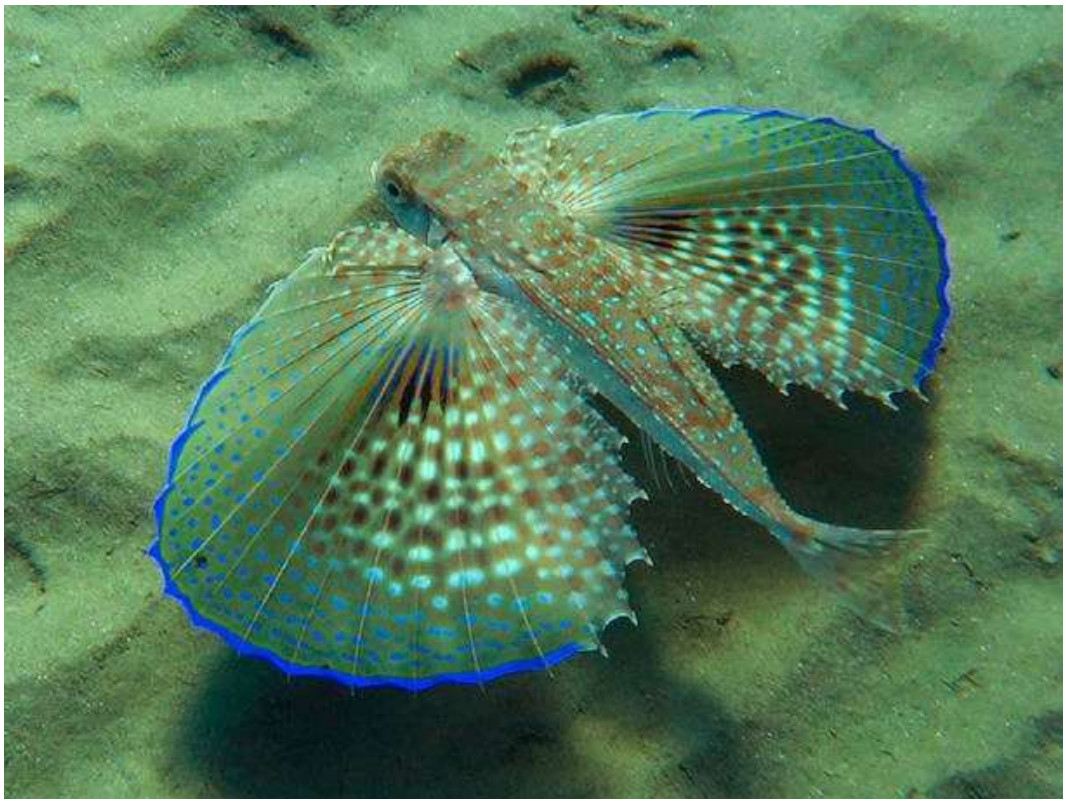

Figure 8. An impressive member of the Chordata, the Dactylopterus volitans spreads its fins and emits a growling sound when approaching it.

Table 4. List of all species found on La Revellata in 2021. Due to the sampling method, the proportion of Chordata (Actinopteri) is very high. The common names were taken from Riedl et al.. The marked names were found on websites listed in the references at the end of the protocol.

Phylum	Class	Species	Common name
Arthropoda	Crustacea	*Maja crispata*	Small spider crab[i]
Arthropoda	Malacostraca	*Diogenes pugilator*	Small hermit crab[ii]
Chordata	Actinopteri	*Atherina boyeri*	Boyers sand smelt
Chordata	Actinopteri	*Bothus podas*	Wide-eyed flounder
Chordata	Actinopteri	*Dactylopterus volitans*	Flying gurnard
Chordata	Actinopteri	*Coris julis*	Rainbow wrasse
Chordata	Actinopteri	*Labrus viridis*	Green wrasse
Chordata	Actinopteri	*Labrus merula*	Brown wrasse
Chordata	Actinopteri	*Symphodus cinereus*	Gray wrasse
Chordata	Actinopteri	*Symphodus tinca*	Doderlein's wrasse
Chordata	Actinopteri	*Symphodus roissali*	Five-spotted wrasse
Chordata	Actinopteri	*Symphodus ocellatus*	Ocellated wrasse
Chordata	Actinopteri	*Symphodus rostratus*	Long-snouted wrasse
Chordata	Actinopteri	*Dicentrarchus labrax*	Bass
Chordata	Actinopteri	*Mullus surmuletus*	Striped mullet
Chordata	Actinopteri	*Chromis chromis*	Blue damsel fish
Chordata	Actinopteri	*Serranus scriba*	Painted comber
Chordata	Actinopteri	*Lithognathus mormyrus*	Striped bream
Chordata	Actinopteri	*Pagellus erythrinus*	Pandora
Chordata	Actinopteri	*Diplodus sargus sargus*	White bream
Chordata	Actinopteri	*Diplodus vulgaris*	Two-banded bream
Chordata	Actinopteri	*Oblada melanura*	Saddled bream
Chordata	Actinopteri	*Diplodus annularis*	Annular bream
Chordata	Actinopteri	*Dentex dentex*	Dogs-teeth
Chordata	Actinopteri	*Sarpa salpa*	Salema
Chordata	Actinopteri	*Sparus aurata*	Gilt-head bream
Chordata	Actinopteri	*Spicara smaris*	Low-body picarel
Chordata	Actinopteri	*Synodus saurus*	Bluestripe lizardfish[iii]
Chordata	Actinopteri	*Trachinus draco*	Greater weever
Chordata	Elasmobranchii	*Dasyatis pastinaca*	Common sting ray
Cnidaria	Anthozoa	*Anemonia sulcata*	Snakelocks Anemone
Echinodermata	Echinoidea	*Arbacia lixula*	Black Sea-urchin
Mollusca	Gastropoda	*Haliotis lamellosa*	Abalone, Ormer
Mollusca	Gastropoda	*Gourmya vulgata*	Common cerith
Mollusca	Bivalvia	*Barbatia barbata*	Hairous ark
Mollusca	Cephalopoda	*Sepia officinalis*	Common cuttlefish
Tracheophyta	Magnoliopsida	*Posidonia oceanica*	Neptune-grass

DISCUSSION

BIODIVERSITY

In our field work we focussed on species that can be seen with the bare eye. Therefore, we snorkelled in the bay and observed the environment. With this technique 37 species were found in one half of a day.

When compared to the previous Calvi course we found nearly the same number of species (44 in 2018 and 37 in 2021). Still, the methods used in the earlier protocols were not the same. For instance, the 2018 course also searched for meiofauna in the substrate, where we did not examine such techniques. Consequently, in 2021 no member of the meiofauna is listed in Table 1 such as e.g., Plathelminthes or Foraminifera.

A meta study, which combined over 100 studies on the ichthyofauna of sandy beaches all over the world, suggests that we are well into the 95% confidence interval for the total fish species found (Figure 3 of Olds et al.). So, to analyse the fish biodiversity from one habitat, the in-situ identification we did seems appropriate (Figure 5). On the other hand, analysing the whole biodiversity of a beach cannot be done with only this method.

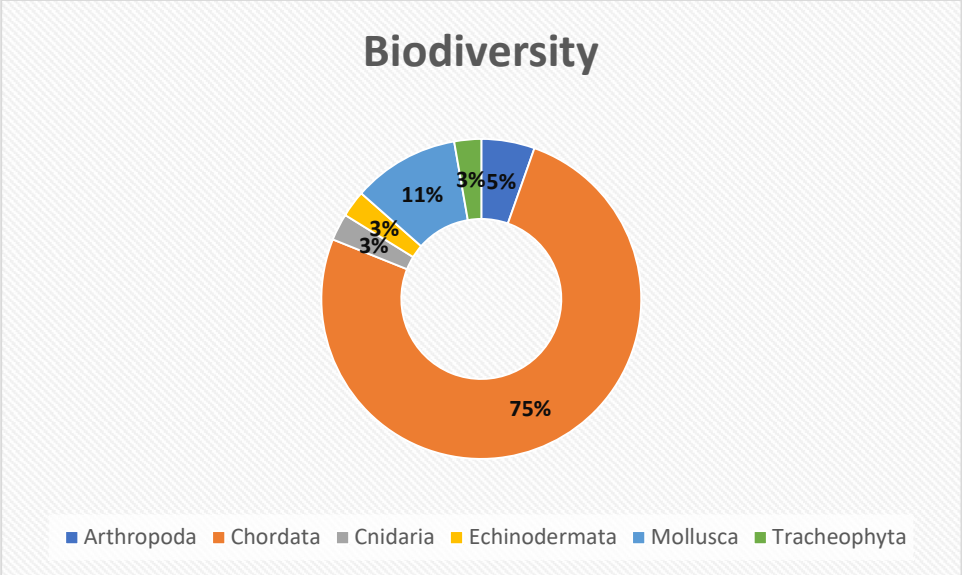

Figure 5. The total species found divided into their taxa. All the Chordata are 'fishes', as listed in table 1.

FISH FAMILY DISTRIBUTION

Looking at the species table, there are two main families represented, the wrasses and the breams. Wrasses usually live on rocky substrate with some algal compartments, or near the adjacent seagrass beds, whereas breams are living more often in shallow waters of the coast, the surf zone. Some breams, such as the striped bream, only occur on sandy beaches because of their feeding habits. Also, harsh oceanic conditions and strong water movement have a bad negative effect on the abundance of wrasses. They need more protected areas to live which 'normal' sandy beaches cannot provide (Treasurer 1994, Skiftesvik et al. 2015 and Louisy).

Analysing the family distribution from our course compared to the last course, some main differences can be seen (Figure 6). First of all, the number of fish species identified was in 2021 nearly twice as high than in 2018. Another quite impressive difference is that the relative abundance of Sparidae is about the same, but the family of Labridae is underrepresented in the course of 2018, although the sampling method was about the same. However, the course of 2021 also looked for fish species in the adjacent areas where seagrass beds and rocky parts occur. In these areas the Labridae are more abundant and maybe in 2018 the students were concentrated only on the sandy parts of the beach. In this area most of the breams occur either directly on the sand or in the water column above the sandy sediment.

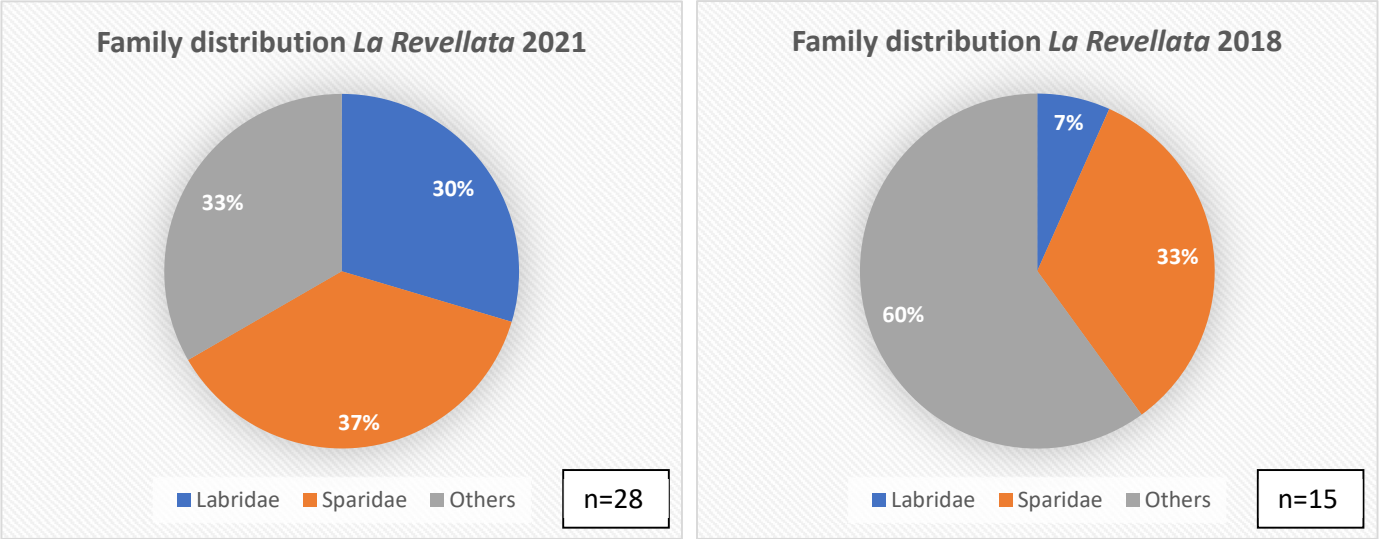

Figure 6. Although the number of fish species found is very different in the two years considered, the relative abundance of Sparidae remains nearly the same. The Labridae, in contrast, are underrepresented in the previous year.

Conclusion

Sandy beaches are often considered as very scarce habitats with only few species abundant. I think that our protocol gives a very different perception. Of course, rocky shores have a higher species abundance, due to the number of niches that can be used by different species. Still, sandy beaches have a lot to offer too, and some species only occur in these areas.

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Coralligène

MORITZ NOWAKOWSKI & ALEXANDER VORLEUTER

INTRODUCTION

First time in 1883, "Coralligène" was described as "producer of coral" and is related to the abundance of the red coral (*Corallium rubrum*) in this type of bottoms. Nowadays, Coralligène is defined as secondary biogenic hard bottom origin mainly produced by the accumulation of red algae, which have calcium deposits in their cell walls making them hard and fractural. It is usually characterized by a clear water phase and being below the zone of providing sufficient light for macrophytes. Although it is more extended in the circalittoral zone, it can also develop in the infralittoral zone, provided that light is dim enough (Fig. 1).

Coralligenous buildups are common all around the Mediterranean coasts, especially the region around Corsica is well known for its particular clear water and coralligène habitats at 60-80 m depths (Laborel, 1961). Many not well-fixed fragments are the result of the counter game between bioerosion and re-cementing or newly formed plaques. Net growing rates are extremely slow with 0.05-0.8 mm per year and almost zero in deeper waters; some coralligène therefore being more than 10,000 years old.

In this type of habitat, light and velocity of water are the most important factors determining the settlement of organisms. The coralligène's biodiversity is considered to be very high and is characterized by mainly sessile organisms. This might be related to the diverse habitats characterized by many fissures and holes of different sizes. Due to the poor light conditions, animals are more important than plants in terms of biomass. Nevertheless, the alga family Corallinaceae comprises the main constructors. While Rodophyta are quite common, only very few Chlorophyta like Ulva can be expected. Further Coralligène builders are calcareous Chlorophyta, Serpulidae, Bryozoans as well as some Crustaceans (Verlaque M. et al., 2003).

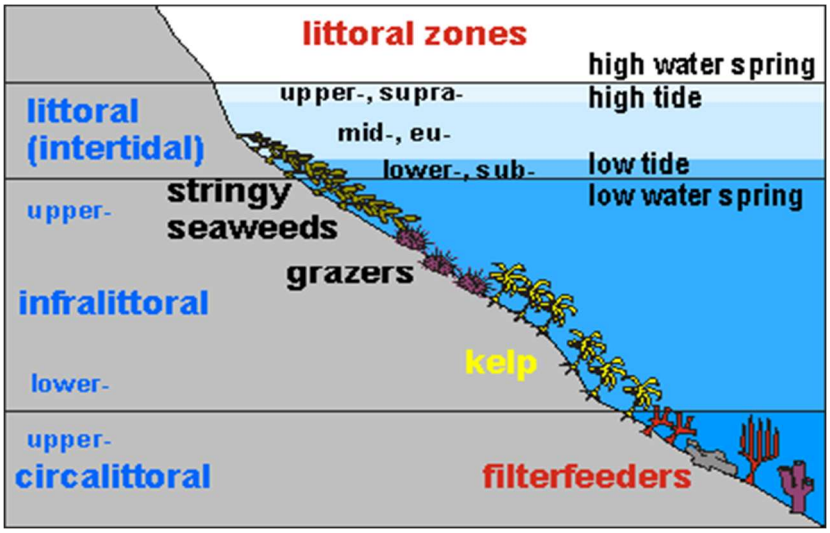

Figure 9: Littoral zones of the coast line. Circalittoral and also infralittoral are the common habitats of Coralligène.

A diverse range of sessile taxa attach to the substratum. Also, certain cnidarians (especially hydrozoans) can be found frequently. Many plathelminthes, nematodes, echiurida, polychaeta, and sipunculida take advantage of the jointed habitat. Mollusca are represented by gastropoda and bivalvia. Among the crustaceans, harpacticoida, amphipoda, and decapoda can be expected. Also, potentially contributing to the calcification process are bryozoans. Since many organisms are hidden within the coralligène, one has to analyze the substratum in detail to detect them.

MATERIALS AND METHODS

The sampling date was the 28th of August 2021. It was the same dredge that was used in the previous years, having the following dimensions:

- Outer door mesh size: 3 cm
- Steert (cod end): 0.5 cm
- Frame opening: 20 cm x 58.5 cm

The dredge is built up like a fishing net with two different mesh sizes and an open heavy-metal frame with jags. The smaller steert is collecting tiny organism and zooplankton, the bigger mesh collecting corals, algae and other organisms. The metal frame with the jags is required to sink to the ground of the sea and graze the coralligène (Fig. 2).

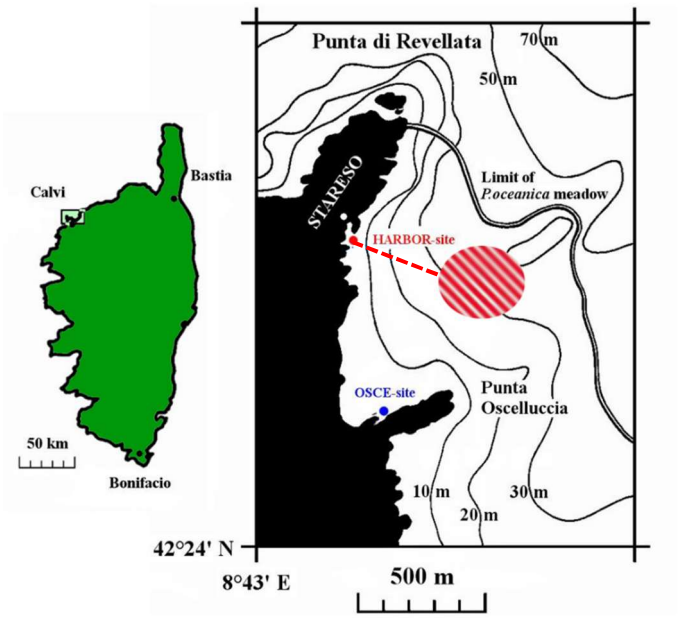

Figure 10: Map of the Coralligène sampling site (white-red area) located in front of STARESO in the Revellata Bay.

With a boat we went out to the sea, around 500-1000 meter away from the Stareso station and the bay of Revellata, and set up the dredge (Fig. 2). Therefore, we released the dredge into the water and waited until it reached the bottom of the sea at around 40-50 meters in depth. With a continuous low speed (1-3 knots) we drove for 10 minutes a straight line, before we pulled the dredge back on the boat. The collectings were placed in a big plastic box filled with sea water. This procedure was repeated once again, before we went back to the station and started to investigate the material.

RESULTS

In total, 59 different species were identified from both dredge sampling runs (Table 1). In four cases only the family of the specimen could be determined. One post-larvae fish, which was caught by the dredge, could not be identified at all and is not shown in table 1. The phyla Mollusca and Arthropoda were the most represented ones with 18 (31 %) and 12 (20 %) different species found, respectively, followed by Annelida with over 6 (10 %) species (Fig. 3). On the part of algae, Rhodophyta is the predominant phylum with 6 (10 %) identified representatives and only two (3 %) Chlorophyta species could be found, including *Caulerpa racemosa.*

Table 5: List of collected species found within the coralligène habitat. Scientific names refer to the World Register of Marine Species (WoRMS, 2021). Names in brackets are currently unaccepted names according to WoRMS but refer to literature used during the course (Riedl, 1983). Names in square brackets are family names for not definable species.

Phylum	Class	Species	German trivial name
Annelida	Polychaeta	*Euphrosine foliosa*	
Annelida	Polychaeta	[Hesionidae]	
Annelida	Polychaeta	*Orbinia sp.*	
Annelida	Polychaeta	*Pilargis verrucosa*	
Annelida	Polychaeta	*Pontogenia chrysocoma*	Schirmchenalge
Annelida	Polychaeta	*Websterinereis sp.*	
Arthropoda	Malacostraca	*Apseudes sp.*	
Arthropoda	Malacostraca	*Athanas nitescens*	
Arthropoda	Malacostraca	*Dromia personata*	Wollkrabbe
Arthropoda	Malacostraca	*Ebalia cranchii*	Cranchs Seespinne
Arthropoda	Malacostraca	*Ebalia tumefacta*	Steinkrabbe
Arthropoda	Malacostraca	*Eurynome aspera*	Erdbeerkrabbe
Arthropoda	Malacostraca	*Galathea nexa*	Furchenkrebs/Springkrebs
Arthropoda	Malacostraca	*Inachus dorsettensis*	Dreieckskrabbe
Arthropoda	Malacostraca	*Inachus thoracicus*	Dreieckskrabbe
Arthropoda	Malacostraca	*Paguristes eremita (Paguristes oculatus)*	Augenfleck Einsiedler
Arthropoda	Malacostraca	*Pagurus sp.*	gemeiner Einsiedler
Arthropoda	Malacostraca	*Pilumnus spinifer*	Rote Borstenkrabbe
Bryozoa	Gymnolaemata	*Porella sp.*	
Bryozoa	Gymnolaemata	*Reteporella grimaldii (Sertella septentrionalis)*	Neptunschleier
Bryozoa	Stenolaemata	*Disporella pristis*	
Chlorophyta	Ulvophyceae	*Caulerpa prolifera*	Kriechsprossalge
Chlorophyta	Ulvophyceae	*Caulerpa racemosa*	
Chordata	Ascidiacea	*Ascidia mentula*	Rosa Seescheide
Chordata	Ascidiacea	*Phallusia mammillata*	Weißwarzige Seescheide
Echinodermata	Asteroidea	*Echinaster sepositus*	Purpurstern
Echinodermata	Holothuriidae		Seegurke
Echinodermata	Ophiuroidea	*Ophiura ophiura (Ophiura texturata)*	Heller Schlangenstern
Mollusca	Bivalvia	*Mimachlamys varia (Chlamys varia)*	Bunte Kammuschel
Mollusca	Bivalvia	*Flexopecten flexuosus (Chlamys flexuosa)*	
Mollusca	Bivalvia	*Glycymeris glycymeris*	Gemeine Samtmuschel

Mollusca	Bivalvia	*Glycymeris pilosa*	Echte Samtmuschel
Mollusca	Bivalvia	*Mactra stultorum*	Bunte Trogmuschel
Mollusca	Bivalvia	*Pecten jacobaeus*	Jakobsmuschel
Mollusca	Bivalvia	[Pectinidae]	Kammmuschel
Mollusca	Bivalvia	*Pseudamussium clavatum (Peplum clavatum)*	Gewellte Kammmuschel
Mollusca	Bivalvia	*Pseudamussium sulcatum (Chlamys bruei)*	
Mollusca	Bivalvia	*Rocellaria dubia*	Europäische Gastrochae-na
Mollusca	Bivalvia	*Talochlamys multistriata (Chlamys multistriata)*	Zwergmuschelse
Mollusca	Cephalopoda	*Sepia sp.*	Sepia
Mollusca	Gastropoda	*Aplysia punctata*	Gemeiner Seehase
Mollusca	Gastropoda	*Cerithium vulgatum*	Gemeine Nadelschnecke
Mollusca	Gastropoda	*Elysia viridis*	Grüne Samtschnecke
Mollusca	Gastropoda	*Felimida krohni*	Prachtsternschnecke
Mollusca	Gastropoda	*Notarchus punctatus*	
Mollusca	Scaphopoda	[Dentaliidae]	Zahnschnecke
Nemertea	Hoplonemertea	*Amphiporus lactifloreus*	Milchweißer Bandwurm
Nemertea	Hoplonemertea	*Carcinonemertes carcinophila*	Krabbenschnurwurm
Nemertea	Hoplonemertea	Gibsonnemertes spectabilis (Drepanophorus spectabilis)	Schnurwurm
Nemertea	Pilidiophora	*Leucocephalonemertes auran-tiaca (Micrura aurantiaca)*	
Ochrophyta	Phaeophyceae	*Arthrocladia villosa*	Zottentang
Ochrophyta	Phaeophyceae	*Stilophora rhizodes*	
Ochrophyta	Phaeophyceae	[Sporochnaceae]	
Rhodophyta	Florideophyceae	*Lithothamnion corallioides*	
Rhodophyta	Florideophyceae	*Lithothamnion sp.*	
Rhodophyta	Florideophyceae	*Mesophyllum alternans*	
Rhodophyta	Florideophyceae	*Osmundaria volubilis (Vidalia volubilis)*	
Rhodophyta	Florideophyceae	*Peyssonnelia squamaria*	Klassische Rotalge
Rhodophyta	Florideophyceae	*Spongites fruticulosa (Lithothamnion fruticulosum)*	

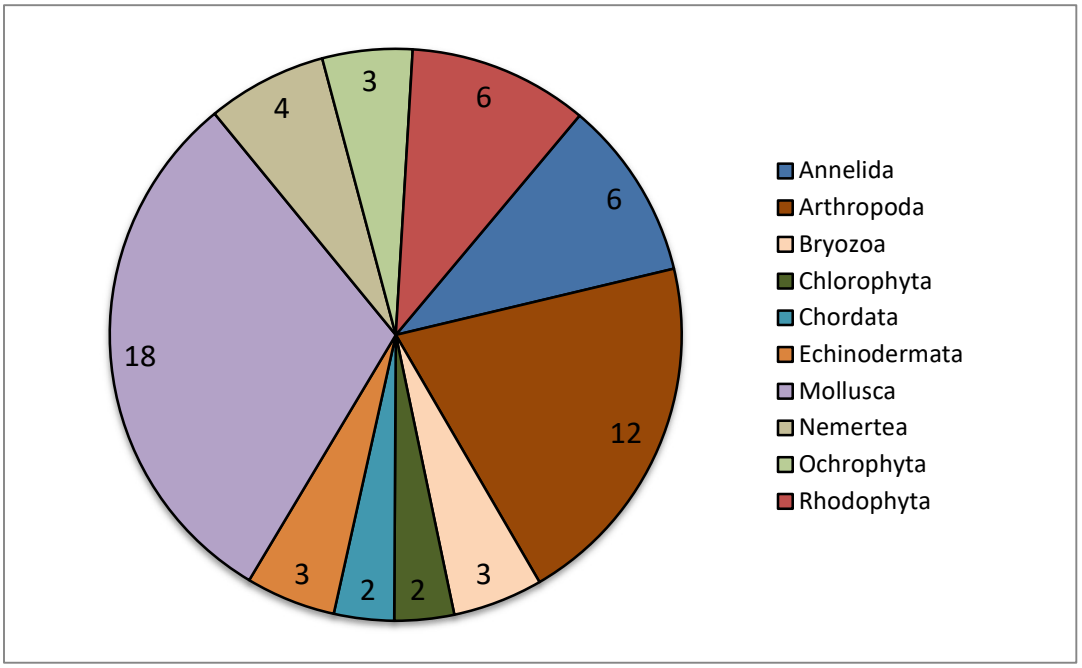

Figure 3: Phylum diversity of all identified species of both dredge samples.

DISCUSSION

Compared to the results of the previous years, a larger variety in different species could be identified. Nevertheless, the composition is very similar with Mollusca and Arthropoda being the predominant phyla, given that the region in front of STARESO harbor is composed of plane plateau coralligène built on sandy substrate. The plane coralligenous fields in the bay of Calvi have shrunk in the past years, but we got lucky with both dredge samples and found a lot of calcareous encrusted substrate. Thus, also a lot of secondary builders, epi- and endofauna could be retrieved and classified. Additionally to the normal calcareous algae composition of coralligène habitats, the green algae *Caulerpa racemose* was highly abundant. It is an invasive species originated from the red sea, meanwhile widespread across the Mediterranean Sea and a danger to the native biodiversity, which needed to be destroyed after finishing identification.

Consolidated, our findings resemble only a fraction of the theoretically possible biodiversity of this habitat. Studies reported of over 900 specimen found in 370 g of algal main builder substrate and up to 1200 species in total (SPA, 2003).

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Girolata & Fango

VERONIKA PEER

GIROLATA

Girolata is in the west of corsica and belongs to the golf of porto together with the natural reserve of the scandola peninsula and the calanche of piana. All of them are part of the regional natural park of Corsica, which was established in 1972 and takes nearly 40% of Corsica. Girolata is only accessable by boat or by foot and since 1983 it is included by UNESCO in the World Natural Heritage List.

The scandola peninsula consists of porphyritic rocks, which are mostly colored red. They were formed more than 250 million years ago by volcanic activity.

In the natural park different habitats can be found in the marine ecosystems, such as sandy shores, rock shores, sea meadows and a lot of underwater caves. The vegetation on land consists mostly of scrubland. These different habitats and the protection of the area give habitat to a lot of species.

Furthermore, it is important to mention that the beaches of Girolata are no high energy beaches like the beach in the Fango delta. The beaches are more protected from waves.

FANGO

The Fango river is in the north-west of corsica and south of calvi. The river flows from an altitude of 2556 m above sea level down in the golf of Galèria. The whole area is part of a UNESCO biosphere Reserve where environmental managers, researchers, residents and tourists coexist. The main problems in the biosphere reserve deal with the management of scarce freshwater resources in this area. The Fango biosphere Reserve has a very rich and unique fauna, especially birds, amphibians and reptiles. The river is shaped by little pools that are filled and give multiple freshwater animals a living space.

Figure 11: Left: Bay in Girolata (https://www.la-corse-autrement.com/girolata-sentier-du-facteur/). Right: Fango River (https://coolcorsica.com/easy-river-hike-in-the-fango-valley/dsc_2477/).

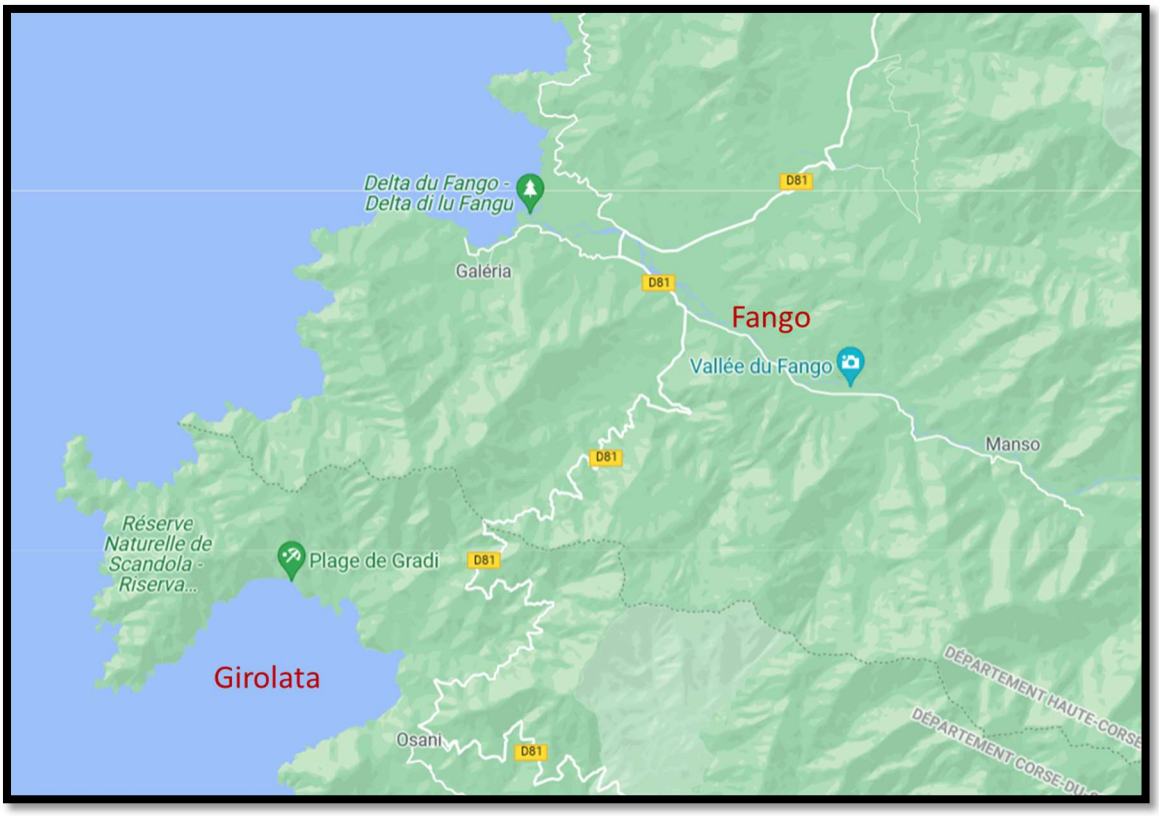

Figure 12: Map of the west of Corsica with Girolata and Fango (from google maps).

MATERIAL & METHODS

For collecting the fauna, nets and plastic bags filled with water were used. Also shells of mussels were collected by hand on the beach. But mostly, fauna and flora of the marine environment in Girolata were only observed, because it is part of the natural park. All animals were released after collection. The focus was set on the fauna of the areas.

RESULTS

GIROLATA

In total 38 species were determined in the bay of Girolata (Table 1). 25 species belong to the group Chordata. Worth mentioning are especially the stingrays and the wide-eyed flounder. They both are mostly found on sandy and muddy soils. Also the barracudas were a highlight to see.

Table 1: Species list of the bay in Girolata.

Phylum	Family	Species	Common name
Chordata	Dasyatidae	*Dasyatis pastinaca*	Gewöhnlicher Stechrochen
Chordata	Sphyraenidae	*Sphyraena sphyraena*	Europäischer Barrakuda
Chordata	Muraenidae	*Muraena helena*	Mittelmeer-Muräne
Chordata	Sparidae	*Dentex dentex*	Zahnbrasse
Chordata	Sparidae	*Diplodus sargus sargus*	Geißbrasse
Chordata	Sparidae	*Diplodus vulgaris*	Zwei-Bindenbrasse
Chordata	Sparidae	*Lithognathus mormyrus*	Marmorbrasse
Chordata	Sparidae	*Oblada melanura*	Brandbrasse
Chordata	Sparidae	*Pagellus erythrinus*	Rotbrasse
Chordata	Sparidae	*Sparus aurata*	Goldbrasse
Chordata	Mugilidae	*Oedalechilus labeo*	Kastenmaul-Meeräsche
Chordata	Pomacentridae	*Chromis chromis*	Mönchsfisch
Chordata	Apogonidae	*Apogon imberbis*	Meerbarbenkönig
Chordata	Serranidae	*Serranus scriba*	Schriftbarsch
Chordata	Atherinidae	*Atherina boyeri*	Kleiner Ährenfisch
Chordata	Atherinidae	*Atherina hepsetus*	Großer Ährenfisch
Chordata	Mullidae	*Mullus surmuletus*	Streifenbarbe
Chordata	Bothidae	*Bothus podas*	Weitaugenbutt
Chordata	Labridae	*Coris julis*	Meerjunker
Chordata	Labridae	*Thalassoma pavo*	Meerpfau
Chordata	Labridae	*Symphodus tinca*	Pfauen-Lippfisch
Chordata	Labridae	*Symphodus ocellatus*	Augenfleck-Lippfisch
Chordata	Labridae	*Symphodus roissali*	Fünffleck-Lippfisch
Chordata	Labridae	*Labrus viridis*	Grüner Lippfisch
Chordata	Tripterygiidae	*Tripterygion tripteronotus*	Roter Spitzkopfschleimfisch
Echinodermata	Asteroidea	*Echinaster sepositus*	Purpurseestern
Echinodermata	Echinoidea	*Paracentrotus lividus*	Steinseeigel
Echinodermata	Echinoidea	*Arbacia lixula*	Schwarzer Seeigel
Echinodermata	Holothuroidea		Seegurke
Arthropoda	Decapoda	*Diogenes pugilator*	
Mollusca	Octopodidae	*Octopus vulgaris*	Gewöhnlicher Krake
Mollusca	Gastropoda	*Columbella rustica*	
Mollusca	Gastropoda	*Luria lurida*	
Mollusca	Bivalvia	*Arca noae*	Arche Noah-Muschel
Mollusca	Bivalvia	*Angulus planatus*	
Porifera	Suberitidae	*Verongia aerophoba*	
Algae	Brown algae	*Cystoseira fimbriata*	

Figure 13: Top left: Angulus planatus. Bottom left: Arca noae. Top Right: Octopus vulgaris. Bottom Right: Luria lurida.

FANGO

In the freshwater river Fango, 5 species were found, whereas the larvaes of the mayflys were only determined to the family-level, as were the mites (Table 2). There were a lot of freshwater blennys and also a european eel was sighted. Different plathelminthes were found, but only one could be identified immediately there and the others unfortunately died of a heat shock in the sun the next day.

Table 2: Species list of the Fango river.

Phylum	Family	Species	Common name
Chordata	Blenniidae	*Lipophrys fluviatilis*	Fluss-Schleimfisch
Chordata	Anguillidae	*Anguilla anguilla*	Europäischer Aal
Arthropoda	Acari		
Arthropdoa	Ephemeroptera		Eintagsfliegen
Plathelminthes	Dugesiidae	Girardia sp.	

DISCUSSION

In the bay of Girolata, there were a lot of different species which are listed in table 1. The focus lay on chordates, which are probably easier to see while snorkelling than arthropods, which we only determined one, as of course other smaller animals. Also, the "sampling" may be limited due to a higher water depth and therefore by the snorkelling skills of the students. In total we determined 38 species, which is compared to the years before much higher (in 2018: 15 species were determined).

In the Fango river, we focused on finding the freshwater Blenny *Lipophrys fluviatilis*, which was there in a high abundance. A special sighting was the European eel, *Anguilla anguilla*. The European eel is a catadromous species, which when earns maturity, leaves freshwater rivers and swim to their spawning place (Sargasso sea believed). The adults die after spawning. Because of the complicated and still mostly unknown life cycle, it is very hard to protect these eels from distinction. Today they are listed as critically endangered species on the IUCN red list status.

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Project reports

Sea urchin development

MATTHIAS ACHRAINER

MICHAEL SODJA

SANDRA BRAČUN

BERT HOBMAYER

Environmental pollutants and how they interfere with the development of sea urchin embryos (*Arbacia lixula*)

MICHAEL SODJA

INTRODUCTION

The pollution of our environment is, besides the climate change (which are co-dependent), one of the main problems of our time. Ironically the equation to solve this problem could not be simpler: producing less waste and recycle more. However, this 'simple' solution is in fact way more complicated and has socioeconomical, demographical as well as biological issues that need to be assigned before. To entangle all this mess that leads to the pollution and climate change itself, has already filled many books and would by far outreach this report. In this protocol, the main focus is about a few ecologic important pollutants that are released into the environment every day. To analyse the effects on the environment, we have used an animal species that is very common in the bay of the STARESO institution, the sea urchin *Arbacia lixula*. (Dürrenmatt et al. p. 960ff.)

Invertebrate Deuterostomia have been used for developmental questions for over a century. The advantages of these animals are that their gametes are normally easy to obtain, and many different larval developments can be observed. From the approximately 7000 species of Echinodermata, sea urchins are the most common when talking about developmental experiments. These animals undergo an impressive metamorphosis from their larval stage to the adult. Only rudiments from the bilateral larvae form the radial symmetric adult. The hydrocoel and the vestibule are the tissues that form the adult body of the sea urchin. Other tissues mostly degenerate. Experiments made on sea urchins have also led to many discoveries, such as regulative development, the role of the nucleus, structure of chromosomes and many more. (Ettensohn et al.)

These points, and the fact that previous Calvi courses also experimented on sea urchins, made it the perfect species to work on during our stay at the STARESO institution. However, in our experiments the focus lays on environmental factors that could interfere with the normal development of sea urchins. Therefore, we have not concentrated on the interaction of genes, as done by the earlier courses, rather than on the effects that can be observed when sea urchin embryos are confronted with different toxicants.

PLASTIC/MICROPLASTIC

In the oceans of the world 5 big accumulations of waste in all forms are known, the garbage patches, which consist to 99% (the great pacific garbage patch) of plastic. The Mediterranean Sea has until now no such garbage patch, however it is still listed as the 6th great accumulation of waste in the world's oceans. Since it has only one connection to the Atlantic Ocean and one small canal that connects it to the Red Sea, the Mediterranean is a concentration pool. By only 1 % of the area of the total ocean surface, the percentage of the global plastic waste distributed in the seawater is 7%.

Recent studies showed (from the book of Hofrichter et al.) that 1.25 million particles per square kilometre of plastic are currently in the Mediterranean Sea with the tendency to go up. The most particles are in the fraction of microplastic, which are particles that are between 5 to 0.0001 mm. This is an important fact if you consider the interactions between substances and its environment. Each plastic molecule has some additives that are used in the production to get the right properties for their specific area of application. These additives can be very volatile and therefore they are likely to get into the environment, and even more so if the plastic particles get smaller. Another important property of microplastics is that they accumulate different toxins on their surface. Still, this would have been too complicated to analyse in the week of our project, therefore we focussed on the additives that are given by production. (Dürrenmatt et al. p. 1019ff.)

CIGARETTES

Another form of litter that is thrown away carelessly all over the world are cigarettes. Following the statement from Slaughter et al., about 4.5 trillion cigarette butts find their way into the environment as they are thrown away. Through many different paths the butts itself or the contained chemicals can get into marine ecosystems, giving them an influence on the health of the marine life.

Earlier studies have shown that cigarettes contain several chemicals that are regarded as carcinogenic for humans. In addition to that, the production of the tobacco itself also influences the environment, due to the use of pesticides, insecticides and so on. Some of the chemicals are released into the environment, as the butts are degraded over time. Through river systems, they can also get to the ocean, even if the cigarette was thrown away on land.

In recent studies these chemicals have shown to be acute toxic to marine wildlife and the paper of Slaughter et al. suggested that the toxicity not only affects non-vertebrate marine species, but also the vertebrate fauna of the oceans (fishes were used as organisms). (Slaughter et al.)

In our experiment, we wanted to see if the sea urchin embryos are also vulnerable to these chemicals, and how the effects vary when using smoked or non-smoked cigarettes.

SUNSCREENS

The last pollutant we experimented with was sunscreen. As the levels of general use of cosmetics rise all over the world, the coastal regions (especially those in warmer regions) are confronted with the problems that sunscreen may cause on different species. Already researched, but still discussed about, is the fact that the ingredients of this product can harm coral reefs, as it interferes with the symbiosis between the reef building corals and their symbionts the zooxanthellae. As a result of the recent studies, laws have been implemented that forbit some chemicals to be used in the sun protection cremes. One of these laws was made in 2018 by the Hawaiian government. In this law sunscreens that contain the chemicals oxybenzone and octinoxate were banned from the market, following studies that considered them to be the most toxic ingredients to corals. (Scanlon)

In our short experiment we wanted to show if the sunscreens that are following this law (without the mentioned chemicals) are still toxic to marine life forms. The fact that in the Mediterranean Sea coral reefs do not occur and the interest if this cosmetic product has also effects on other species, led us to an experiment with the embryos of sea urchins. Our tests were performed on the developing embry-

os after fertilization of the *Arbacia lixula* to see if their growth is in some way depressed or if a chemical in this cosmetic interferes with the normal development and leads to deformation.

MATERIALS AND METHODS

PLASTIC/MICROPLASTIC

One of the sources of marine plastic/microplastic are so called 'ghost nets' and other ropes that are released from fisher boats and float in the ocean. Hence, we used 2 ropes with a diameter of 5mm and the length of 50 cm. These ropes were then cut into 5 pieces and given into 150 mL of fresh sea water. For one experiment the 5 pieces were cut with a scalpel to get some microplastic, the other 5 pieces were not further processed.

A second approach was made with 2 plastic bottles, where 9x13,5 cm^2 were cut out of each and sliced into 4 pieces. These pieces were also put into 150 mL of fresh seawater, and for one approach the 4 pieces were again scratched on the surface with a scalpel to get microplastic. The other pieces were left as they are.
The four solutions stayed from 5pm 24 hours outside to soak into the water and to be heated by the sunlight.

Figure 14. The workflow of getting embryos from adult sea urchins. (A) The sea urchin is put upside down on an open vessel, so the madropores are under water. The then injected solution leads to a release of gametes (here sperm indicated by the arrow. (B) Petri dishes are prepared with oocytes. The next step would be adding of the sperms.

Fertilization

On the next day the fertilization was prepared. For this we used the most common sea urchins in the bay, *Arbacia lixula*. To get the gametes we used the same method as described in Banyuls 2001 with only one exception; we injected up to 2 mL of 0.5M KCl (which was prepared before) into the sea urchin when upside down. Therefore, we only injected once and not twice with a good result. The next step was to fertilize the eggs. The eggs were put in a petri dish with a few replicas (normally 8 to 12). Then 5 drops of sperm were added with a 5 mL Pasteur pipette. The eggs with the sperm were shaken softly and distributed again with the tip of the pipette. For each fertilization, one approach was checked upon if the eggs were in fact fertilized.

The embryos were then incubated in the petri dishes. After 1.5h the embryos were incubated with the test solutions. For each approach one replica was made and one control for the whole experiment. In the evening after the first counting the embryos were transferred in fresh solutions from the same kind as before. After the second counting the experiment was finished.

Analysis

To analyse the embryos, they were shaken softly in a spiral movement to concentrate them in the middle of the petri dish. Then one or two drops were put on a specimen slide with hollow grinding with a 1mL Pasteur pipette and looked upon in a microscope. The embryos were differentiated by their developmental stadium (1-cell, 2-cell, 4-cell, 8-cell, cleavage, blastula, gastrula, prism, pluteus) and counted. This was made for the first experiment after 9 hours and 21 hours of incubation. For the second one the counting was after 10 and 23 hours of incubation.

This experiment was remade with the same precautions, but the solutions stayed for 24 hours inside, because the temperature differences of the 4 approaches from the first experiment were too high and could not be controlled. However, the remade experiment only featured the rope design from the first one. In addition to the first experiment, in the second one we added solutions that were filtered with a 100 µm mesh size filter before transferring the embryos into the solution. Hence, we had again 4 different approaches: rope without processing, rope with processing, rope filtered without processing, and rope filtered with processing. A control was made for the whole experiment with fresh sea water.

Cigarette

For the cigarette experiment we adapted the method from Slaughter et al., as we took 5 non smoked cigarettes with tobacco into 1 L and let it stay for 21 hours. Afterwards we filtered it with 100 µm mesh size, so the tobacco particles are no longer in the solution. Then the solution was diluted into the concentrations 5 cigarettes per 10^3L, and 10^4L. The same preparations were made for the smoked cigarette butts with tobacco, but only two stumps per litre were used, following the instructions from the paper. The solution was also diluted into the same concentrations as mentioned above (2 butts per 10^3L, and 10^4L).

The collection and fertilization of the gametes were conducted as described above, as well as the incubation with the test concentrations. For each concentration two replicas were made with an extra two control replicas.

The embryos were analysed in the microscope after 8 and 23 hours, with the same criteria as described above.

SUNSCREEN

For the experiment with the sunscreen, we started again with the production of the solution. For this 4 mL of SunKiss® sunscreen was filled up to 100 mL with fresh seawater. This stock was then diluted into our 4 experiment concentrations, always using 5 mL from the foregoing stock, and adding 45 mL of fresh seawater, leading to the concentrations of 4 mL, 400 µL, 40 µL, and 4 µL of sunscreen in 1L fresh seawater.

The collection and fertilization of the gametes were conducted as described above, as well as the incubation with the test concentrations. For each concentration two replicas were made with an extra two control replicas.

The embryos were analysed in the microscope after 9 and 21 hours, with the same criteria as described above.

RESULTS AND DISCUSSION

PLASTIC/MICROPLASTIC

The results for the first experimental design showed after 9 hours a clear effect between the different treatments and the control embryos. This analysis was made only qualitative and not quantitative, because of small embryo numbers. Therefore, we did not count the different developmental stages to maintain the number of embryos necessary for the second analysis. After 21 hours of incubation in the test solutions the embryos were analysed for quality and quantity (Table 1). The result shows that the most visible effect was in the group where the rope was processed with the scalpel (microplastic), although the non-processed rope embryos had still a slower development than the control group (all pluteus). The approach with the processed plastic bottle showed nearly the same outcome as the control, whereas the unprocessed plastic bottle embryos were nearly all degenerated or dead.

Due to the unexpected outcome of the plastic bottle experiments, where the unprocessed approach killed nearly all embryos, and the processed approach had hardly any effect, we assumed that there had to be a mistake in the working process as we conducted the experiment (maybe the temperature variation explained above). Hence, these two designs are not further regarded in the discussion.

Following the first experiment, our idea was to test if the particles itself had a negative effect on the embryos or if the higher desorption from the additives led to the slower development in the embryos incubated with the processed rope solution. As explained in the method section we made therefore a filtered and non-filtered solution of the different approaches. The second experiment conducted with microplastic/plastic showed in general less effect on the embryos. After 7 hours only the unfiltered solution of the processed approach tended to have a small influence on the development of the embryos. However, after 20 hours all the test designs seemed to have the same outcome, whereas the control embryos developed a little faster.

In the first experiment the result indicated that the processing of the materials influenced the development of the embryos, but in the same experimental design two approaches seemed to fail, which led to the conclusion that a mistake was made. Therefore, no assumptions could be made regarding the first experiment. The harsh effect of the first design could have something to do with the UV-light of the sun and the higher temperature in the production of the test solutions, because it is known that these two factors desorption of additives from plastic molecules (Dürrenmatt et al.). This could also explain why the effects were not this high in the second experiment where the solutions stayed inside with more or less constant temperatures and no direct sunlight. However, this is only a possible explanation and needs to be further researched in more controlled conditions.
The statement that microplastic is more harmful than normal sized plastic, could not be confirmed in our experiment. Although the processed and unfiltered rope showed at first a higher effect on the embryos, the later analysis showed no such effect. Still an effect can be seen when the experimental designs are compared to the control.

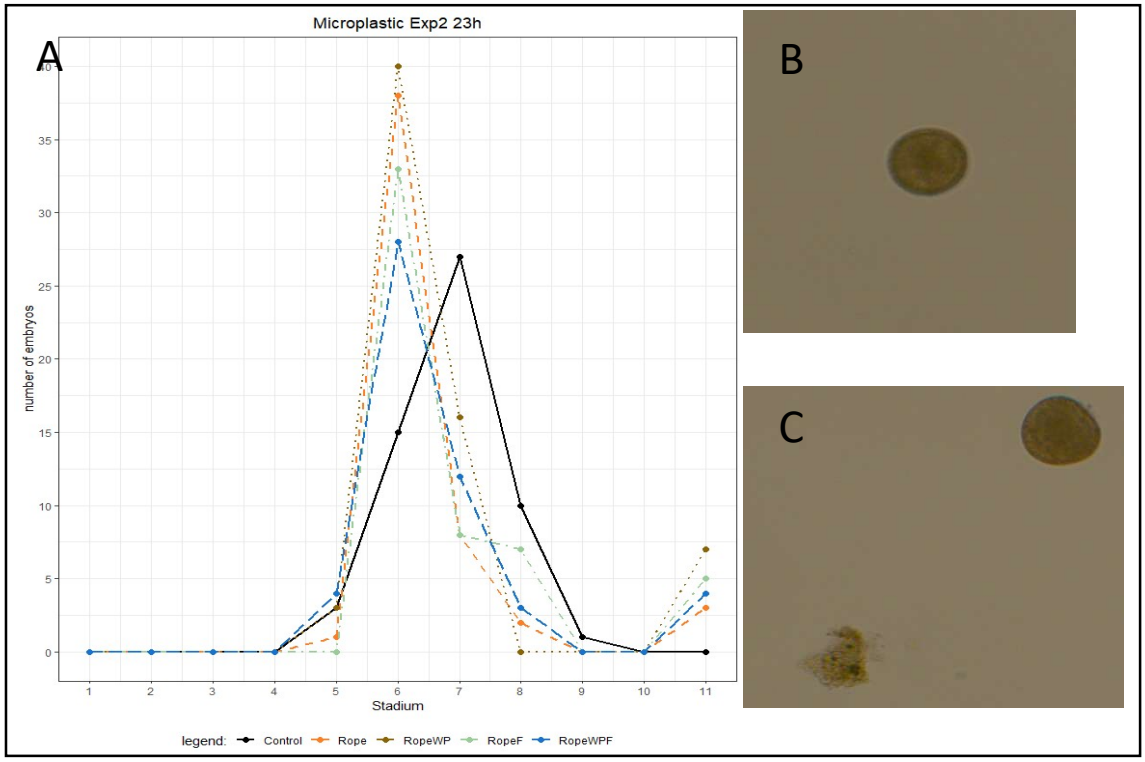

Figure 15. (A) The graph shows the distribution of the developmental stages after 23 hours into the experiment. The effect on all of the test designs was nearly the same, and a main part of the specimen were in the blastula stage: (B) a typical blastula stage from the filtered, but none processed rope design, and (C) an early gastrula stage in vicinity to a degenerated cell bulk.from the unfiltered and processed rope experiment. The numbers on the x-axis represent the different developmental stages stated in the analysis part. 10=exogastrula and 11=degenerated.

Following our analysis, the fact that plastic influences the development of sea urchin embryos can be carefully confirmed. However, our experiment contained only a close time frame of the life cycle of sea urchins, and the effect that may occur in the adults could not be seen. The assumption that smaller particles of plastic lead to a higher desorption of additives and therefore a higher toxicity on life forms (Dürrenmatt et al.) could not be confirmed in our experiments, still more controlled experiments would have a higher validity than ours.

Cigarettes

The experiment with whole unused cigarettes and used cigarette butts resulted in some differences for the test designs where unused cigarettes were soaked into fresh sea water. At 7 hours into the experiment the first analysis was made. At this point the embryos of the control group were in the same developmental stage as the two approaches with the cigarette butts. Here no visible effect for the different concentrations were observed. The first small shift in development could be seen for the 1:10.000 concentration of whole cigarettes. Another shift lays between the two concentrations for the whole cigarettes, where the higher concentration led, as expected, to a slower development. Interestingly, the analysis after 20 hours drew a different picture. In this counting session the control group was nearly in the same developmental stage as the different concentrations of the butt solutions, as well as the lower concentration of whole cigarettes. The higher concentration, on the contrary, was still in the cleavage stage (Table 1).

In the study, which was the template to our experiment, from Slaughter et al., the researchers stated that around 1 cigarette butt/l has an LC50 for topsmelt. For our experiment, we diluted the solution up to 2 cigarette butts per 10^3L, respectively 10^4L, because of the higher vulnerability in the developing stages. The results show that in this case the dilution could have been too high, as the embryos showed nearly the same developing speed as the main part of the controls (few of the embryos were farther developed), with only some of them degenerating after 20 hours of incubation (Figure 3). This suggests that if a further experiment is taken, the test concentrations can be higher than the one we performed.

Adapted from the same paper, we wanted to show if unsmoked tobacco is harmful for marine life, or if the smoking leads to a higher toxicity. In this case the tested embryos showed some effect on the test solutions. When looking at especially the second analysis, a clear inhibition of development can be observed. The fact that nearly all the embryos were stuck in the cleavage stage, led to the assumption that the chemicals in cigarettes have an influence in the pathways necessary for the transition between the cleavage stage and the Blastula. (Slaughter et al.) This finding can be very interesting in further experiments, when gene expression is also measured and visualized in the embryos.

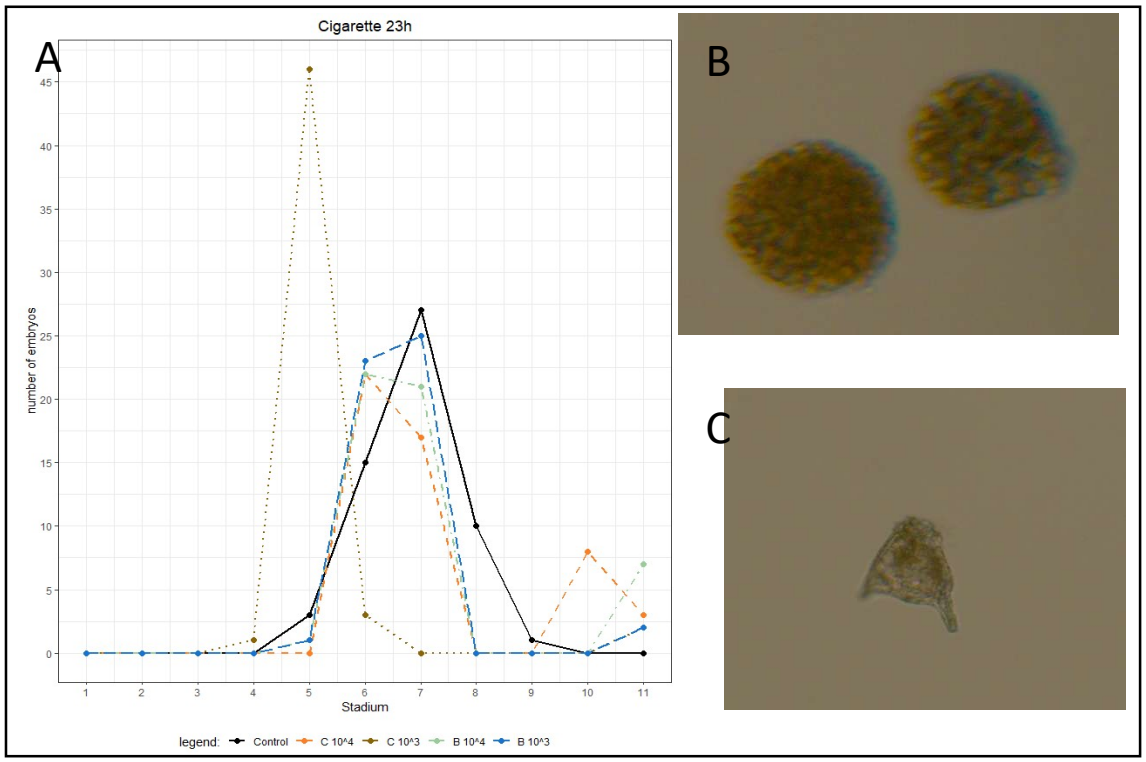

Figure 16. In the graph (A) note that the treatment with the higher cigarette concentration is showing a clear shift. When compared to table 1, you can see that these test objects remained mostly in the cleavage stage (B). C shows a pluteus larvae from the control group. The control group was the only one with prism and pluteus stages over the whole experiment. The numbers on the x-axis represent the different developmental stages stated in the analysis part. 10=exogastrula and 11=degenerated.

SUNSCREENS

Our experiment with the sunscreen as a possible stressor for the sea urchin embryos, showed a very surprising result. The quality analysis suggested that all of the concentrations contained similar developmental stages, with the range form late cleavage to even some pluteus larvae. When counting the embryos under the microscope this was confirmed, as the main distribution of the embryos was between the blastula and the prism stage.

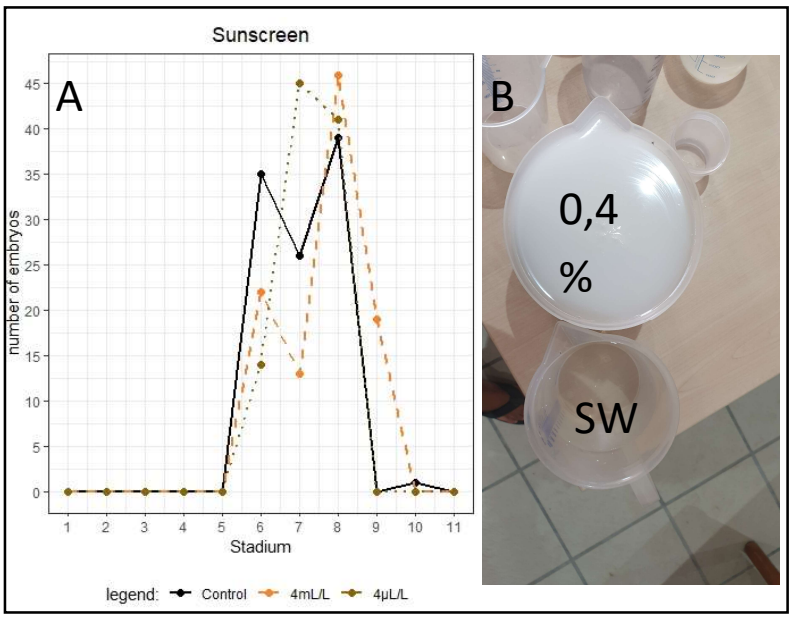

Figure 17: The sunscreen treatment showed no visible shift or effect on the sea urchin embryos. When looking at the graph, the different concentrations and the control group were nearly in the same stages (A). In (B) the highest concentration in which the embryos were incubated is portrayed against normal seawater. The numbers on the x-axis represent the different developmental stages stated in the analysis part. 10=exogastrula and 11=degenerated.

Sunscreen causes in coral reefs the whitening of the corals and is a main stressor to these reefs. Because of this, we believed that the toxic effect on other marine species, such as sea urchin embryos would be also significant. However, our results show that the embryos are much more resilient than we expected, with even the highest concentration had no effect on the development of the larvae. In the paper from Danovaro et al. the main driver of coral bleaching, and therefore the most toxic to the reefs are the viruses within the hard corals, when they get in contact with the UV filters from the sunscreen. This leads to an induction of the lytic cycle of the viruses and the damaging of the hard corals.

The fact that the highest concentration with 4 mL sunscreen in 1 L fresh sea water had no effect on the sea urchins, suggests that those interactions between viruses and the embryos are not that common in this species, or if they occur then at least not that harmful. For example, the hard corals bleach within 96 hours when the concentration is as low as 10µL/L seawater. (Danovaro et al.)

Furthermore, the chemicals in the sunscreen seem to have no direct effect on the embryo's development. This finding contradicts the research of Corinaldesi et al., who tested three different sun-

screens with much lower concentrations on another sea urchin. In their results every sunscreen tested showed some result. The only difference was the sea urchin species (*Arbacia lixula* vs. *Paracentrotus lividus*). An explanation could be that *Arbacia lixula* is more resistant than other species, however, this is only a vague statement, and maybe this can be tested in the next Calvi course.

CONCLUSION

Overall, this protocol of our project in Calvi in 2021 gives us a small insight in the dangers that can result from the ongoing pollution of our oceans. In our experiments the main effect on the sea urchin embryos could be seen in the microplastic and cigarette experiment. Although the sunscreen does not affect the development of this species to the point of our research, we cannot say that other species may act as resilient against this cosmetic product.

This field of environmental toxicology is very important for the future as increasing portions of pollutants especially microplastic, nanoplastic) are getting into the world's oceans. The sea urchin embryo is an interesting model organism for that, and I think many experiments can be conducted with this species.

Table 6: The quantitative analysis for each of the experiments conducted in the week. Note that the controls that were made for the single experiments is always in the first row. The second experiment of Microplastic as well as the cigarette experiment were conducted at the same time with the same gametes, therefore the control was counted only once.

	Microplastic 1				Microplastic 2 first analysis				Microplastic 2 second analysis			
Stadium	Control	Rope	RopeWP	Control	RopeF	RopeWPF	Rope	RopeWP	Control	RopeF	RopeWPF	Rope	RopeWP
1-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
2-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
4-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
8-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
Cleavage	0	4	40	2	3	8	6	29	3	0	4	1	3
Blastula	0	13	57	48	50	62	58	25	15	33	28	38	40
Gastrula	0	16	2	7	4		1	0	27	8	12	8	16
Prism	0	46	0	0	0	0	0	0	10	7	3	2	0
Pluteus	100	1	0	0	0	0	0	0	1	0	0	0	0
Exogastrula	0	0	0	0	0	0	0	0	0	0	0	0	0
Degenerated	0	15	9	0	0	0	0	0	0	5	4	3	7
	Cigarettes first analysis						Cigarettes second analysis				Sunscreen		
Stadium	Control	C 10^4	C 10^3	B 10^4	B 10^3	Control	C 10^4	C 10^3	B 10^4	B 10^3	Control	4mL/L	4µL/L
1-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
2-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
4-cell	0	0	0	0	0	0	0	0	0	0	0	0	0
8-cell	0	0	0	0	0	0	0	1	0	0	0	0	0
Cleavage	2	24	42	4	0	3	0	46	1	1	0	0	0
Blastula	48	26	13	49	54	15	22	3	22	23	35	22	14
Gastrula	7	1	0	6	9	27	17	0	21	25	26	13	45
Prism	0	0	0	0	0	10	0	0	0	0	39	46	41
Pluteus	0	0	0	0	0	1	0	0	0	0	0	19	0
Exogastrula	0	0	0	0	0	0	8	0	0	0	1	0	0
Degenerated	0	0	0	0	0	0	3	2	7	2	0	0	0

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Inhibition of sea urchin development by the invasive alga *Chrysopheaum taylorii*

MATTHIAS ACHRAINER

ABSTRACT

Invasive species can have a huge impact on the ecosystem. Here we describe, how the invasive microalga *Chrysophaeum taylorii* among other algae can inhibit embryonic development of various sea urchin species. The produced mucilage of these algae seems to be based on the zoöspore stage of the alga. Further we show, that the zoöspores are mobile units and have the ability to self-organise by their own movement and by hitchhiking on other organisms.

INTRODUCTION

Invasive species in the Mediterranean sea are an increasing problem, causing a loss of biodiversity as well as being able to cause substantial economic problems. One of these invasive species in the Mediterranean sea is the microalga *Chrysophaeum taylorii*, which is known to produce mucilage. *Chrysophaeum taylorii* belongs to the family of Pelagomonadaceae and is described as a microalga, where the plants are filamentous. The filaments are exceedingly delicate, and the plant is between 1-3 cm in size. The reproductive form of the alga is a single-celled zoöspore. These cells are pyriform, ovoid or nearly round and have two short flagella at their anterior end (Taylor 1951).

Chrysophaeum taylorii can growth on rocks, on other algae, as well as on *Posidonia,* and is known for his bloom also from other spots in the Mediterranean sea, like in Sardinia (since 2007) or at Elba (since 2005) (https://www.researchgate.net). Although the effect of the mucilage on the environment and the ecosystem is not yet clear, studies showed that the bloom of *C. taylorii* can lead to a depletion of other macroalgae (Caronni et al. 2019). Density and growth of the microalgae are controlled by stressors like nutrient enrichment, mechanical disturbance, benthic organisms, and hydrodynamics (Caronni et al. 2017). Beside its role as an invasive species, the microalga is also known to produce antibiotic chrysophaentins (Davison und Bewley 2019).

Sea urchins have a long history as models in developmental biology. High numbers of synchronously developing embryos can be obtained quickly and easily, the embryos are transparent and can be treated with small molecules (Ettensohn 2017). The fertilized egg develops in around one day into a pluteus larva, allowing fast readouts during the experiment. Genomes (Sodergren et al. 2006) as well as CRISPR/Cas protocols (Oulhen und Wessel 2016) are available.

Based on previous observations, in this project we want to analyse the influence of the mucilage on the development of embryos in two different sea urchin species, *Arbacia lixula* and *Sphaerechinus granularis*.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Animals of the species *Arbacia lixula* and *Sphaerechinus granularis* were collected in the harbour of the STARESO station. Samples from *Chrysopheum taylorii*, *Halopteris sp*. and *Padina pavonika* were collected in the bay of Calvi (S.Fig 1).

FERTILIZATION

In general we followed the protocol published by Ettensohn (2017). Animals were put upside down in a reservoir, filled with seawater, as high that the madropore plate is still in contact with the sea-water. Further, 2 mL of a 0.5M KCl-solution were injected in the floor plate of the animals, leading in the release of sperm or eggs into the water of the reservoir. In the case, that a sea urchin did not released any material, we reinjected 2ml. Eggs were transferred into petri dishes, filled with sea-water. Sperm were added to start the fertilisation and synchronise the experiment. One drop of embryos were put on a slide and counted under the microscope. The embryos were analysed by their developmental stadium (2-8-cell, early cleavage, blastula, gastrula, prism, pluteus) and counted.

CREATION OF CONDITIONED MEDIA

Algae's were collected as described above and incubated in sea water. After around 30min, algae were taken out of the sea water and squeezed by hand 4-5 times. The exiting water was collected separately and was used to produce the conditioned media (CM). To produce filtered CM, CM of *Chrysophaeum sp.* was used and filtered with a 100µM filter 2-3 times. Fertilized sea urchin eggs (*Arbacia lixula*) were incubated in 6-well plated with filtered or unfiltered CM of *Chrysophaeum* and the different developmental staged were counted and compared 7h after the fertilization.

To produce different diluted CMs, the pure exiting water of squeezed *Chrysopheum sp*. or *Halopteris sp* was used. The unfiltered CM was diluted with fresh sea water to a final concentration of 5%, 10%, 25%, 50% and 100%, pure sea water was used as control. Fertilized sea urchins of the species *Sphaerechinus granularis* and *Arbacia lixula* were incubated in 6-well plates with the different diluted CMs of either *Chrysophaeum* or *Halopteris* and the developmental stages were counted and compared 24h after the fertilization.

REAGGREGATION EXPERIMENT

Single cell solution was produced as described above, including 1-3 filter steps. Ten to twenty ml of these solution were transferred into a 50ml Falcon tube and incubated at RT for 1-4h without moving or touching the tube. The arising aggregates were analysed by microscopy.

TIME LAPS MOVIES

One drop of the filtered 100% single cell solution were transferred to a hollow ground microscope slide. For timelaps 1 the shot-interval was 2min, for timelaps 2 2min.

RESULTS

Chrysophaeum taylorii conditioned media inhibit sea urchin development in a dose-dependent manner

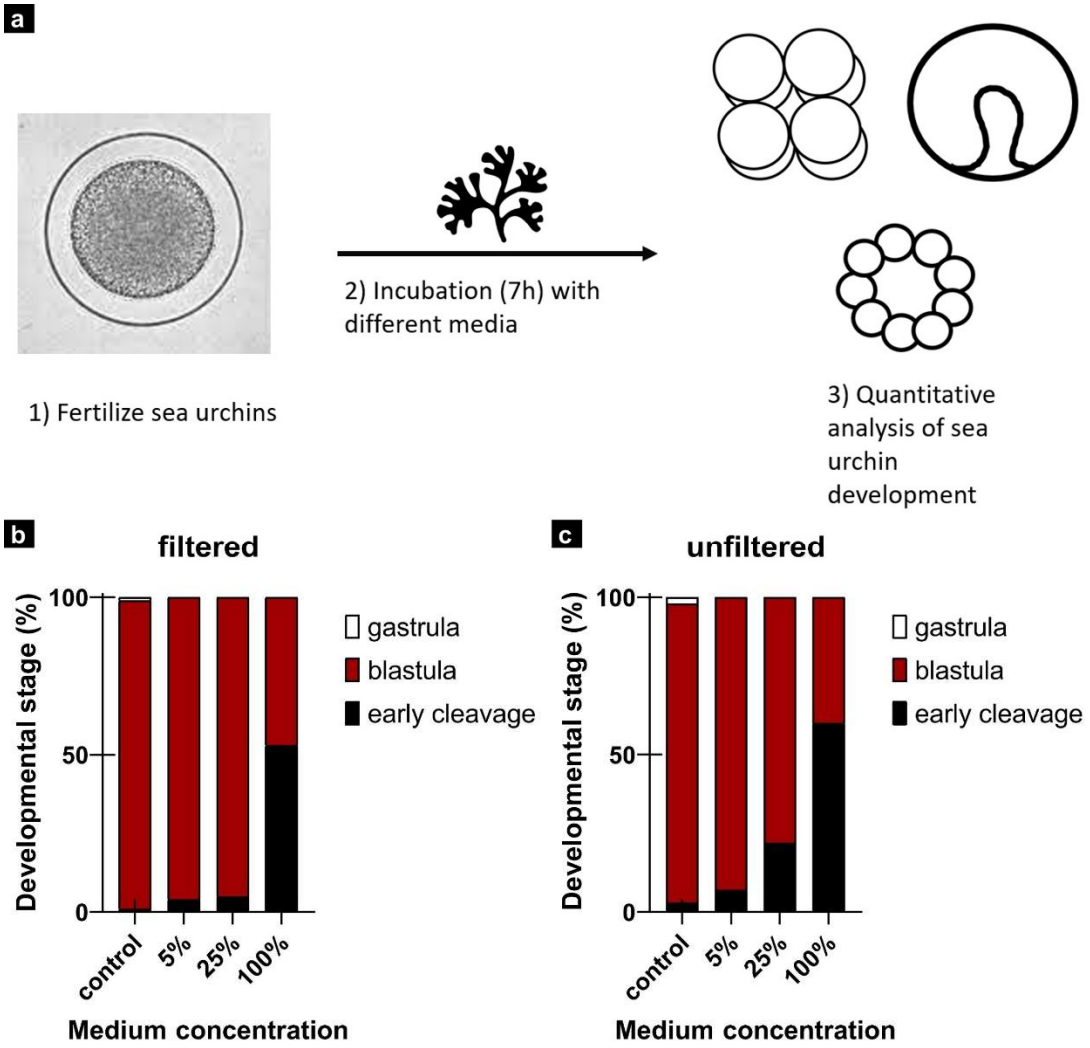

Figure 18: Chrysophaeum taylorii slows down sea urchin development. (**A**) Fertilized eggs were incubated in seawater containing either filtered or unfiltered Chrysophaeum CM. (**B**) Embryos, treated with 0%, 5% or 25% CM were mostly at the blastula stage after 7h, while embryos treated with 100% CM were mostly in the early cleavage stage. (**C**) Embryos, treated with 0%, 5% or 25% unfiltered CM were in most cases at the blastula stage after 7h, while embryos treated with 100% unfiltered CM were mostly in the early cleavage stage.

To investigate the influence of the invasive microalgae on embryonic development of sea urchins, we first collected sea urchins and *Chrysophaeum* samples in the bay of STARESO (see sup. Fig. 1A). We squeezed the collected mucilage and collected the resulting medium. Out of this 100% conditioned medium (CM), we created different dilutions in seawater. Sea urchins were forced to spawn by the injection of KCl and the collected eggs and sperm were put into Petri dishes to allow fertilization. Embryos then developed either in 0% (control), 5%, 25% or 100% CM, filtered or unfiltered (**Fig. 1A**). To analyse possible developmental defects or delays, we counted the total numbers of embryos

which were at the 2-8 cell stage, the early cleavage, the blastula-, the gastrula- the prism or the pluteus larva stage.

Nearly all control embryos (n=100) were in the blastula stage after 7h. We observed only few examples (<1%), which were early cleavage or later stages (**Fig. 1B-C**). Embryos, treated with 5% or 25% filtered CM showed no significant difference to the control group; more than 95% of all embryos were in the blastula stage. However, in contrast, embryos witch developed in 100% filtered CM showed a clearly slowed or inhibited development. While 47% of the embryos were in the blastula stage, 53% of the embryos were in the early cleavage stage (**Fig 1B**). Embryos treated with 0% or 5% unfiltered CM were with more than 95% in the blastula stage. The unfiltered 25% CM embryos, however, developed slower, 25% were in the early cleavage stage. Again, 64% of the embryos treated with unfiltered 100% CM were in the early cleavage stage (**Fig. 1C**).

Our data suggest, that the CM obtained from *Chrysopheum taylorii* can slow down or inhibit embryonic development of sea urchins. Unfiltered media seem to have a dose-dependent, stronger effect than filtered media.

Other algae also inhibit embryonic development of sea urchins

In order to clarify the specificity of how *C. taylorii* inhibits sea urchin development, we extracted CM media of microalgae, filtered it and created 6 different dilutions (0, 5, 10, 25, 50 and 100%). To proof that the developmental inhibition effect is *Chrysophaeum* specific, we used two different endemic algae as controls, *Halopteris sp*. and *Padina pavonika* (Padina data are not shown). We created CM from these algae and let the sea urchin embryos develop for 24h either in CM of *Chrysophaeum* or control algae (Fig. 2A). *Arbacia lixula* embryos of the control groups (0% CM) developed after 24 in the most cases (78%) into a pluteus larva (Fig 2B). In another data set, 98% of all *A. lixula* embryos were in the larva stage after 24h (data not shown). This differences in development can be caused by fluctuating temperatures in the lab, but we can conclude that the majority of all embryos should have developed to pluteus larva after 24h. In the case of *A. lixula* embryos treated with 5% *Halopteris* CM, 76% of all embryos were in the larva stage as well as 96% of all 10% *Halopteris* CM samples (Fig. 2B). Also embryos treated with small concentrations of the *Chrysophaeum* CM (0, 5, 10%) were with more than 75% in the larva stage (Fig. 2C).

Embryos treated with 25% *Halopteris* CM reached the larva stage only in 46% of all counted embryos. 28% were in the prism stage, and 28% in the gastrula stage. At higher CM concentrations, embryos developed in the majority only till the gastrula stage (50%-Halopteris-CM) or till the blastula stage (100% *Halopteris* CM; Fig .2B).

As shown before, small concentrations of the *Chysophaeum* CM have no (big) influence on the development of the sea urchins. However, also embryos treated with 25%-*Chrysophaeum* CM showed a slower development with only 40% larva-stage animals and more than 20% of all embryos were still in the blastula stage. The incubation in 50% Chrysophaeum CM leads in 58% of all counted samples in a blastula-stage, embryos treated in 100% *Chrysophaeum* CM arrived in 95% only the blastula stage with embryos, which are still in the early cleavage phase (Fig. 2C).

In summary, we showed that *A. lixula* embryos developing in different CMs show a clear developing defect, leading to a slower or completely abrogated development. The process seems not to be algae-specific, and it is working in a dose-dependent manner.

The developmental inhibition is a sea urchin species-independent process

To investigate if the negative influence of alga CM is independent of the sea urchin species, we collected also *Sphaerechinus granularis* animals in the bay of STARESO and let embryos develop in different CMs from *Halopteris sp.* and *Chrysophaeum taylorii*. As the S. *granularis* develop slightly slower then the *A. lixula* species, we counted only the embryos from 2-8 cell stage until the gastrula stage.

After 24h, the majority of all embryos had arrived at the gastrula stage in the control groups. Again, small concentrations of the CMs, either *Halopteris* or *Chrysophaeum* seems not to slow down or influence the development of the *Sphaerechinus* embryos (Fig. 2D-E). However, embryos developing in the 50% Halopteris CM showed a shift in the development, the majority of all embryos arrived only the blastula stage, with specimens that were still in the early cleavage stage. The treatment of the embryos with 100% *Halopteris* CM leads in 90% of all cases to a stop in the early cleavage stage, while only few embryos were able to develop further (Fig. 2D).

Again, low concentrated *Chrysophaeum* CM (0-25%) seems to have no effect on the development of the *Sphaerechinus* embryos, the majority of all embryos arrived the gastrula stage (Fig. 2E). Embryos treated with 50% *Chrysophaeum* CM developed in equal parts to the blastula or gastrula stage, while the incubation in 100% *Chrysophaeum* CM lead in the most cases to a developed blastula, with some examples that arrived only the early cleavage stage (Fig. 2E).

Our data indicates, that the inhibiting effect of the algae seems not to be restricted to one species, and is more a general effect on sea urchin early development.

The zoospore as the functional unit of the mucilage

Further, as we saw that the algae can influence the development of sea urchins and with that the ecosystem, we wanted to take a closer look on the mucilage. In general, the mucilage seems to be a big net/smear, based on some polysaccharide-like structure that connects the whole system (Fig. 3A). In this big net, a big community of species like nematodes or planktonic species can be observed.

In comparison to older descriptions of the microalga, we only could observe the zoöspore of *Chrysophaeum taylorii* in the mucilage. No other parts/form of the alga were observable. The zoöspore seems to have a polar design with chloroplasts included (**Fig. 3B**).

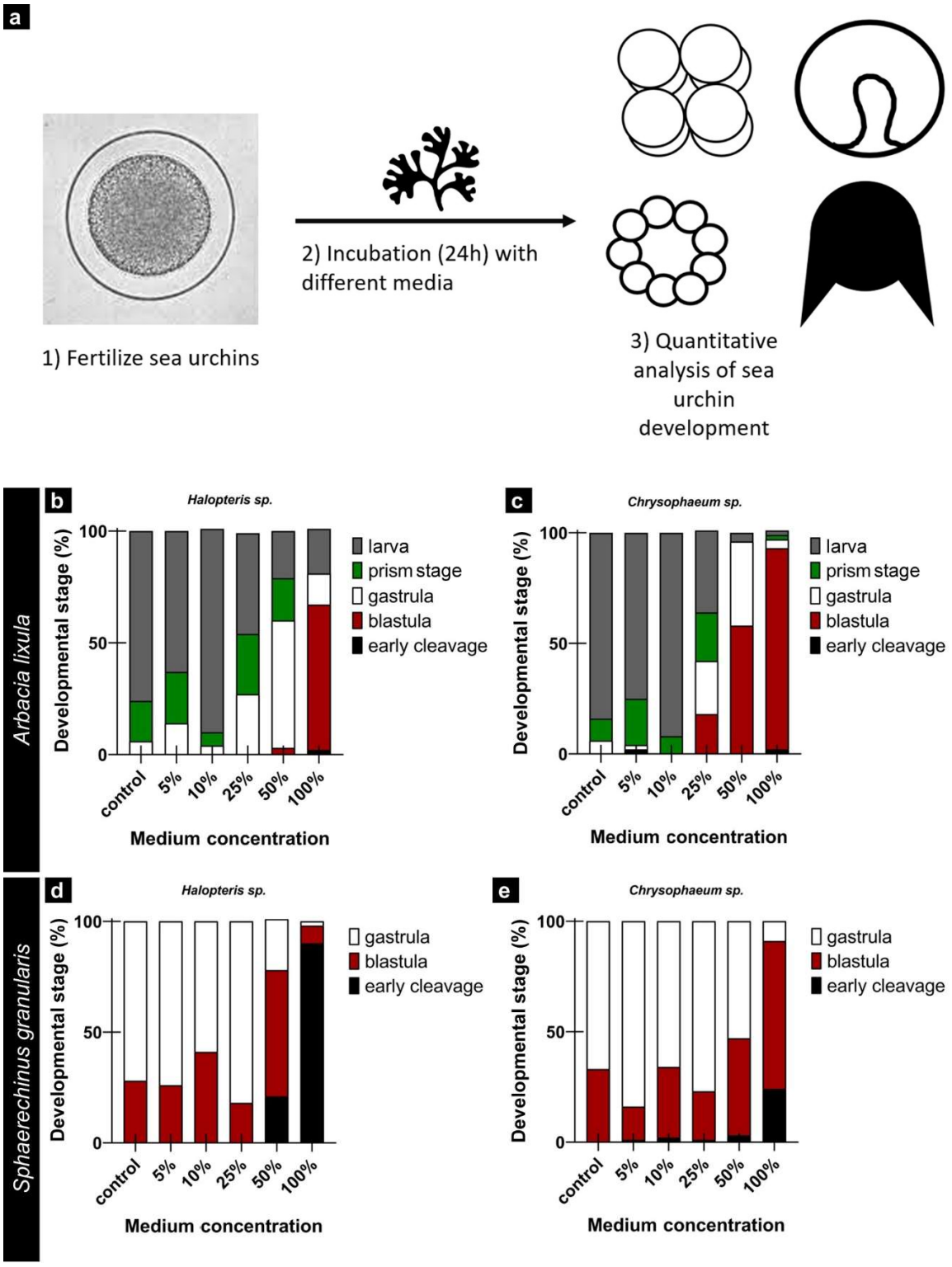

Figure 19: Different algae species inhibit sea urchin development in a sea urchin species-independent way: (A) Sea urchin embryos were incubated with algal CM from Chrysophaeum taylorii and Halopteris for 24h. The CM of Halopteris sp. and Chrysophaeum inhibits the development of Arbacia lixula embryos (B-C), as well as the development of Sphaerechinus granularis embryos in a dose-depending manner (D-E).

Chrysophaeum taylorii shows self aggregation behaviour

Interestingly, we observed in our highly concentrated CM of *C. taylorii* cell clusters and aggregates of the microalga. To proof this potential self aggregation process, we dissociated the mucilage with a pipette, shaked and filtered it, which resulted in a single cell algae solution (Fig. 3C). We incubated the single cell solution in falcons over time.

After one hour, big clusters of aggregated and connected zoöspores were observable in the falcons, while single cells were less present (Fig. 3D). This process and the size of the clusters were increasing over time, indicating a ongoing aggregation over time (data not shown).

Chrysophaeum taylorii is a mobile organism

To investigate, if it is possible that the zoospores can move by their own, we created again a single cell solution of zoöspores and performed time-laps movies over two hours (see supplementary movie 1). We observed that the zoospores seem to pump solution through their body to move from one point to another (Fig. 3E-F). Different zoospores in the video move into different direction, indicating that movement is not caused by environmental factors like small shaking or chemotaxis. It is unclear to what extent active movement occurs in natural environment, where waves and the tide have maybe a bigger influence on the distribution of the microspores.

Zoospores can hitchhike nematodes for aggregation

To further visualise the self aggregation process, we created again single cell solution with a high density of cells. Notably, in one of these timelapses we observed that the zoöspores stuck to a nematode (Fig. G; supplementary movie 2). Over time, the nematode tried to move and with this movement collected more and more zoospores, leading to a increasing *Chrysophaeum* aggregate (Fig. 3H).

Our data indicated, that the self aggregation process of *Chrysophaeum* can be caused by their own movement/behaviour as well as by other organisms like nematodes.

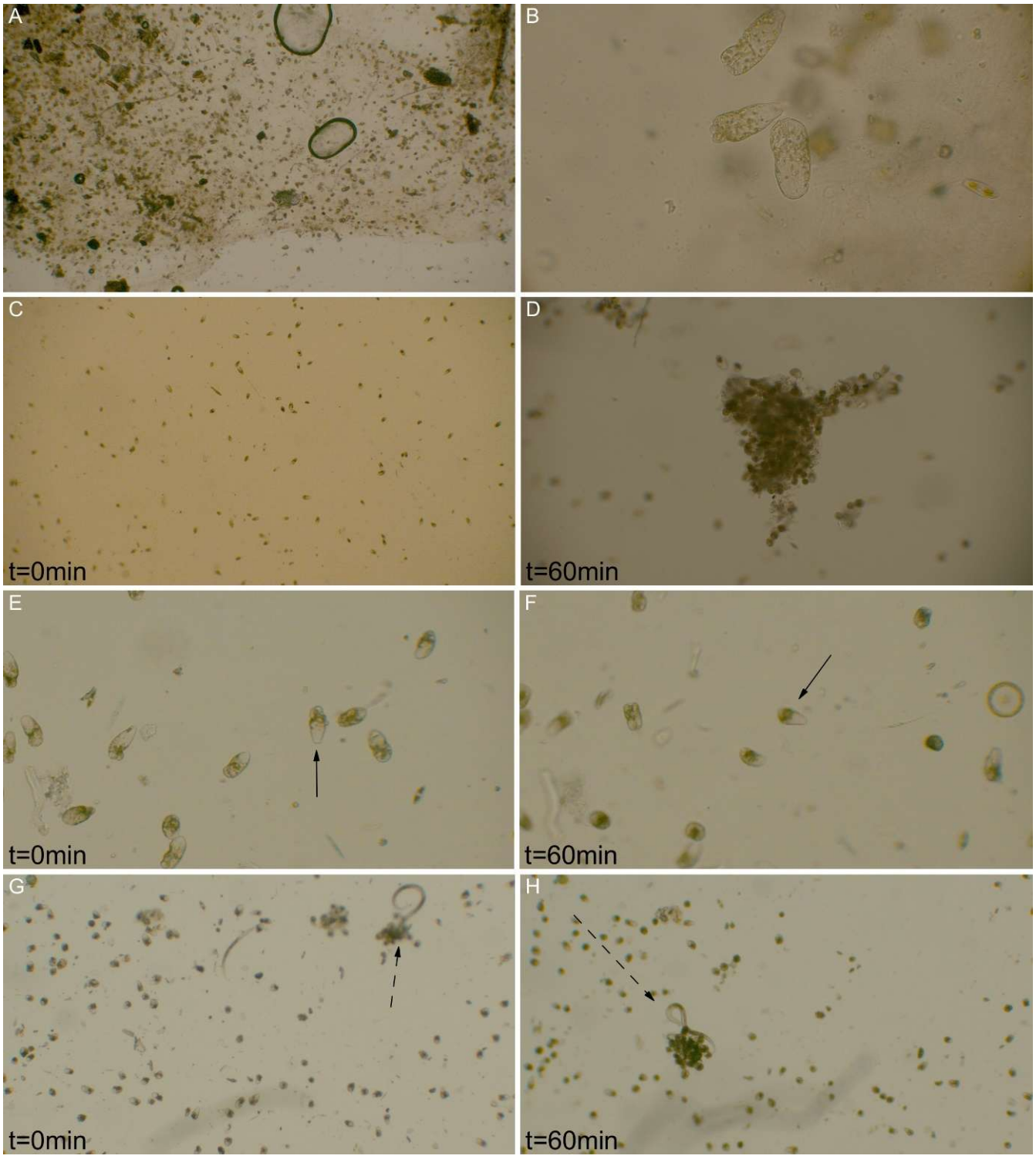

Figure 20: Structure and self-organisation of Chrysophaeum taylorii. The mucilage of the algae is based on a big connected net (**A**), while the bases of the mucilage is the zoospore form of the alga (**B**). C. taylorii can aggregate over time (**C-D**), move by their own (**E-F**) and use nematodes for the aggregation process (**G-H**). Arrows indicate the same zoospore, moving over time. Dashed arrows indicate nematode-based larger aggregates.

DISCUSSION

Previous studies showed the wide and complex influence of invasive species on their new environments (Bradley et al. 2019; Linders et al. 2019). Here we report, how the presence of an invasive algae can interfere and inhibit the development of sea urchins (**Fig. 1-2**). Surprisingly, also native algae species effects the development in a negative way (**Fig. 2**). These may have evolved as a natural strategy to control the sea urchin density, as some sea urchin species also use algae as food source. As both species, *A. lixula* and *S. granularis*, were negatively affected by the algae, we can consider that these effects are not species-specific. However, all our results are lab-experiments; field experiments are needed to proof our data.

While there are several reports that molecular factors (Kumano und Foltz 2003) as well as pollution like microplastic (Rendell-Bhatti et al. 2021) can inhibit or interfere with the development of sea urchins, our observations strongly indicate different algae can do the same. The underlying molecular mechanisms are not known. The data of *Chrysophaeum* CM at higher concentration showed that most embryos only reached the blastula stage in both species (**Fig. 2C; E**). This indicates that the microalga produces small molecules or something else, which inhibits or interferes with the gastrulation process. Whether the algae stop or slow down embryonic development is not yet clear. More elongated observations are necessary to clarify this issue.

Further experiments showed that *C. taylorii* can move independently over short distances and reaggregate at the spore stage (**Fig. 3E-F**). The ability to self-organise could be highly relevant, if we think about spreading of the mucilage. Single spores could be distributed by high wave activity, aggregate and colonize new areas.

In summary, here we reported for the first time that algae can inhibit the development and embryogenesis of sea urchins, and we showed that single zoospores of *Chrysophaeum* may be capable of reaggregation and self-organisation.

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SUPPLEMENTARY MATERIAL

Supplementary figure 1: Sample-places of sea urchins and algae's. White star indicates sample places of different sea urchins, white plant indicates the sample place of the different algae's.

Supplementary movies 1 and 2

Fish surveys

NICOLAS BIOLY

PAMELA NENNING

MARIE-LUISE CONTALA

MORITZ NOWAKOWSKI

VERONIKA PEER

ALEXANDER VORLEUTER

REINHOLD HANEL

Fish Diversity and Abundance – Transect

NICOLAS BIOLY & PAMELA NENNING

INTRODUCTION

With an average depth of 1 460 meters (maximum 5 267 meters) and an area of 2 969 000 km² the Mediterranean Sea is the largest landscape enclosed basin on Earth and represents the home of more than 17 000 marine species (Coll *et al.*, 2010). The fauna and flora is mainly of Atlantic origin but about 15-16% of the species are endemic (Tortonese, 1985). Therefore the Mediterranean Sea is a biodiversity rich environment which includes many different habitat types.

Since 1992 the University of Innsbruck together with the Christian-Albrechts-University Kiel and the GEOMAR Helmholtz-Centre for Marine Research biannually conduct biodiversity surveys around STARESO station along Revellata peninsula in the Bay of Calvi.

One of the main research interests during these courses is the assessment of fish species diversity and abundance in the vicinity of the research station. This assessment is conducted by transect swimming to avoid destructive methods like fishing surveys since the littoral zone along the Revellata peninsula is a protected area (Thanopoulou *et al.*, 2018). Even though the results may highly depend on the observer's experience and also the season (Williams *et al.*, 2006), visual assessment surveys provide a good opportunity for long-term investigation of fish species diversity, abundances and even biomass estimations as well as fish behavioral patterns in different habitats. Azzurro *et al.*, 2007 e.g. reported activity patterns of different fish families based on visual inspection and described groupers (*Serranidae*) to be more active during the daytime while cardinalfishes (*Apogonidae*) tend to be more night-active. During a 24 hours transect observation such diurnal patterns of different species could probably be investigated in Corsica as well.

A further aim was to recognize changes in the macroalgae coverage of hard substrates within the transect compared to previous years. Algae could be indicators for environmental changes caused by ocean warming or acidification, since previous studies already showed that impacts of human activities are proportionally stronger in the Mediterranean than in any other sea of the world (Tew, 2010). Therefore it can be concluded that the Mediterranean biodiversity is strongly influenced by natural and anthropogenic impacts (Coll *et al.*, 2010). So substrate surveys and an investigation of algae coverage was performed not only to get an over-view of habitat changes over time but also to assess possible linkages between substrate quality and fish diversity and abundance.

MATERIAL AND METHODS

The survey area around STARESO Station not only provides boulder fields, which are influenced by both terrestrial nutrient sources and by coastal aquatic phytoplankton production (Levinton, 2018) but also wide-ranging seagrass meadows of endemic Posidonia oceanica which represents an endangered habitat. Hence the area surrounding the bay of Revellata has been a marine protected area for more than 30 years.

To determine the local fish diversity and abundance around STARESO, a transect line in form of a colored rope was laid out on the ground over 155 meters around the harbor wall of the station, covering different substrate types. The transect was divided into four sections according to the different habitat types (Figure 1A). The beginning and end of each section was marked by self-constructed buoys (empty milk bottles), which at the same time marked the later breakpoints dur-ing transect swimming to discuss and note the observed findings. Labeled stones were used to flag the observation area of the transect, which was 3 meters on both sides of the rope, resulting in a corridor of 6 meters width. To obtain a depth profile of the entire transect the depth measure-ments were carried out every 5 meters along the rope using the PLASTIMO ECHOTEST II device (Fig-ure 1B), revealing the harbor entrance with fields of seagrass as the deepest part (around 8 meters) of the transect.

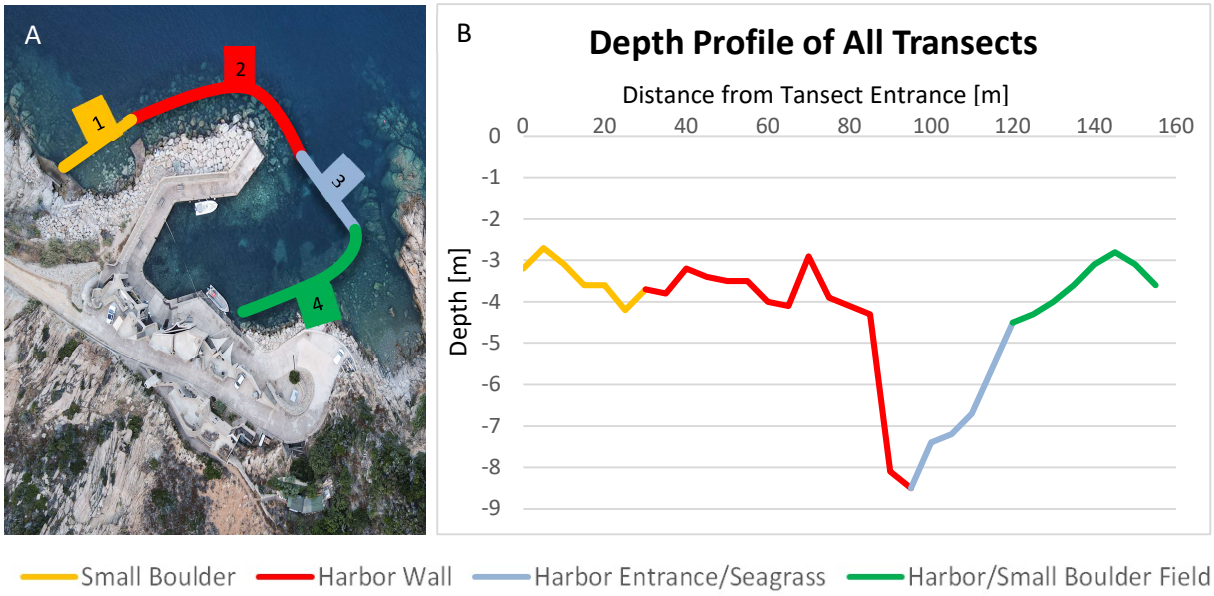

Figure 21: (A) Scheme of coast around STARESO station showing the different parts of the line transect. (B) Depth profile of all transect parts. (1, orange): Small boulder containing the entrance and therefore the starting point of the transect. (2, red): Harbor wall with bigger boulder field. (3, blue): Harbor entrance with fields of seagrass and sand. (4, green): Harbor entrance of STARESO station with small boulder field containing the last point of the transect.

To better define the different habitats, the diameter of 5 representative stones per transect section were obtained using a common measuring tape (Figure 2). To determine algae diversity and cover-age, 4 representative algae species were collected within each transect section and identified using general identification literature (Riedl, 1983). Additionally 3 1 m² frames were placed on the ground of each transect part (Figure 3) to measure algal coverage by picture documentation. The depths of the laid out frames were determined using the PLASTIMO ECHOTEST II device. The following scale was used to rank the contributions of the algae species to the total algae coverage:

A = <5%, B = 5-15%, C = 16-25%, D = 26-50%, E = 51-75%, F = 76-100% (Table 1).

One species could not be identified with certainty which is referred to as “unknown alga” within the subsequent analysis.

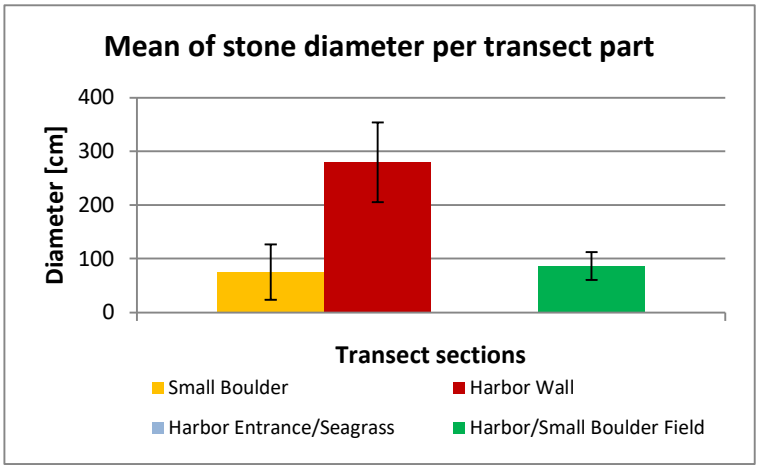

Figure 23: Mean and standard deviation of stone diameter per transect section measured by a common measuring tape. (n = 5)

Figure 22: Representative square on the ground of transect part 1 to determine algae coverage and concentration.

Transect observation was conducted every 3 hours within a 24 hour observation period from 12:00 to 12:00, resulting in a total of 9 observations. Therefore two people snorkeled for about 30 minutes at the surface along the transect line with constant speed and without turning around or diving down to prevent disturbance of the fish communities. After every section of the transect, the observation team paused to compare sightings and document the fish counts of each species on a diving slate which was prepared in advance. Abundance estimations were performed according to the following categories:

A = 1, B = 2-5, C = 6-30, D = >30 (Table 1).

For analysis these categories were replaced by defined values (A = 1, B = 3, C = 20, D = 60, Table 1) and all observed species were categorized by families to provide comparability to previous investigations. A closer look was given to wrasses (Labridae), seabreams (Sparidae) and smelts (Atherinidae), since these families were already reported to show major differences in circadian behavior patterns in previous studies.

Table 7: Categories of abundances and chosen values for later analysis

Category	Counted fish number	Analysis fish abundance	Analysis algae coverage
A	1	1	<5%
B	2-5	3	5-15%
C	6-30	20	16-25%
D	>30	60	26-50%
E	-	-	51-75%
F	-	-	76-100%

Subsequently, the average bayesian weight per species was calculated using the average length according to Fishbase (Bailly, Froese and Pauly, 2021) and if no data were available according to further literature (Sousa, 1979; 'NOAA National Center for Research on Aquatic Invasive Species (NCRAIS)', 2021; Elshakh *et al.*, 2021; Jonna, 2021; Mazza and Marion Beltramini, 2021; Onay, 2021). Hence the biomass per species per m² was calculated using the entity of all observations per species within 24 hours.

RESULTS

ALGAL COVERAGE:

The results of the algae growth in the different transects were evaluated (Table 2). These results were also interpreted graphically in Figure 4 to present a better overview of the results.

Table 8: Shows the percentage of algae growth in the various transects and the depth at which the measurements were carried out. Whereby the letters indicate the following degree of coverage: A<5%, B=5-15%, C=16-25%, D=26-50%, E=51-75%, F=76-100%)

Transect section	Replicate	Depth in [m]	Total Cover	*Padina pavonica*	*Dictyota dichotoma*	*Chrysophaeum taylorii*	*Halopteris scoparia*	*Jania rubens*	*Dictyota linearis*	*Posidonia oceanica*	"Unknown alga"
Small Boulder	1	3	100	D	A	C	B	0	A	0	D
	2	2,8	95	C	A	C	B	0	B	0	D
	3	2,9	50	B	0	A	B	0	A	0	C
Harbor Wall	1	3,3	100	B	0	D	B	A	0	0	C
	2	2,9	100	B	0	E	B	0	A	0	B
	3	1,9	95	B	B	B	D	A	A	0	C
Harbor Entrance	-	-	100	0	0	0	0	0	0	F	0
Harbor / Small Boulder	1	3,9	100	D	A	C	B	A	A	0	D
	2	2,5	100	D	0	B	D	0	A	0	D
	3	2,9	100	D	A	A	D	0	B	0	D

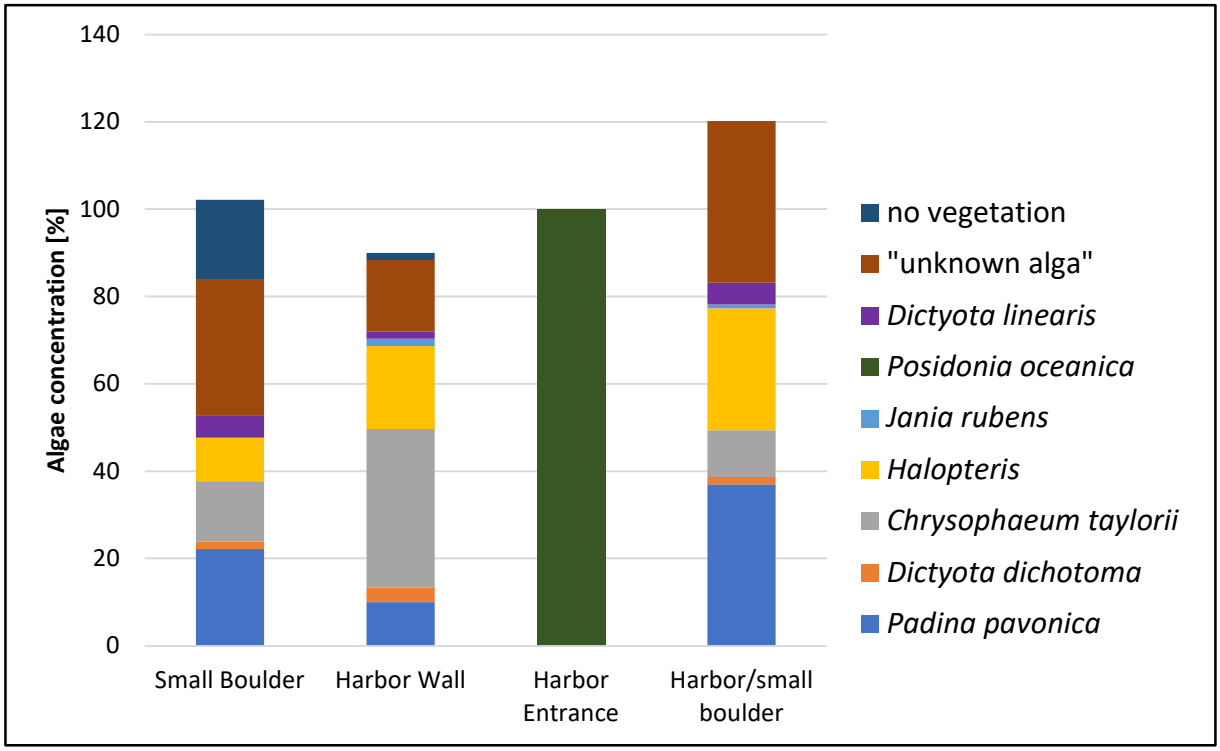

Figure 24: Shows the different algae concentrations from Table 2 in the respective transect. In addition it must be mentioned that the concentrations shown here, are fixed numbers and not a framework as in Table 2. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the algae concentrations shown here.

Figure 25: This species could not be identified with certainty which is referred to as “unknown alga” within subsequent analysis.

In the first transect section from Figure 4, the most dominant algae were "unknown algae" (Figure 5), Padina pavonica and Chrysophaeum taylorii. In the “Harbor Wall” sector, this composition is already changing, so the concentration of Chrysophaeum taylorii increased by more than double. Also there is an increase in Halopteris scoparia. In contrast, the frequency of “unknown alga” and Padina pavonica drops significantly. No major changes were found in the remaining algae species. If you take a closer look at the third section of the transects, you will see that there are no more algae at all. This is the case because the whole area is covered with Posidonia oceanica. The last section of the transect, “Harbor/small boulder”, has a similar concentration of algae species as the first section, with the exceptions of Halopteris scoparia and Padina pavonica. However, this difference is not as strong as it initially appears, because the percentage from the fourth transect, it is well over one hundred. Therefore, the algae species concentrations are more likely to be somewhat below the mean value of the concentration range used. Hence, it can be stated that transect one and four have very similar algal species concentrations. Furthermore, the data from Table 2 were compared with the data from the marine biology excursion 2018, which carried out the same measurements (Figure 6). Species of the genus Halopteris and Dictyota were grouped under their genus in order to enable a comparison with the old data. On closer inspection of the algae composition of the first transect “small boulder”, it becomes apparent, that a noticeable decline in Padina pavonica can be observed in this area over the years. In contrast to this, an increase in Chrysophaeum taylorii, Halopteris and other algae can be observed. Jania rubens could not be observed in 2021. In the second transect section “Harbor Wall” a clear decline in the number of Halopteris species can be observed. In contrast, there is an enormous increase in Chrysophaeum taylorii. For the other algae, no change in density from 2018 to 2021 could be determined in this area. The third transect, "Harbor Entrance", was not dealt with in Figure 6, since then as now it has 100 percent coverage of Posidonia oceanica. The general growth of algae in the fourth transect has increased considerably over the years, so that the entire habitat is now covered by algae. The concentration of Halopteris and Chrysophaeum taylorii increased, too. The rest of the algae displays a similar concentration like in the year 2018. With the exception of Jania Rubens which was only observed to a small extent in 2021.

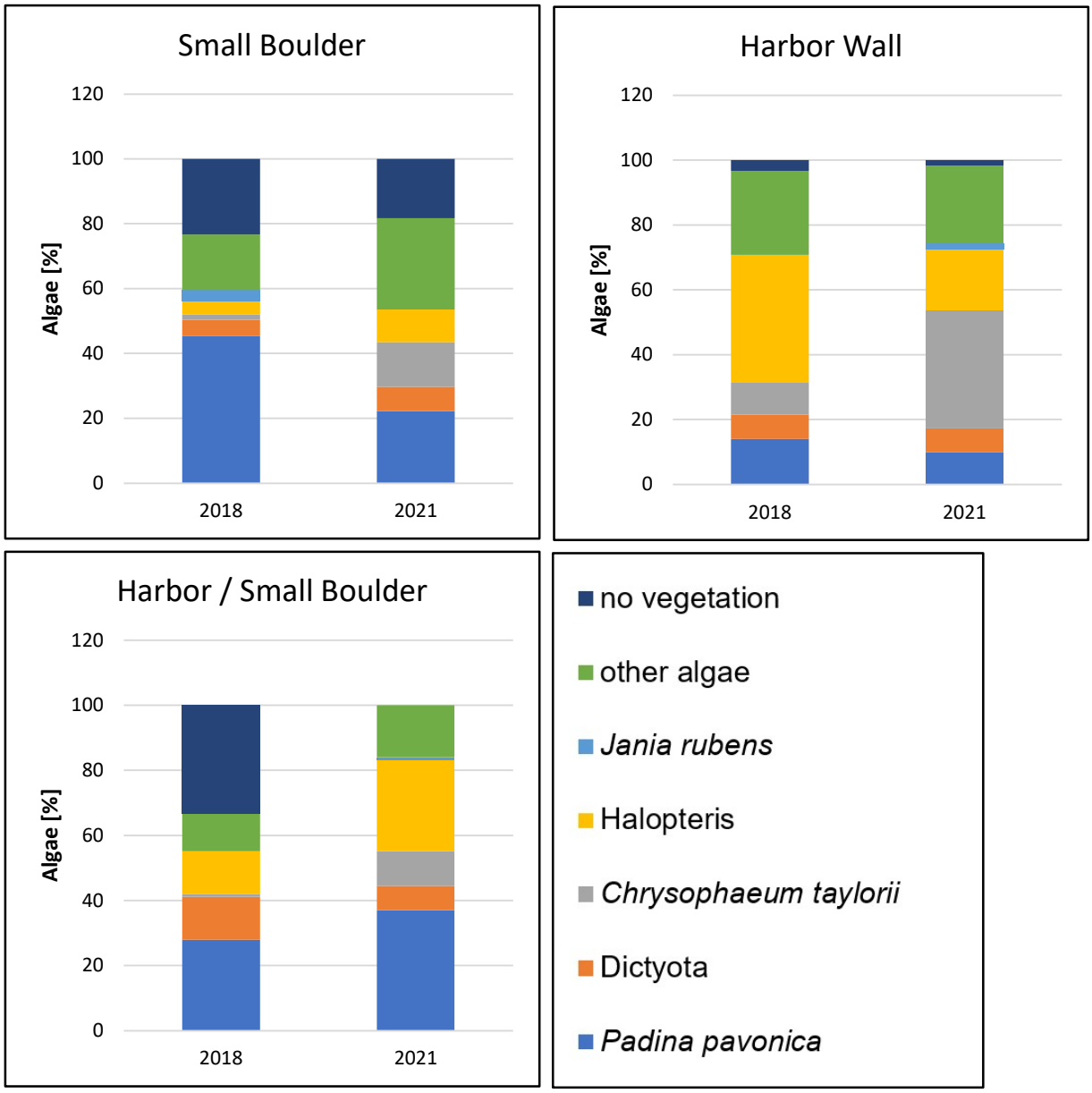

Figure 26: Indicates the algae concentration of different species or families in three different transects. The data from the Marine Biology course 2018 are shown as well as the data obtained in this study (Table 2). In addition it must be mentioned, that the concentrations shown here are fixed numbers and not a framework as in Table 2. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the algae concentrations shown here.

Fish Transect

A total of 27 species out of 10 families were observed during the transect observations. Figure 7 shows how many individuals of the respective family were sighted in all transects over the entire monitoring period. This shows, that the most common species belong to the families Sparidae, Atherinidae, Labridae, Mugilidae and Pomacentridae. A closer look at the 2018 transect data (Figure 8) shows that these five families were also the most abundant at that time. The earlier transect data also show how many and which fish families were present per transect in the study area in 2018. This

was also evaluated in this study. See Figure 9. In addition, comparing the 2018 and 2021 data shows that the ratio of fish sighted to transects is the same in both studies. Most fish were counted in the second transect and the fewest in the third transect. The second most fish were counted in the fourth transect. It is also striking that the ratio of fish family concentration to each other in the four transect sections is about the same in both studies.

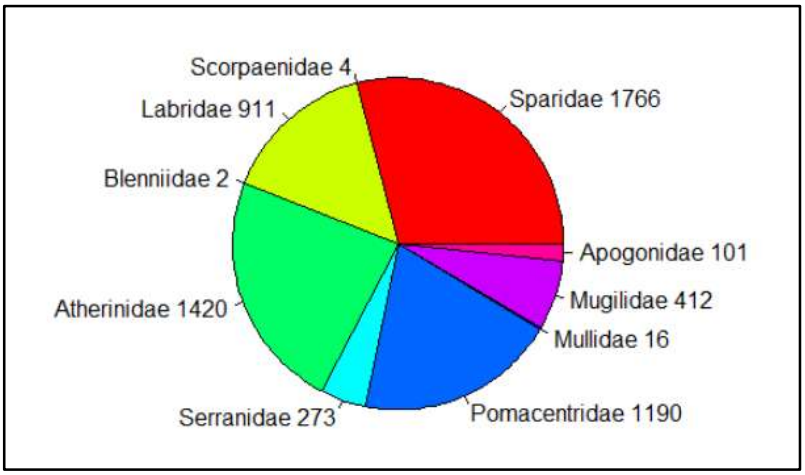

Figure 27: Indicates the number of sighted species of the respective families in the line transect within 24 hours. In addition it must be mentioned that the data shown here are fixed numbers and not a framework. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the fish numbers shown here.

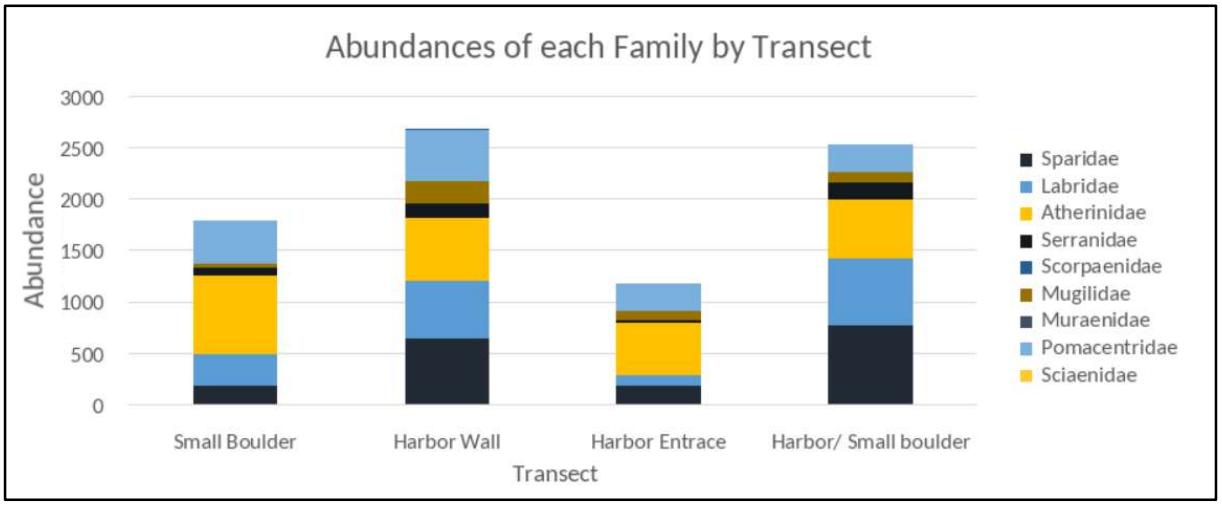

Figure 29: Abundance of each family by transect from the marine biology excursion 2018.

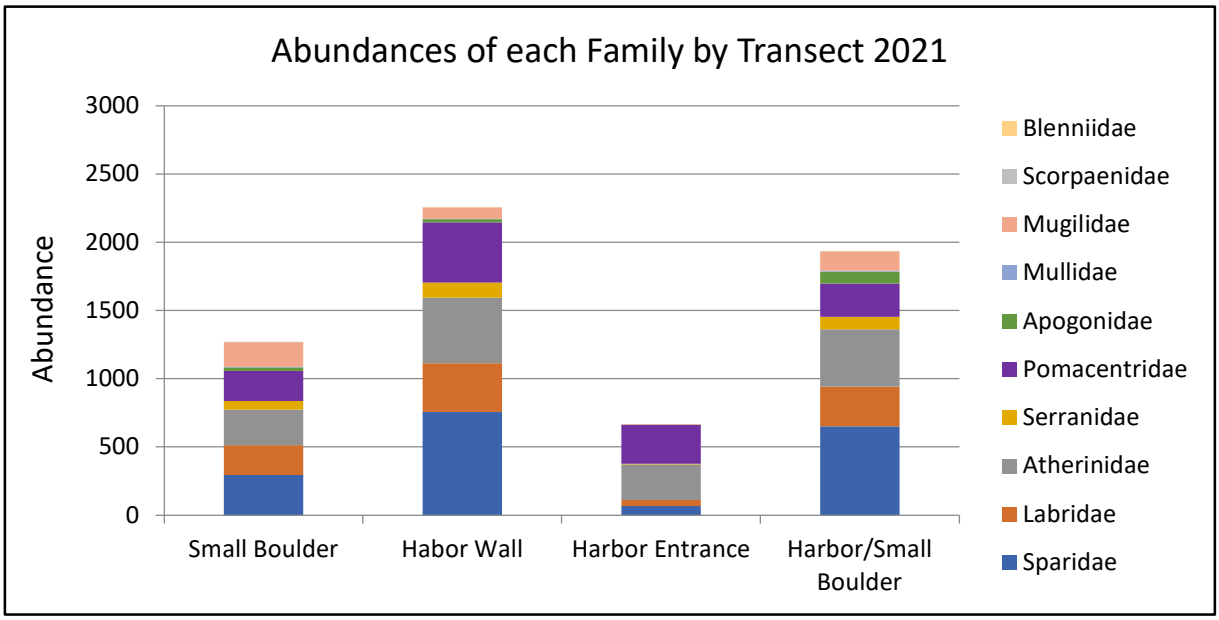

Figure 28: Abundance of each family by transect over 24 hours from 2021. In addition it must be mentioned that the data shown here are fixed numbers and not a framework. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the fish numbers shown here.

Furthermore, the abundance of the fish was evaluated per m^2, since the four transects were of different sizes (Figure 10). It is noticeable, that there were most fish per m^2 in the first transect. Followed by the fourth transect with the second most fish in it. The second transect comprised the third most fish and the third transect included the fewest fish.

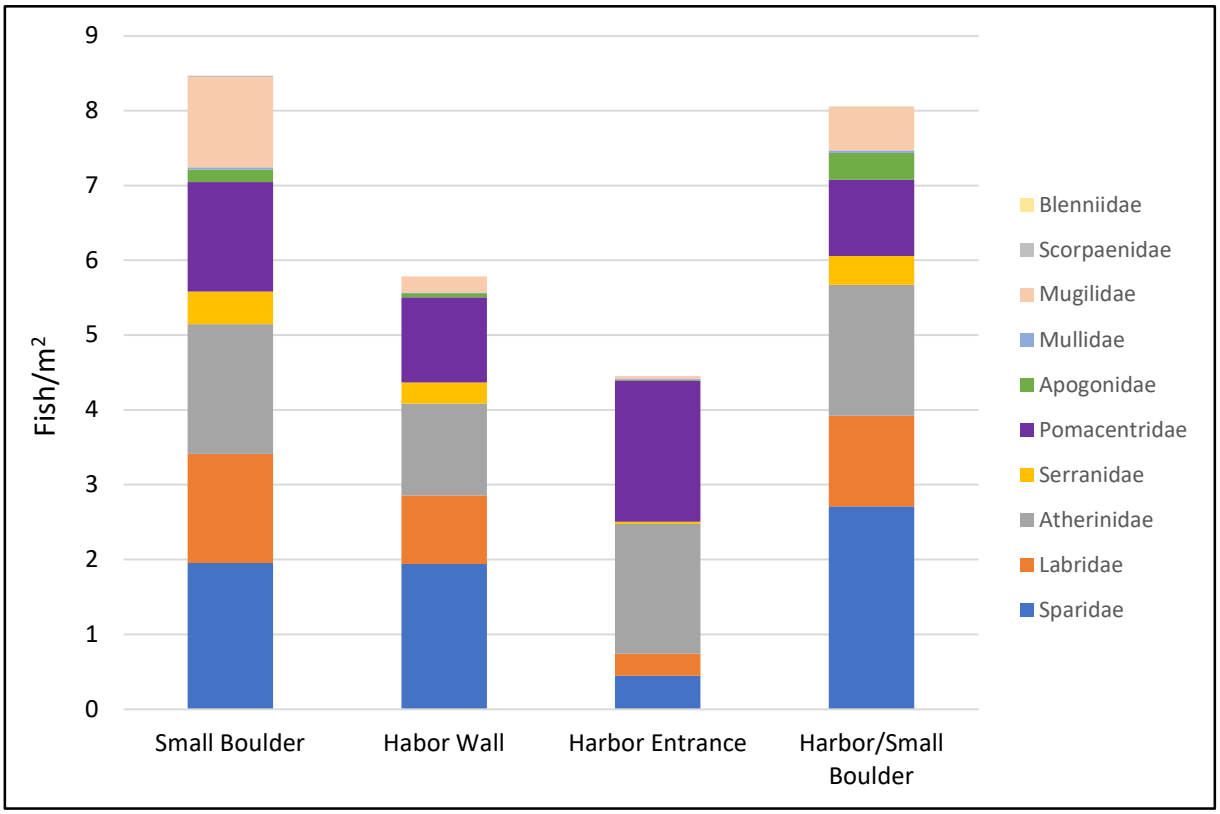

Figure 30: Fish per m^2 of different families by transect over 24 hours from 2021. In addition it must be mentioned that the data shown here are fixed numbers and not a framework. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from

However, the previous data only provided total numbers of species and did not show the ratio of biomass of fish in the transect areas. Therefore, from various sources and publications, the average weight of the respective fish species was used to calculate and display the biomass per m^2 (Figure 11). The bayesian weight was used in the calculation. However, it must be considered, that most of the fish in the studied area have a lower weight than the bayesian weight, that was used for the calculation and therefore the values for most of the fish are slightly lower. On closer look at Figure 11, you can see that the majority of the biomass is made up of the *Sparidae* and *Labridae* familys, followed by *Serranidae* and *Pomacentridae*. It can also be seen that the largest part of the biomass is in the transect "Harbor/small boulder" and the second most biomass is in the first transect "small boulder". The fewest amount of biomass can be found in the third transect, “Harbor Entrance”.

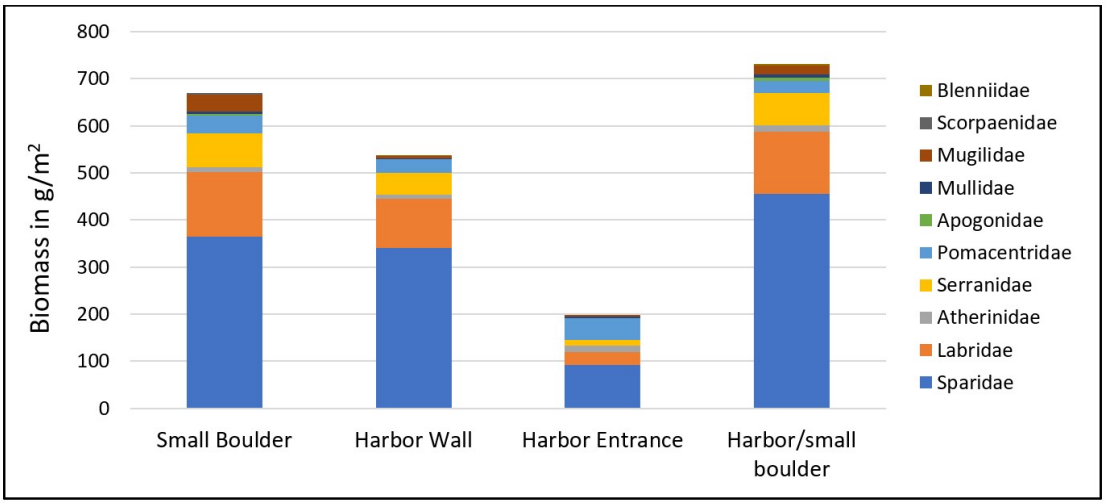

Figure 31: Indicates the biomass of the investigated fish families in grams per m² in the various transects within 24 hours. In addition it must be mentioned that the data shown here are fixed numbers and not a framework. Therefore, this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the fish numbers shown here.

Ultimately, the transect data obtained can also be used to look at the behavior of the fish according to the time of day (Figure 12). At Nr. 1 it is noticeable, that the Atherinidae were seen more at night. In addition, no time-of-day-dependent behavior could be determined in the *Serranidae* and Apogonidae. Looking at Nr. 2 it is evident that *Scorpaenidae* were also mainly sighted at night. Many *Mullidae* were observed during the day, but there was a clear peak at midnight. The *Blenniidae* only show a peak at 12:00 o'clock. Nr 3 in Figure 12 shows the *Sparidae*, *Labridae* and *Mugilidae* which all display a similar behavior patterns. During the day they were clearly represented and the further it got night, the less this was the case until an increase could be observed again at dawn. The *Pomacentridae* show this behavior patterns, too, but they were still strongly represented until 9 p.m. before they were no longer seen. They also could be seen in large numbers from 6:00 a.m.

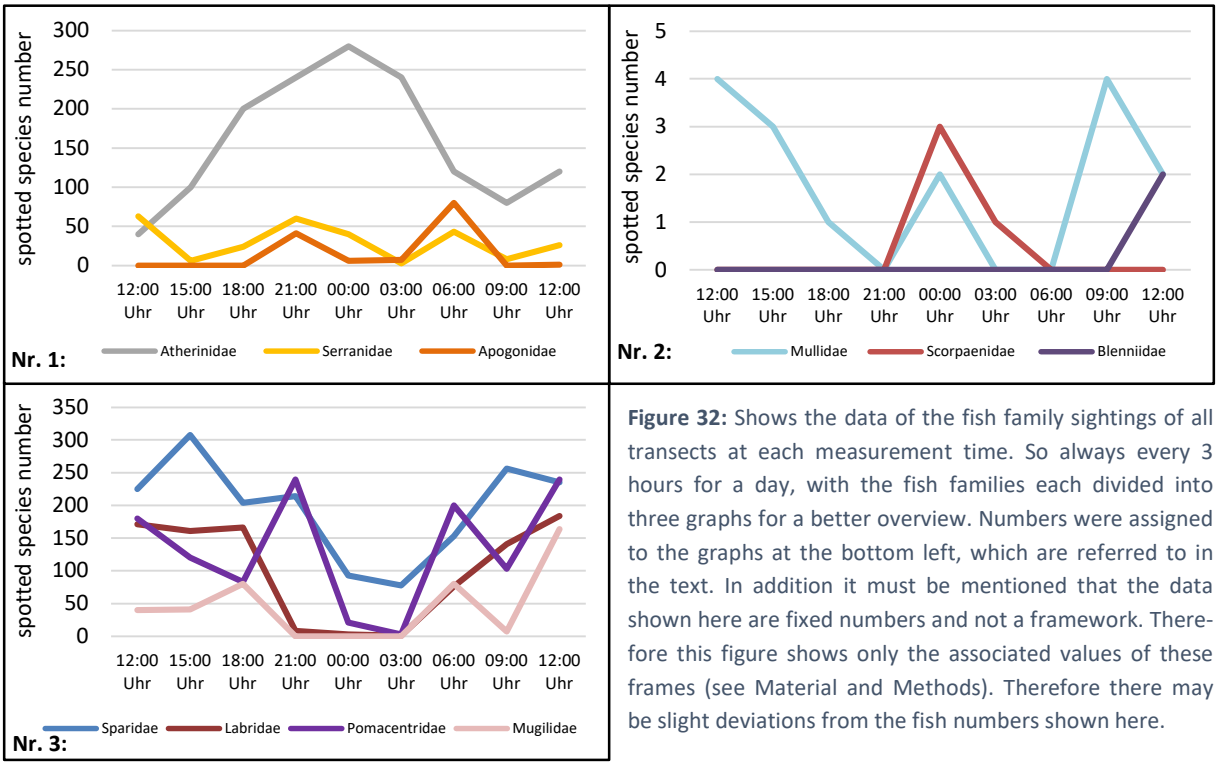

Figure 32: Shows the data of the fish family sightings of all transects at each measurement time. So always every 3 hours for a day, with the fish families each divided into three graphs for a better overview. Numbers were assigned to the graphs at the bottom left, which are referred to in the text. In addition it must be mentioned that the data shown here are fixed numbers and not a framework. Therefore this figure shows only the associated values of these frames (see Material and Methods). Therefore there may be slight deviations from the fish numbers shown here.

DISCUSSION

HABITAT COMPOSITION

First of all, it becomes apparent that the habitats through which the transect runs are very different. This is clearly illustrated by Figure 1 and 2. This becomes even clearer, if you consider Figure 4, because there it is visible, that each transect has a very specific algae composition, except the third transect, which is completely overgrown by *Posidonia oceanica*. Only the first and fourth transects seem to be similar in terms of the algae composition. This is not too surprising, as they initially have the same stone sizes, as can already be seen from Figure 2 and are also quite similar in terms of depth (Figure 1) and position. Both transects lie close to the shore. These factors seem to be the perfect habitat for *Padina pavonica* and the "unknown alga". This assumption is supported by the fact, that they mainly appear in transects one and four. In the second transect, the main algae are *Halopteris* and *Chrysophaeum taylorii*. It seems, that these algae prefer larger areas of stones as habitat, as they are found in greater concentration in transect two. The study by Caronni et al. 2015 shows that this assumption applies to *Chrysophaeum taylorii*. If you take a closer look at the change in algae concentration over the years (Figure 6, "Small Boulder") it becomes apparent that *Padina pavonica* is significantly less present in the first transect in 2021, than it was the case in 2018. In the case of *Chrysophaeum taylorii*, *Halopteris* and other algae, it is exactly the other way round. They were much more represented in 2021. In the second transect a decline of *Halopteris* can be noticed. In contrast to *Chrysophaeum taylorii*, which has spread incredible strong. In the fourth transect it becomes clear that there are much more *Chrysophaeum taylorii* than in 2018. An increase in *Halopteris* is also visible. It can be determined, that much more area is overgrown with algae than in 2018. These changes over the years could have several reasons. At first, various factors could have caused this behavior in the algae, such as a lack of nutrients, increased solar radiation or changed temperatures. The most credible explanation for this behavior of the algae is, however, that the alga *Chrysophaeum taylorii* massively displaced the alga *Halopteris* in the second transect. The strong decrease in *Halopteris* in the second transect would speak for this, as does the strong increase in *Chrysophaeum taylorii*. This would also explain, why more *Halopteris* were found in transects one and four, as the algae had to spread to other areas in order to survive. This would also match with previously obtained data, as it has already been shown in other works, how aggressively *Chrysophaeum taylorii* spreads in the Mediterranean sea (Aktan and Topaloğlu, 2011). On the other hand there can be high incidence of this species in small areas, too (Aktan, Dede and Ciftci, 2008). Ultimately, the development of *Chrysophaeum taylorii* must be closely monitored over the next few years, as it can massively change ecosystems and already has it in our studied area, if you take into account the already massive change in the composition of the algae (Figure 6) (Blasi and Caronni, 2015; Caronni *et al.*, 2015, 2017). In addition the influence of algae on various organisms is also particularly problematic. In this study, it could be proven that the development of sea urchins was strongly influenced to the negative, when they came into contact with the mucus produced by *Chrysophaeum taylorii* (See chapter: Influence of algae treatment on sea urchin development: Results, Discussion). It is therefore of the utmost importance to continue to investigate, in detail, the influence of this alga on the ecosystem in order to identify any negative consequences at an early stage and, if necessary, to initiate countermeasures.

FISH TRANSECT

Figure 7 already made it clear that the main fish families, that occur in the study area are the *Labridae, Sparidae, Mugilidae, Atherinidae* and *Pomacentridae*. These five fish families were also the most dominant ones represented in the previous course reports. The results showed a decrease in fish sightings from 2018 (Figure 8) to 2021 (Figure 9), which could indicate a decline in fish populations. However, if considering the data from the 2016 Marine Biological Course, significantly fewer fish, than 2018, can be determined. The comparability of these results must therefore be questioned. There are other reasons for this assumption, too. Initially, very inexperienced persons are responsible for these monitoring. Furthermore, the monitoring were carried out by several people, which leads to further measurement errors, as each person is differently qualified in species recognition and thus each measurement differs from person to person. Another reason is, that these monitoring projects do not take place at the same time in the year. It could be possible, that at certain times of the year some fish species are more and others less represented in the study area. Ultimately, therefore, the comparability to previous course measurements must be viewed as limited. However, despite these discrepancies, some interesting conclusions can still be drawn from comparing the two results. For example it was clear, that the sighted fish in both works were distributed in the same proportion to the transects. This can be compared as the 2018 and 2021 transects were near the same size. In addition this distribution could be found in other course reports, too. For this reason, this distribution shows a good picture, that there have been no major changes in the way of life of the fishes. This fact is reassuring, especially with regard to the strongly reproducing alga *Chrysophaeum taylorii*. Despite this massive spread of this alga, no change in the behavior of the fishes could be detected. Another reason which supports this fact, is that the fish family concentration to each other in the different transects of 2018 and 2021 were roughly the same.

Looking at the distribution of fish families per m^2 (Figure 10), it becomes clear, that the first transect section is the area in which most of the fish per m^2 are located. Followed by the fourth transect and then the second. The fewest fish were sighted in the third transect area. This behavior was ascertained in the marine biology course 2016, too. In this study, based on the data obtained and the work of Bonaca and Lipej 2005, it was assumed that the number of species on *Posidonia oceanica* is lower than on other habitats. However, this conclusion must be viewed critically, since in these studies different families were assessed, than in the studies of the marine biological excursion. Furthermore, the work by Frau et al. 2003 speaks against this theory. In this work it was investigated, whether fishes from the *Sparidae* and *Labridae* families tend to live in rocky areas or in seagrass meadows with stones. No preferred area could be determined. These results are also supported by the work of Jadot et al. 2006 where it was observed that *Sarpa Salpa* occurs in areas with *Posidonia oceanica* in approximately the same concentrations as in habitats with algae. Ultimately, these works show, that there is no preferred habitat for these two fish families. The fact, that in the present study and previous studies the fewest fish per m^2 was found in the third transect can have several other reasons. For Example, it is difficult to recognize species in the depth of this transect and furthermore it is very hard to spot fishes between seagrass, which could explain the lower measurements in this area. On the other hand the fewest sighted family in comparison to other transects in this area was *Sparidae* and this species is quite easy to spot and identify, therefore this theory is doubtful. Another reason could be, because the third transect is the harbor entrance, which is often crossed by boats, and this could deter the fishes. In addition, wave action, lack of hiding places or lack of prey in this area could

also be the reason for this behavior. Finally, further studies have to be carried out to clarify exactly why this area is being avoided.

Looking at the biomass of the sighted fish families, (Figure 11) it becomes evident that most of the biomass is possessed by the *Sparidae*. This is not surprising, as they occur in very large numbers in the study area and tend to be relatively large compared to the other fish present in the study area.

Finally, the fish sightings were viewed in relation to time (Figure 12). This made the day night rhythm of some fish families visible. It became clear, that the *Atherinidae* are more observable at night. This is the case, because during the night they hover motionless in the column of water. By doing this, they avoid swarm behavior, so that they are not easily targeted by predators. No preferred time could be determined for the *Serranidae* in this study. The same result can be found for the *Apogonidae*. The data from Figure 12 Nr. 1 tend to be more towards night activity, but it could not be clearly established. However, based on the work of Azzurro et al. 2007 it could be shown that *Serranidae* are more active during the day and *Apogonidae* tend to be more active at night. That *Apogon imberis* is commonly seen at night can be explained by the fact, that they usually hide in caves during the day and only come out at night to eat (Bailly, Froese and Pauly, 2021). The fact, that *Serranidae* tend to be diurnal is not surprising, as it was often observed during this study, how they ate during the day. The fact, that they were also spotted often at night may be due to the fact, that they do not have too many predators in the study area and therefore do not have to retreat to certain regions for protection. At Figure 12, Nr. 2 it can be seen, that the *Scorpaenidae* were increasingly sighted at night. It is strongly supposed, that the two monitored species are nocturnal. However, it must still be viewed critically on the basis of this data, since very few Scorpaenidae have been sighted, so that no clear statement can be made on the basis of the data obtained here. Including other sources and reports, it becomes clear that the species *Scorpaena notate* and *Scorpaena porcus* are nocturnal hunters (Mazza and Mario Beltramini, 2021; Mazza and Marion Beltramini, 2021). The data obtained cannot be used to make any clear statements about the *Blenniidae* either, since too few individuals have simply been sighted. The few individuals were seen during the day. For this reason a daytime activity is assumed. This assumption is supported by already mentioned studies (Azzurro *et al.*, 2007). The prioritized time of *Mullidae* is most likely the daytime. Although some were seen at night, most of them rested on the ground. This explains why the peak is not as high at night as it is during the day, because it is much more difficult to see the ground at night and resting fishes draw less attention to them. In addition, the species *Mullus surmuletus* was also actively observed during the day while moving and eating. The measurements in Azzurro et al. 2007 suggest that this family was active during the day. At Nr. 3 in Figure 12 it can be clearly seen, that they are day active fishes that look for places to rest at night. This has already been shown for the *Sparidae* family at *Sarpa salpa* in a study by Jadot et al. 2006. There it was shown, that some *Sparidae* species are looking for completely different habitats to spend the night there. In addition, there were also some fish, that did not look for other habitats, but spent the night where they were found during the day. This matches with our results. On the one hand, a decrease in *Sparidae* was observed during the night. On the other hand, there were also individuals who remained in the transect and could be observed there during the night. Since this behavior of strong occurrence during the day and strong fall off at night was observed in other *Sparidae*, in this study too, it is assumed that the behavior of the other species of the *Sparidae* family is similar to that of *Sarpa salpa*. In order to be able to prove this, however, individual studies would have to be carried out on the various species. The next family are the *Pomacentridae*

and the *Labridae*, these could not be observed at all during night. This is, because they retreat into caves and crevices at night and observation is not possible due to the restricted view. They show this behavior to hide from predators at night. This also makes it clear, that these two families are diurnal fishes (Hillyer, Beale and Shima, 2021; Jonna, 2021). In the last family, the Mugilidae, a diurnal activity was also determined on the basis of the obtained data. This assumption was made, because this family was not sighted at night, which suggests that these fish either retreat to other areas or hiding spots at night. The data from Azzurro et al. 2007 and the fact that the species *Liza ramada*, *Liza saliens*, and *Chelon labrosus* are diurnal, also suggest that the species in this family are mainly active during the day (Gisbert, Cardona and Castelló, 1997). In addition, the credibility of the data from Figure 12 should once again be emphasized as the study by Azzurro et al. 2007 also examined this daily rhythm of different fish families and came to the same results as in this study.

CONCLUSION

Ultimately, no major deviations in fish behavior could be determined in this study compared to previous marine biological excursions. It can therefore be assumed that the ecosystem has not been severely impaired over the last years. These are promising results. Nevertheless, these studies must continue to be carried out over the next few years in order to check the stability of this ecosystem in the future. In addition the invasive alga *Chrysophaeum taylorii* should be treated with caution, as this alga has spread massively in the port over the past three years and could become a problem for the ecosystem in the future.

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Fish diversity in dependence of human presence on the north-east coast of Corsica

MARIE-LUISE CONTALA & MORITZ NOWAKOWSKI

INTRODUCTION

The Mediterranean Sea is one of the richest and most diverse marine habitats for various plant and animal species. Due to its special location, the Pangaea continental drift (150 million years ago) and Quaternary ice age (started about 2.4 million years ago), many different species developed through local adaptation to special climatic conditions (Li *et al.*, 2015; Loïc, Heine and Albouy, 2017). Furthermore, this plate tectonic and environmental changes shaped the global biodiversity gradients and contributed to allopatric emergence of new species across straits and the development of endemic species (Loïc, Heine and Albouy, 2017).

Since 1992, the University of Innsbruck together with the University of Kiel conduct a biodiversity survey every two years at STARESO research station on the Island of Corsica (France). These student courses provide an opportunity for a long-term assessment and monitoring of the fish species diversity in the surrounding habitats. Thereby, species and abundance changes caused for example by environmental changes (including ocean warming and acidification) or anthropogenic disturbances (including pollution and eutrophication) may be discovered (Tugrul, Ozhan and Akcay, 2019). So, with this time series investigation we can establish a dataset important for understanding the system under the underlying environmental pressures.

However, despite repeated observations in the area, there could be a systematic bias in species assessment due to anthropogenic disturbance caused by the presence of snorkelers or divers. Previous studies discovered a clear human impact on the result of fish monitoring studies, leading to differences in species composition (Degerman *et al.*, 2001; Tibbetts, 2009; Rabalais, 2015). Fish monitoring without human presence can affect changes in abundance estimations and has different consequences for the animals living in this habitat. So, one of the issues in this year's fish assessment was to test new technical approaches to exclude a systematic bias in fish biodiversity and abundance estimations caused by human presence.

Within this course we wanted to continue the qualitative fish species monitoring time-series investigation, focusing also on possible environmental changes that might be responsible for gains and losses of fish diversity over time. Furthermore, we wanted to investigate the possible links between fish and human behavior and their potential effect on abundance estimations.

In addition, we wanted to attract so far undetected fish species with different baits in the absence of humans to eventually further widen the marine field course check-list for the fishes of the Bay of Calvi (Corsica, France).

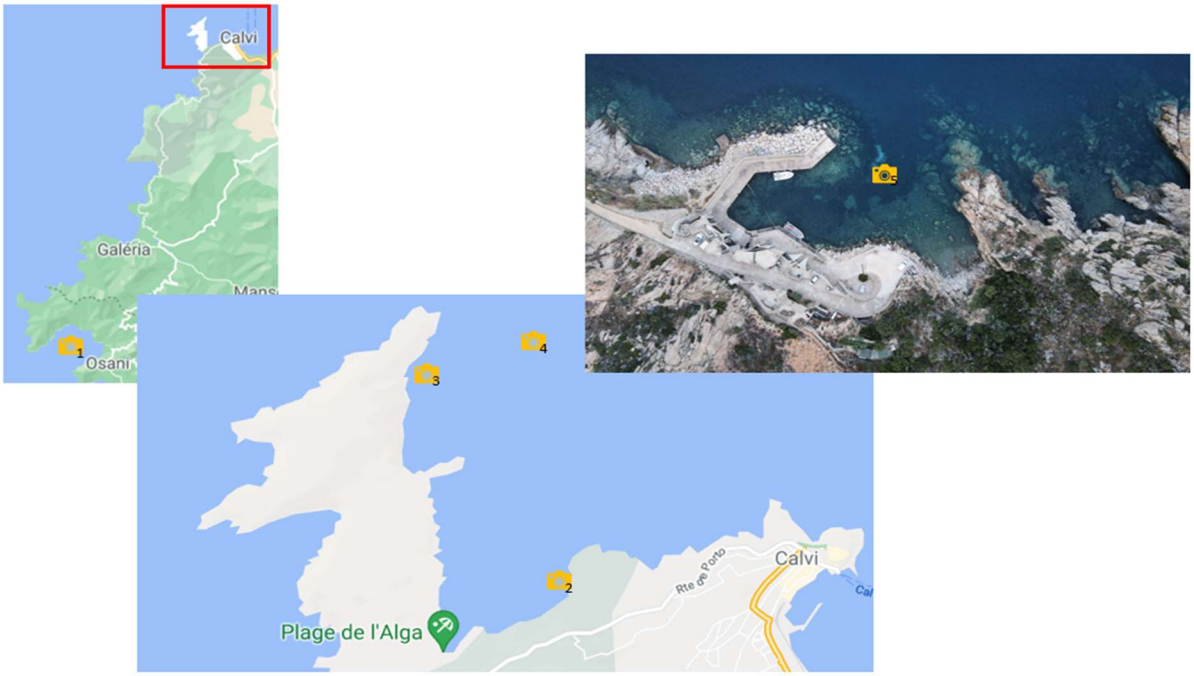

Figure 33: Sampling/ documentation (snorkeling and diving) sites of fish: 1) Pebble beach, 2) sand beach, 3) rocky coast (STARESO), 4) corraligene (50 m depth), 5) seagrass.

MATERIAL AND METHODS

FISH SPECIES LIST

To generate the species list, five different habitats (pebble beach, sandy beach, rocky shore, secondary hard substrate (corraligene to about 50 m depth) and seagrass) were visually inspected and also sampled within one week (Figure 1; Table 1). The different fish species occurring in these habitats were observed, identified and if possible documented by photography. Only specific fish were caught to determine them more precisely and to better get to know their species-specific characters. Some individuals were analyzed in more detail (protocol reference: gut content) whereas most specimens were released immediately after catch and species identification. As a final result, the fish list, existing since 1996, was updated and revised by adding the 2021 records. Two so far unknown species for the area were sequenced for the mitochondrial COI gene at the Thünen Institute of Fisheries Ecology, Bremerhaven, Germany, to confirm morphological species identification and subsequently added to the list (marked orange within Table 3). The species found in each pertain habitat can be found in protocols from different students (Table 1).

Table 9: Sampling site descriptions as well as the linkage to the specific protocols including specific fish species of the pertain habitat. The protocol of algae was done by Nico but not listed here because algae occur within multiple habitats of this table.

Sites	Habitat	Latitude	Longitude	Protocol by
1	Pebble beach	42.339384504474985	8.628369493178672	Veronika
2	Sandy beach	42.56512202181785	8.73610707297597	Michael
3	Rocky shore	42.58011383159004	8.724697304901127	Pamela
4	Corraligène	42.58135405832141	8.730518311830828	Alex & Moritz
5	Seagrass	42.580195164410014	8.72436449074145	Marie

GoPro experiments

To investigate fish abundance and fish behavior in the absence or presence of humans we established GoPro- based experiments. Therefor we used a GoPro hero 8 black edition plus an underwater housing. To provide stable conditions, the GoPro was attached at the bottom to a 2 kg piece of a diver´s weight belt, so that the GoPro couldn't drift away by currents. We also added a small air-filled buoy, which was connected to the GoPro and helped to detect the GoPro after the monitoring. We tried two different settings for the monitoring: First option was to do a proper time lapse where the GoPro took every 5 seconds a single photo for a predetermined amount of time. The second option was a continuous single shot filmed in 4k and 25 fps (frames per second), as described in Table 2.

Table 10: GoPro settings we used in the described experiment (red box).

Settings	Video	Time lapse (Photo)
Resolution	4K (3840 x 2160)	12 MP
Frames per second / Intervall	25	Every 5 seconds
Aspect ratio	16:9	4:3
Angle	Wide (16-34 mm)	Wide (16-34 mm)
Color profile	Flat	Flat
Recording time	30 min	2 h

Both settings had advantages and disadvantages. For example, the time lapse mode needs less battery and can therefore shoot over a longer time period with a comparable resolution. On the other hand, the continuous shot provides a fluent video, which makes it easier to study fish behavior. We tried both settings, but decided to go with the 4K video regarding to our scientific goal we wanted to achieve.

We placed the GoPro at five different locations to cover diverse habitats and a significant amount of data. Some were placed in the harbor of the station, some outside and in varying depths (Figure 2, Table2). We also tried to attract fish with different baits (Table 2), e.g. fish (*Symphodus tinca)* attached to a piece of plumb and a small buoy (see picture). For other attempts we used crushed sea urchins (*Arbacia lixula*) (Figure 3).

Figure 34: Recording locations (Go Pro) to document the behavior of fish in the absence of humans.

Table 3: Locations of recording as well as if the experiments were implemented with (X) or without (-) bait as well as witch kind of bait. At one location a maximum of 3 records were made (no bait, sea urchin and fish).

Location	Depth (m)	No Bait	Bait – Sea urchin	Bait – fish
1	-	X	-	-
2	-	-	X	-
3	4.80	X	X	X
4	5.20	X	X	-
5	2.30	X	X	-

Figure 3: Location 4 without bait (A). Collected sea urchin, which is placed in front of the GoPro (B). With a rock the sea urchin was smashed and opened up (C).

RESULTS

FISH SPECIES LIST

Within the Mediterranean Sea, around 777 species of fish species were recorded to date (Fishbase, 2012b). 35% (424 species) are recorded off the coasts of Corsica (Fishbase, 2012a; Figure 4A). About one fifth of these species (20%; Figure 4A) was found within the course (1996-2021), corresponding to 108 documented species. During this year's course, more than half of the species listed between 1996-2021 were rediscovered or newly discovered (Figure 4B). We were able to record a total of 66 species, whereby four fish species were found for the first time and had not yet been included in the course species list: *Gaidropsarus cf. mediterraneus,* the larva of *Crystallogobius linearis, Odondebuenia balearica* and *Sphyraena sphyraena* (Table 4; orange marked). Species like *Dactylopterus volitans, Parablennius incognitus* and *Gouania wildenowi* were only found this year as well as only one or two times more within the time span since 1996 (Table 4; marked in rose). Species which were not found this year but in the years before were *Phycis phycis, Seriola dumerili, Xyrichthys novacula* and *Coryphoblennius galerita.* In contrary, sixteen fish species were always present since 1996 (Table 4; marked in blue).

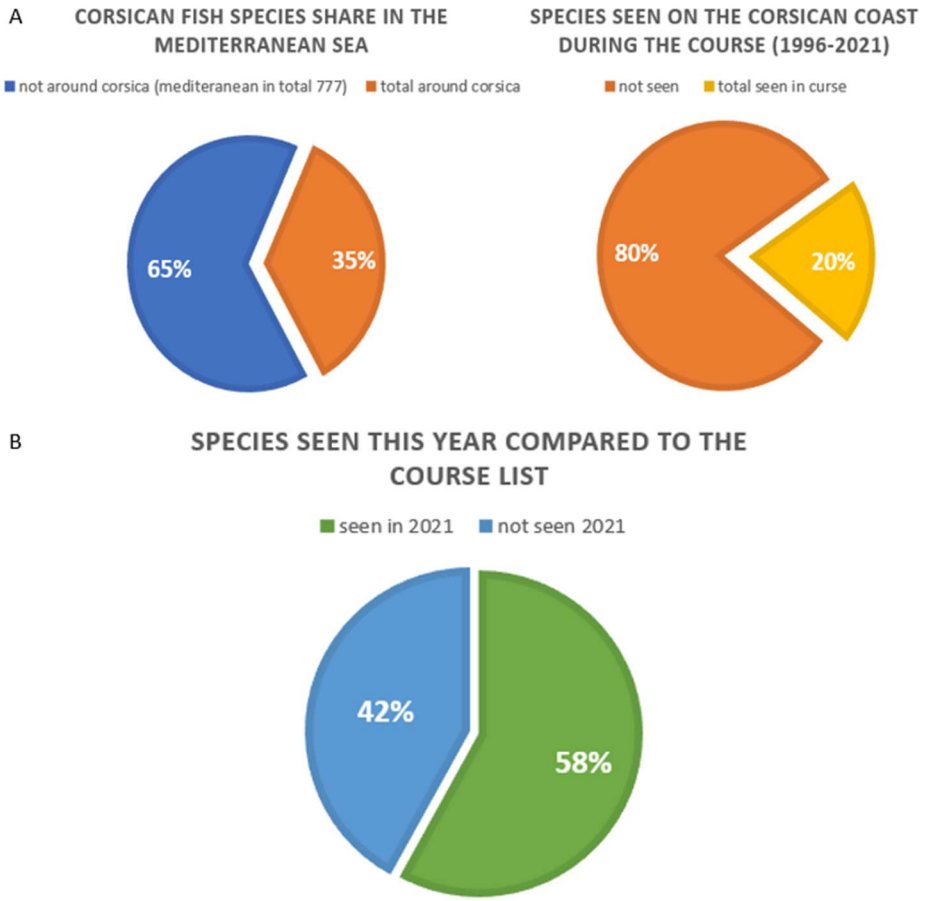

Figure 4: Species distribution from scale from Mediterranean Sea (A) to fish species seen within the course 2021 (B).

Table 11: List of all fish species identified within the scope of the marine biological excursion of the University of Innsbruck and the Christian-Albrechts University Kiel since 1996. Marked species were found the first time.

Art	2021	2018	2016	2014	2012	2010	2008	2006	2004	2002	2000	1998	1996
Torpedo marmorata						x							
Dasyatis pastinaca	x	x	x	x		x	x	x	x	-	-	-	-
Pteroplatytrygon violacea								x	-	-	-	-	-
Myliobatis aquila								x	x	x	-	-	-
Muraena helena	x	x	x	x	x	x	x	x	x	x	x	x	-
Conger conger	x			x			x	-	x	-	x	x	-
Anguilla anguilla	x	x						x	x	-	x	x	-
Engraulis encrasicolus		x		x				x	-	-	-	-	-
Synodon saurus	x			x		x							
Phycis phycis		x	x	x				x	-	x	-	-	-
Gaidropsarus cf. mediterraneus	x												
Oedalechilus labeo	x	x	x	x	x	x	x	x	x	x	-	-	-
Liza aurata	x	x			x	x	x	x	x	x	x	x	-
Atherina boyeri	x	x	x	x	x	x	x	x	x	x	x	x	-
Atherina hepsetus	x	x	x	x	x	x	x	x	x	-	x	-	-
Belone belone	x	x	x	x	x	x	x	x	-	x	-	-	-
Hippocampus guttulatus								-	-	-	-	x	-
Syngnathus typhle								-	-	x	-	-	-
Dactylopterus volitans	x					x							
Scorpaena porcus	x	x	x	x	x	x	x	x	x	x	x	-	-
Scorpaena notata	x	x	x	x		x	x	x	x	x	x	x	-
Scorpaena scrofa			x	x		x	x	x	-	x	-	x	-
Chelidonichthys sp.								-	x	-	-	-	-
Dicentrarchus labrax	x		x			x		-	x	-	-	-	-
Epinephelus marginatus	x	x	x	x	x	x	x	x	x	x	x	x	-
Serranus cabrilla	x	x	x	x	x	x	x	x	x	x	x	x	-
Serranus scriba	x	x	x	x	x	x	x	x	x	x	x	x	-
Anthias anthias					x			-	-	x	-	-	-
Apogon imberbis	x	x	x	x	x	x	x	x	x	x	x	x	-
Seriola dumerili		x	x	x				x	x	-	-	-	-
Trachinotus ovatus		x						x	-	-	-	-	-
Trachurus mediterraneus				x				-	x	-	-	-	-
Pomadasys incisus						x							
Boops boops	x	x	x	x	x	x	x	x	x	x	-	-	-
Dentex dentex	x	x	x	x	x	x	x	x	x	x	-	x	-
Diplodus annularis	x	x	x	x	x	x	x	x	x	x	x	x	x
Diplodus puntazzo	x	x	x	x	x	x	x	x	x	x	x	x	x
Diplodus sargus sargus	x	x	x	x	x	x	x	x	x	x	x	x	x
Diplodus vulgaris	x	x	x	x	x	x	x	x	x	x	x	x	x
Lithognathus mormyrus	x	x	x	x	x	x	x	x	x	x	-	x	-
Oblada melanura	x	x	x	x	x	x	x	x	x	x	x	x	x
Pagellus acarne	x	x	x	x	x		x	-	x	x	-	-	-

Pagellus erythrinus	x	x	x	x			x	-	-	x		x	-
Pagrus pagrus								-	-	x	-	-	-
Sarpa salpa	x	x	x	x	x	x	x	x	x	x	x	x	x
Sparus aurata	x	x	x	x	x	x	x	x	x	x	x	x	x
Spondyliosoma cantharus	x	x	x	x	x	x	x	x	x	x	x	-	x
Spicara maena		x			x		x	x	-	x	x	-	-
Spicara smaris	x		x	x		x							
Sciaena umbra	x	x	x	x	x	x		x	x	x	-	-	-
Mullus barbatus								x	x	-	-	-	-
Mullus surmuletus	x	x	x	x	x	x	x	x	x	x	x	x	-
Chromis chromis	x	x	x	x	x	x	x	x	x	x	x	x	-
Coris julis	x	x	x	x	x	x	x	x	x	x	x	x	x
Labrus viridis	x	x	x	x	x	x	x	x	x	x	-	x	x
Labrus merula	x	x	x	x	x	x	x	x	x	x	x	x	x
Symphodus cinereus	x	x	x	x	x	x	x	x	x	x	-	x	x
Symphodus doderleini								-	x	-	-	-	-
Symphodus mediterraneus	x	x	x	x	x	x	x	x	x	x	x	-	-
Symphodus melanocercus	x	x	x	x	x	x	x	x	x	x	x	x	x
Symphodus ocellatus	x	x	x	x	x	x	x	x	x	x	x	x	x
Symphodus roissali	x	x	x	x	x	x	x	x	x	x	x	x	x
Symphodus rostratus	x	x	x	x	x	x	x	x	x	x	x	x	x
Symphodus tinca	x	x	x	x	x	x	x	x	x	x	x	x	x
Thalassoma pavo	x	x	x	x	x	x	x	x	x	x	x	x	-
Xyrichthys novacula		x	x	x									
Gymnammodytes cicerelus			x										
Trachinus araneus				x				-	x	-	-	x	-
Trachinus draco	x	x	x	x	x	x	x	x	-	-	-	-	x
Trachinus radiatus				x									
Uranoscopus scaber		x						x					
Tripterygion tripteronotus	x	x	x	x	x	x	x	x	x	x	-	x	-
Adiablennius sphinx	x	x	x	x	x	x	x	x	-	x	-	x	-
Coryphoblennius galerita		x	x	x		x	x						
Lipophrys trigloides			x					x	x	-	-	-	-
Microlipophrys dalmatinus							x						
Microlipophrys nigriceps				x		x	x	x	x	-	-	-	-
Parablennius gattorugine	x	x	x	x				x	x	x	-	x	-
Parablennius incognitus	x						x						
Parablennius pilicornis						x	x						
Parablennius rouxi			x		x	x	x	-	x		x	x	-
Parablennius sanguinolentus	x	x	x	x	x	x	x	x	x	x	-	x	-
Parablennius zvonimiri	x	x	x	x	x	x	x	-	x		x	x	-
Salaria fluviatilis	x	X	x	x	x	x	x	x	x	-	-	-	-
Salaria pavo			x					-	x		x	x	-
Clinitrachus argentatus								-	x	-	-	-	-
Crystallogobius linearis (larva)	x												
Gobius bucchichi	x		x					x	x	x	-	x	-
Gobius cobitis	x			x				x	x	x	-	-	-
Gobius fallax	x	x	x										
Gobius paganellus			x					x	-	-	-	-	-

Gobius geniporus			x					-	x	-	-	-	-
Pomatoschistus minutus				x		x	x	x	-	-	-	-	-
Odondebuenia balearica	x												
Gouania wildenowi	x		x			x							
Lepadogaster bimaculatus			x	x									
Lepadogaster candollei							x	x	x	x	-	x	-
Lepadogaster lepadogaster	x		x			x							
Opeatogenys gracilis					x			x	x	-	-	-	-
Callionymus pusillus	x	x	x										
Sphyraena sphyraena	x												
Sphyraena viridensis	x	x	x	x	x	x	x	x	x	-	-	-	-
Scomber colias		x											
Bothus podas	x	x	x	x	x	x		x	-	-	-	-	-
Phrynorhombus regius						x	x	-	x	x	-	-	-
Arnoglossus kessleri								-	-	-	-	x	-
Solea lascaris							x	x	-	-	-	-	-
Pegusa nasuta			x										
Pegusa impar		x											
Ballistes capriscus							x	-	-	-	-	x	-
Total Number: 108	66	62	68	64	49	61	60	66	65	53	35	46	18

GO PRO EXPERIMENTS

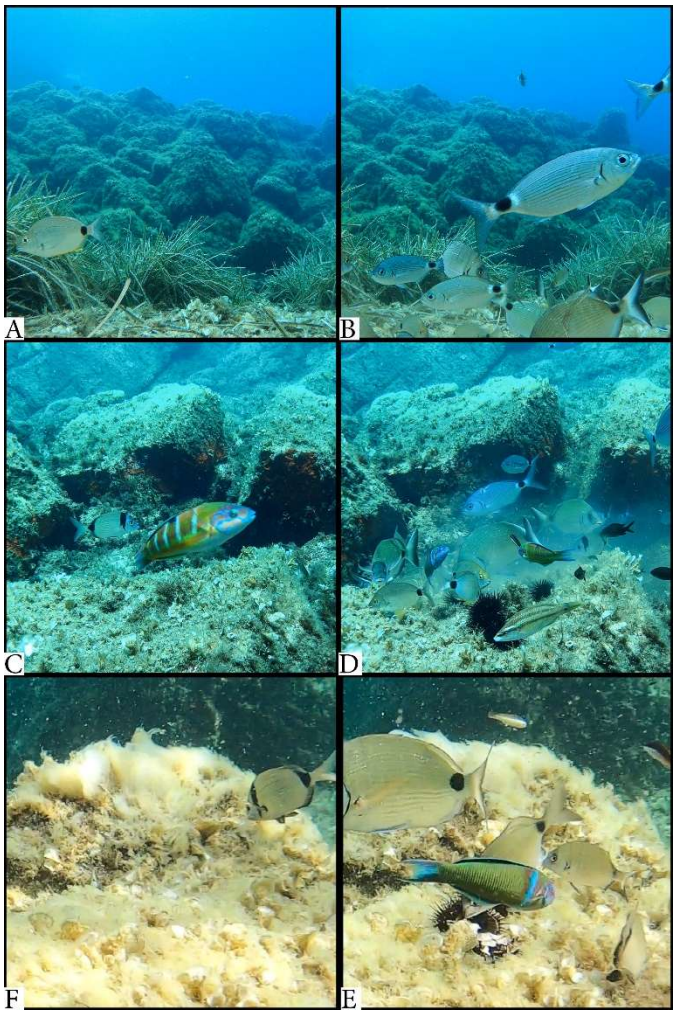

Figure 35: The GoPro was placed at three different locations once without a bait and a second time with smashed sea urchin. Photo A/B is from location 3, Photo C/D from location 4 and photo E/F from location 5 shown in Figure 2.

For the experiment we used the settings described in Table 2 and placed them at three different locations to investigate fish abundance with and without the presence of bait to get a significant outcome. In Figure 6 screenshots from the original videos were taken as examples for the effect of bait on fish bevavior. Photo A, C and F shows the three different locations without bait, B, D and E after we placed crushed sea urchins in front of the GoPro. Location 3 represented a habitat of seagrass, sand and smaller rocks. Location 4 shows slightly larger rocks and location 5 in a depth of 2.3 meters also rocks with brown algae. The presence of bait significantly increased the abundance of fish on a certain spot (A, C, F). Among the most attracted species were the saddled seabream (*Oblada melanura*), annular seabream (*Diplodus annularis*) and the common two-banded seabream (*Diplodus vulgaris*). Also, the ornate wrasse (*Thalassoma pavo)* and damselfish (*Chromis chromis*) were observed. The footage also shows a dusky grouper (*Epinephelus marginatus*) at location 4, which was never seen there before. He observed the bait from a distance, hiding at the rocks and the seagrass, but never approached to it (not shown in the Figure 6).

Figure 36: Photo A-C shows a mediterranean moray (Muraena Helena) observing the pray (Bait: Symphodus tinca) before it attacks it.

At Location 3 we also placed a fish bait to see what kind of fish will be attracted. The Mediterranean moray (Muraena helena) lives in coastal area distributed all around the Mediterranean Sea. In Figure 7 it is observing the prey, first from the seagrass in the back of the photo, then it is crossing the scene from left to right (A), before it is getting closer to the fish (B) and attacks the bait (C). Because the fish is connected to a buoy and a piece of plumb through a rope, the moray is not able to bite through the rope and escape with the bait. It is wrapping it´s whole body to get the most strength on the rope till the moray managed to bite through the rope, bolt the fish and disappeared (In the appendix the original video shows the full hunting scene).

Figure 37: A: Location 1: A green wrasse (Labrus viridis). B: Location 2: We placed a bait trap to attract fish. C: Location 4: Diver crossed our experimental set-up.

In Figure 8 we showed some interesting things we also investigated. Photo A: First of all, we tried to find the right settings and location to observe the fish abundance and behavior. We placed the Gopro at location 1 outside the harbor of the stareso station. A place with seagrass and rocky habitats near to the open sea. An undisturbed area without a lot interference with humans, where we can investigate if the fish abundance and behavior is changing. We didn't use a bait. Interestingly is that the green wrasse (Labrus viridis) was showing up and crosses several times in front of the Gopro. A behavior, we couldn't see when we were snorkeling, because there it was swim near the ground, hiding. Also, another dusky grouper (Epinephelus marginatus) showed up at this location, which we never saw there when we were snorkeling. In the second photo (B) we tried to attract the fish with bait, therefore we placed some sea urchin in the fish trap and let it sink to the seabed. No fish was attracted via the fish trap. In photo C, at location 4, some divers crossed our approach with sea ur-

chin. In comparison to Figure 6 (photo C/D) the fish disappeared during the stop of the divers above the bait. It was not always easy to set up the experiment right from the start. In this case, it needed time to find the right spot, the best bait and even than the experiment could be interrupted unexpectedly.

DISCUSSION

FISH SPECIES LIST

Out of a total of 777 fish species presently recorded for the Mediterranean sea (Fishbase, 2012b), the compiled fish species list of the joined Marine biology courses of the Universities of Innsbruck and Kiel since 1996 now comprises a total of 108 species. This is astonishing, given that fish observations during the course are done in a narrow coastal fringe in the vicinity of the research station. Therefore, deep areas (> 20 m depth) and pelagic species are generally excluded from observation. Within this year's course, we found more than half of the listed species from the previous years. In addition, four species were found for the first time and could be added to the list.

Changes in the occurrence of species over the years could imply alterations in environmental conditions caused by global warming or anthropogenic disturbance due to pollution or eutrophication (Coll et al., 2010). However, caution should be exercised for any such interpretations, since observations are restricted to rather small area over a relatively short period of time. Information is available about habitat loss caused by anthropogenic impacts, like anchoring, sewage, pollution, on and around the Corsica (Coll et al., 2010; Kalogirou et al., 2010; Habibullah et al., 2016). This could explain the absence of some vulnerable species, like the eagle ray Myliobatis aquila in the present records (Table 4). However, the rediscovery of rarely found species such as Dactylopterus volitans, Parablennius incognitus and Gouania wildenowi shows, that shifts in the list are more parsimoniously be explained by changes in observation effort than in short-term environmental alterations (Table 4 – marked in rose). Sixteen species, mostly seabreams and wrasses, marked in blue in Table 4 are almost ubiquitously found every year, including various Diplodus and Symphodus species. They are also known for their large variance for individual reproductive success linked with adaption on stress factors (Lande and Shannon, 1996; Planes and Lenfant, 2002).

Species depletions are often caused by a single factor of exploitation, habitat loss, eutrophication, introduced predators, diseases, or disturbance (Coll et al., 2010). Some species depletions and extinctions are also caused by multiple causes whereby Corsica was classified as 'not severely affected' by extinction or threat for marine fish in comparison to other locations within the Mediterranean Sea (IUCN, 2005). Nevertheless, changing factors are also leading to immigration or emergence of new species not normally found in this habitat or possibly more common within the habitat.

With regard to the newly recorded species, *the shore rockling Gaidropsarus cf. mediterraneus* is normally found within rocky habitats from the sea level down to 450 m and is hiding at shallow depths (Fishbase, 2021c). This year the species was observed during night snorkeling in very shallow depth. Since it could not be caught, a proper species identification was not possible. The crystal goby *Crystallogobius linearis* is frequently found in the eastern Atlantic but relatively rare in the Mediterranean Sea where it inhabits coastal waters, over shell, sand or muddy bottoms. Eggs are laid in emp-

ty tube-worms and are guarded by the male (Fishbase, 2021a). The larva of the crystal goby has not yet been described. The coralline goby *Odondebuenia balearica* is often found in the Mediterranean Sea (Fishbase, 2021b). Additionally, the genus occurs offshore, on coralline and other coarse grounds as well as on sand with rocky outcrops. The European barracuda *Sphyraena sphyraena* has a wide distribution range but is commonly seen in coastal and offshore waters within the Mediterranean Sea (Fishbase, 2021d). So, three of the four newly recorded species are either cryptobenthic (the two gobiid species *Odondebuenia balearica* and *Crystallogobius linearis*) or night active (*Gaidropsarus cf. mediterraneus*) and thus difficult to spot. To distinguish between the two barracuda species *Sphyraena sphyraena* and *Sphyraena viridensis* is difficult for inexperienced observers.

In summary, the fish list includes a main portion of the diversity which can be found around the Corsican Island giving us a good insight into the biodiversity of marine fish. Furthermore, the long-term study provides information on possible environmental influences or other stress factors that could possibly pose a threat to the individual species and their distribution. Vulnerable species for example are suffering from global warming and anthropogenic disturbance whereby sixteen fish species show a stable distribution over the years suggesting reproduction success and adaption on stress factors. Newly identified species, I our opinion were found easier due to observation effort than in short-term environmental alterations that can lead to immigration or emergence. The following years of this study and thereby of the student course will provide more insights for this long-time project.

GO PRO EXPERIMENTS

GoPro-based experiments with and without bait to study fish behavior in the absence of humans were first tried in this year's course. The absence of humans or, more generalized, changes of environmental parameters can lead to altered fish behavior due to differences in stress levels (Masud et al.; 2005). To measure and quantify fish behavior, many systems have been developed. Technology provides a huge variety of possibilities to observe the marine wildlife without disturbing it. For more than a decade now, action cameras like the GoPro´s are inevitable for monitoring. They are small, cheap, almost undestroyable, easy-to-handle and offer high resolution up to 5.3 K (5120 x 3840 pixel; GoPro hero 10) (Zarco-Perello, S., Enríquez, S.; 2019). 5.3 K refers to a horizontal display resolution of approximately 5000 pixel, which means a higher pixel rate leads to higher resolution and a more detailed video. Video recording has been used for the remote observation in real time, and also for the offline reproduction of fish activity through video playback. Experiments may last many hours, even days, making video systems an invaluable tool in behavioral study of fish (Widmer, L. et al.; 2019). One disadvantage is the long duration of the recorded video, which requires equal hours of observation from a person, making analysis very hard to be performed. It´s also not easy to show the massive amount of data, even if it´s here in a protocol nor in the appendix.

In general, we found out that sea urchin bait works better than fish bait (Figure 6, Table 3) to attract the common, carnivorous fish species in coastal areas around the STARESO station. We also observed that the fish bait attracted bigger predators (like moray eels) and no smaller fishes. The fish trap didn't work out as we thought. We observed a different behavior in fish movement and we also saw fish species at locations, where they were never detected before during the course (figure 8, A), which leads us to the hypothesis that the presence of human has a disturbing effect on fish behavior (Diogo S. M. Samia et al.; 2019).

OUTLOOK

With our experiments we showed that video recording is a powerful and absolute useful tool to investigate fish diversity and abundance. Furthermore, we set the scene for future experiments, which can be adapted in many different directions. Baited experiments can be expanded for bait size and quality (e.g. fish blood), water depth and daytime. Video observation can be helpful to complement the fish species list and to support species identification. Since investigations and experiments can be conducted every second year in Corsica, it is suggestive to continue with it as a long-term study. Furthermore, it would be interesting to assess how fish behavior changes in the presence or absence of humans, maybe also of humans carrying a spear gun (Diem Samantha C. Tran et al.; 2016). But this is up to the next group of students.

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Gut content analysis and age determination of the peacock wrasse (*Symphodus tinca*) and the brown wrasse (*Labrus merula*)

VERONIKA PEER & ALEXANDER VORLEUTER

INTRODUCTION

The peacock wrasse (*Symphodus tinca*) and the brown wrasse (*Labrus merula*) belong both to the family of Labridae and the tribe (subfamily) Labrinae, which is endemic to the North Atlantic, including the Mediterranean Sea. *S. tinca* is distinguishable for its duckbill like mouth and *L. merula* is recognizable by its blue fin hem. Wrasses are characterized by a generally rather short intestine and the lack of a true stomach. The feeding behaviour of the two species is very different. While *S. tinca* searches for food often in groups, regularly associated with other species, like *Symphodus ocellatus* and *Symphodus rostratus*, *L. merula* lives solitary and obviously avoids foraging in association with other species. In addition, while *L. merula* attacks and picks individual targets, *S. tinca* is known to grasp pieces of algae. These algae are sucked in and spit out to filter out the animals that are living within the algae (Koblmüller et al., 2003). Both wrasses are active during daytime and can reach similar lengths (*Symphodus tinca*: 44 cm maximal standard length, *Labrus merula*: 45 cm maximal standard length). With these feeding observations, different questions were addressed in this course. First, are *S. tinca* and *L. merula* food competitors and has *S. tinca* more plant material in its guts? Second, are there differences in the diet of younger/smaller animals to older/larger animals?

To analyse the gut content, fishes were collected and the gut was dissected. Gut content analysis is an important method to assess the diet composition of fishes and reconstruct their feeding behaviour. Further this is important for trophic studies to understand the biology and ecology of fish. There are different approaches to analyse gut contents. The most robust and accurate method nowadays is the frequency of occurrence (%F) method for diet composition. It is cheap, fast and has a minor loss of information compared to other more detailed methods (Le et al., 2019).

With the dissection of the otoliths, the age could be determined. Otoliths, which are known as “earstones” consist of calcium carbonate and are located behind the brain of bony fishes. These earstones help the fish to balance and hear in the water. There are three types of otoliths that can be found: Sagitta, Asteriscus and Lapillus. Each of them comes in pairs. The Sagittae are the largest ones (Popper & Lu, 2000). The shape and sizes of otoliths varies within species. Under a binocular microscope the growth rings can be seen: the darker translucent zones, which show a fast growth period and the white opaque zones, which show a slower growth period (**Fig. 1**). By counting the opaque zones (annuli) the age of the fish can be determined (Boughamou et al., 2014).

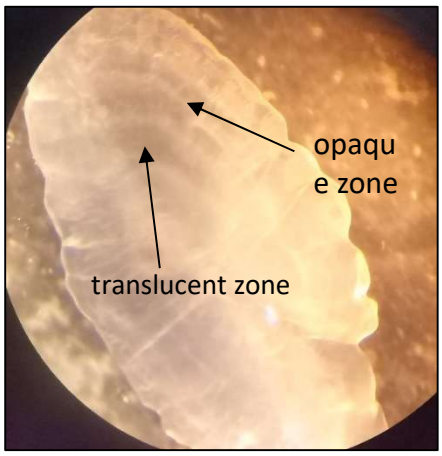

Figure 12: Otolith of the wrasse *Symphodus tinca.*

MATERIALS AND METHODS

To analyse gut content and age, 10 individuals of different sizes of the two species *Symphodus tinca* and *Labrus merula* were caught with a hand net or with the help of a spear gun. The fishes were caught in the vicinity of the Station de Recherches Sous-Marine et Océanographique "STARESO" along the Revellata peninsula near Calvi, Corsica. Fishes were immediately frozen and kept for subsequent preparation of gut content and otoliths.

Prior to dissection, animals were measured, weighted and photographed. The total length and the standard length of fish were recorded. The gut contents were inspected under a binocular microscope and the percentages of specific plant and animal taxa as well as the undistinguishable, heavily digested material were estimated. Sagittal otoliths were viewed under a binocular microscope, after extraction through a transverse cut on the posterior dorsal part of the head behind the eyes (**Fig. 2**).

Figure 2: Left: Extraction of otoliths from Labrus merula. **Right:** Labrus merula (08).

RESULTS

In total, ten *Labrus merula* and eleven *Symphodus tinca* specimens have been caught during the week. The findings of the fish gut content analysis as well as body measurements are shown in **Table 1**. In addition, sagittal otoliths were excised, if retrievable, and the age of the individuals was determined by counting opaque zones. Different maturity, body length and age are shown in a proper variety among both species. *Symphodus tinca* is reported with a maximal age of 15 years, has a common length of 25 cm SL (standard length) and reaches maturity at a length of 12 to 15.2 cm. *Labrus merula* is reported with a maximal age of 27 years, has a common length of 40cm SL and reaches maturity at a length of 15 to 20 cm. Furthermore, one specimen each of the two species *Serranus scriba* and *Scorpaena porcus* was caught and investigated for comparison. It is also important to mention, that in *L. merula* 06 and *S. tinca* 10 parasites were found in their guts.

Table 1: Dissected specimen caught, analysed for body measurements, gut content and age.

Fish ID	length [cm]	standard length [cm]	weight [g]	gut content [%]	age [years]	date of catch
Labrus merula 01	23,2	19,1	163	85% Crustacea	1 otolith: 2-3 years	Monday (2.8.21)
				10% Gastropoda		
				5% Polyplacophora		
				single algea fila-ments		
Labrus merula 02	17	14	66,9	100% Crustacea	-	Monday (2.8.21)
Labrus merula 03	8	6,7	5,7	95% Crustacea	-	Monday (2.8.21)
				5% Gastropoda		
				single algea fila-ments		
Labrus merula 04	16	12,7	49,2	90% Crustacea	-	Tuesday (3.8.21)
				10% Gastropoda		
Labrus merula 05	6,6	5,4	2,9	100% Crustacea	-	Tuesday (3.8.21)
Labrus merula 06	17,3	14	65	100% Crustacea	1 otolith: age unknown	Thursday (5.8.21)
				single algea fila-ments		
Labrus merula 07	15,5	12,6	42,2	40% Crustacea	1 otolith: 2 years	Thursday (5.8.21)
				48% digested		
				10% Algae		
				2% Mollusca		
Labrus merula 08	16,2	13,4	50,2	40% Crustacea	2 otolith: 3 years	Thursday (5.8.21)
				45% digested		
				10% Mollusca		
				5% Gastropoda		
				single algea fila-ments		
Labrus merula 09	26,3	21,5	220,3	60% Crustacea	1 otolith: 3 years	Thursday (5.8.21)
				30% Mollusca		
				10% digested		
Labrus merula 10	23,3	19,3	156,8	40% digested	1 otolith: 3-4 years	Thursday (5.8.21)
				30% Crustacea		
				15% Bivalvia		
				15% Gastropoda		
Symphodus tinca 01	22	16,9	118,2	40% digested	1 otolith: 4 years	Tuesday (3.8.21)
				40% Crustacea		
				10% Algae		

	10% Gastropoda					
Symphodus tinca 02	17,3	13,7	62,9	74% digested	-	Tuesday (3.8.21)
	13% Polychaeta					
	10% Algae					
	2% Mollusca					
	1% Crustacea					
Symphodus tinca 03	16,9	13,4	64,7	60% digested	1 otolith: 2 years	Tuesday (3.8.21)
	20% Algae					
	10% Crustacea					
	5% Polychaeta					
	5% Gastropoda					
Symphodus tinca 04	18,7	15,4	81,9	48% digested	1 otolith: 3 years	Tuesday (3.8.21)
	45% Crustacea					
	5% Algae					
	2% Mollusca					
Symphodus tinca 05	18	14,2	78,7	40% Crustacea	-	Tuesday (3.8.21)
	20% digested					
	20% Mollusca					
	15% Algae					
	5% Polychaeta					
Symphodus tinca 06	13,7	11,5	31,4	40% digested	-	Tuesday (3.8.21)
	30% Crustacea					
	15% Bivalvia					
	10% Echinodermata					
	5% Algae					
Symphodus tinca 07	13,2	10,6	31,9	68% digested	1 otolith: 2 years	Tuesday (3.8.21)
	20% Crustacea					
	12% Algae					
Symphodus tinca 08	5,4	4,1	1,7	80% digested	1 otolith: < 1 year	Tuesday (3.8.21)
	10% Crustacea					
	10% Algae					
Symphodus tinca 09	14	11,2	36,1	75% digested	1 otolith: 3 years	Tuesday (3.8.21)
	20% Crustacea					
	5% Algae					
Symphodus tinca 10	12	9,6	24,3	50% digested	1 otolith: 3 years	Tuesday (3.8.21)
	20% Polychaeta					
	20% Crustacea					
	5% Mollusca					
	5% Foraminifera					

Symphodus tinca 11	11,5	9,6	18,4	30% digested	1 otolith: age unknown	Tuesday (3.8.21)
				25% Crustacea		
				15% Bivalvia		
				15% Algae		
				5% Echinodermata		
				5% Polychaeta		
				5% Gastropoda		
Serranus scriba 01	18	14,7	69,3	100 % Crustacea - Pilumnus sp.	1 otolith: 5 years	Monday (2.8.21)
Scorpaena porcus 01	14,5	11	60,9	100% Crustacea	2 otolith: 3 years	Wednesday (4.8.21)

Gut content analyses revealed, that *Labrus merula* mostly fed on crustacea, while *Symphodus tinca* had a much broader range in diet (**Fig. 3** and **4**). Molluscs as prey play a similar role with 11% and 7% in *L. merula* and *S. tinca*, respectively, which could be partially identified as gastropoda or bivalvia. Despite some single filaments in a few *L. merula* specimens, no algae could be found in their guts, while *S. tinca* exhibit an average of 10% plant (algae) material throughout all specimens except for *S. tinca* 10. Heavily digested organic material, which could not be accurately identified, contributed to up to 53% in the *S. tinca*. When comparing younger and older individuals of *S. tinca*, only slight differences could be seen compared to the younger and older individuals of *L. merula* (Fig. 5). The young *S. tinca* had in addition polychaetes and also Echinodermata in its gut. The younger individual of *L. merula* had mostly crustaceae in the gut, whereas the older one had also Bivalvia and digested food. For this analysis, specimen *L. merula* 03 (8 cm, 5.7 g, no otolith) and 10 (23.3 cm, 156.8 g, 3-4 years old) as well as *S. tinca* 01 (22 cm, 118.2 g, 4 years old) and 11 (11.5 cm, 18.4 g, age unknown) were used.

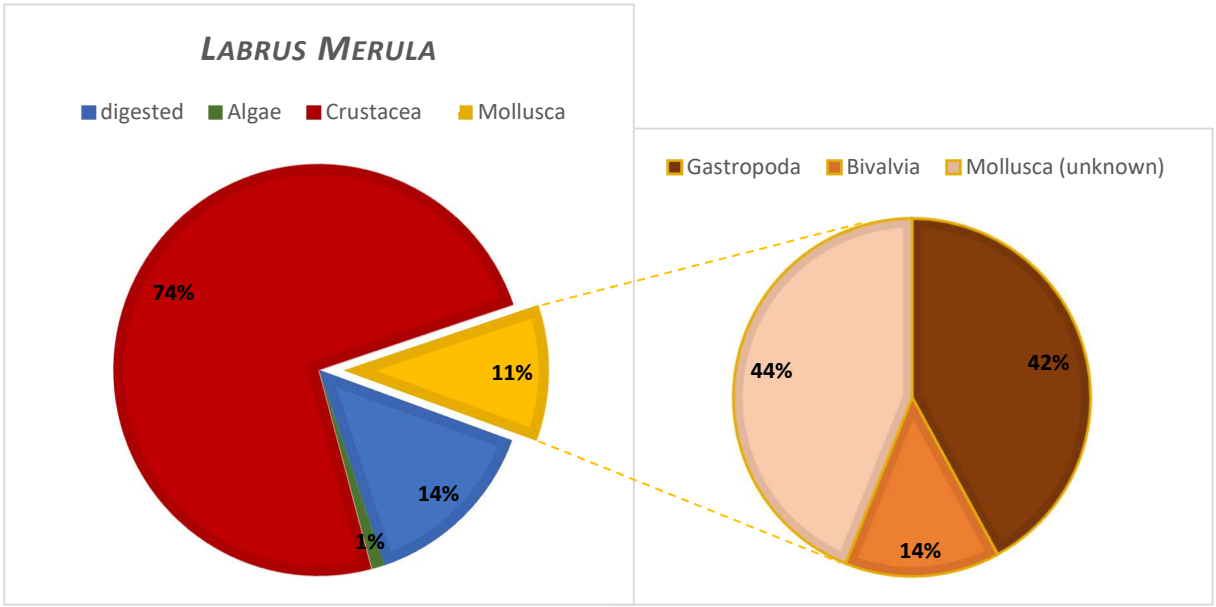

Figure 3: Total food distribution in percentage of gut content analysis of all *Labrus merula* specimen.

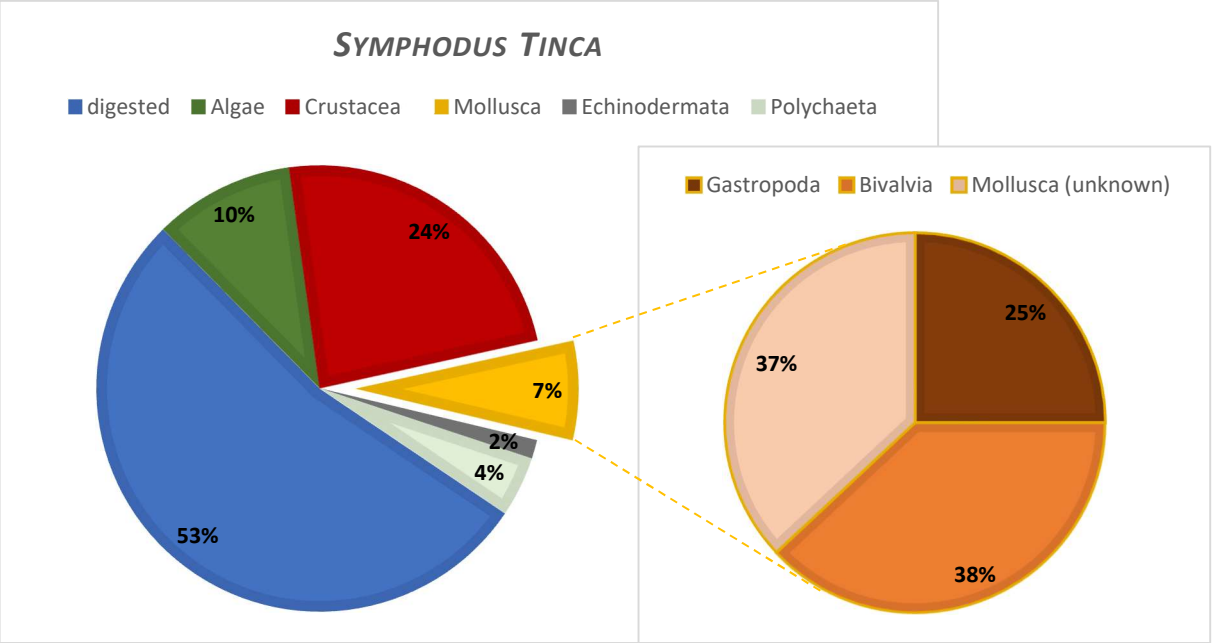

Figure 4: Total food distribution in percentage of gut content analysis of all *Symphodus tinca* specimen.

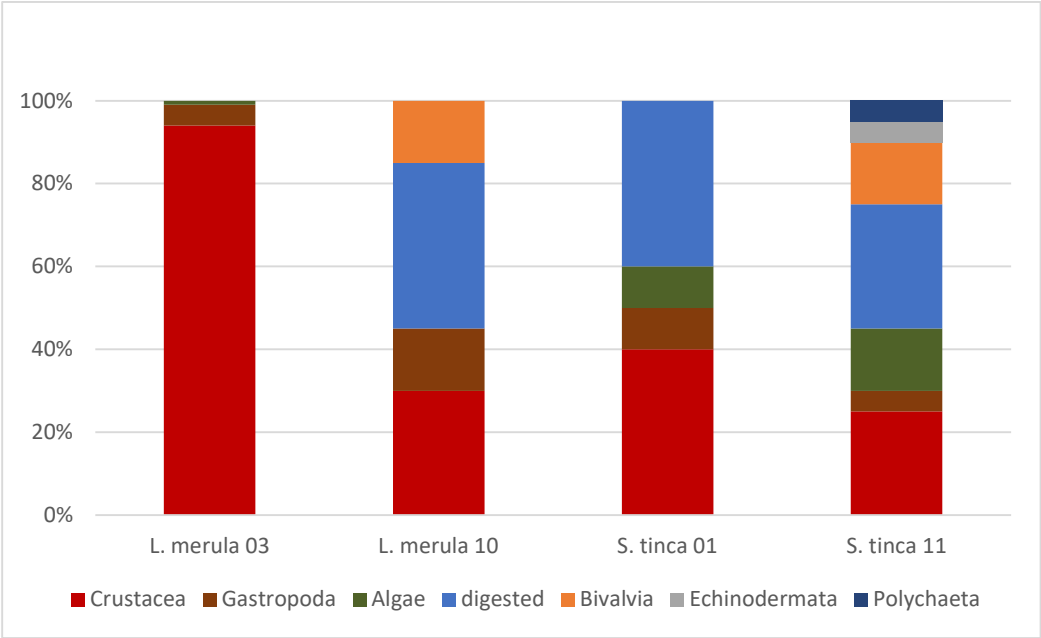

Figure 5: Comparative food distribution between *Labrus merula* and *Symphodus tinca* specimens in percentage.

DISCUSSION

Our data show a clear difference in the diet composition of *Labrus merula* and *Symphodus tinca*. The rather predatory *L. merula* predominantly feeds on crustaceans and small molluscs by foraging and directly picking its prey. This is reflected in an overall proportion of 75% of the gut contents being crustaceans. Only single algae filaments could be found in four out of ten individuals. *S. tinca* on the other hand shows a very different feeding behaviour, with the tactic of sucking in and spitting out portions of algae to extract infauna organisms. This is reflected in higher amounts of algae in their gut, showing that parts of it are swallowed in combination with their actual prey. If this plant material could also have a nutritional benefit, is unclear. However, due to a relatively short gut in this species and the fact, that the plant material does not show and sign of digestive degradation, this is rather unlikely. Crustaceans again represent the principal component of the identified diet. Unfortunately, more than half of all guts of *S. tinca* were filled with heavily digested and undefinable material, which could, if identifiable, change the overall results.

Gut contents within a species varied a lot and final conclusions if the diets of younger and older fish differ could not be made. Only within *L. merula* a trend was visible that smaller fish feed mainly on crustaceans and bigger ones show a broader diet range including more molluscs. However, since this analysis is based on a relatively small number of individuals and a very short period of time in the summer season, it cannot be regarded as fully representative. Seasonal variation in the diet of fish species needs to be considered. Ouannes-Ghorbel and Bouain (2006) showed for example a big difference of seasonal prey composition for *S. tinca* during one year.

In summary, our findings are largely in agreement with previous diet analyses of the two species (Dulčić, 1999; Ouannes-Ghorbel and Bouain, 2006). Other species within the foraging group of *S. tinca* were not examined like in previous field trips. Furthermore, differences in the diet composition between males and females of one species were not compared in our studies and might also contribute to a better understanding of the feeding behaviour of Mediterranean wrasses.

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