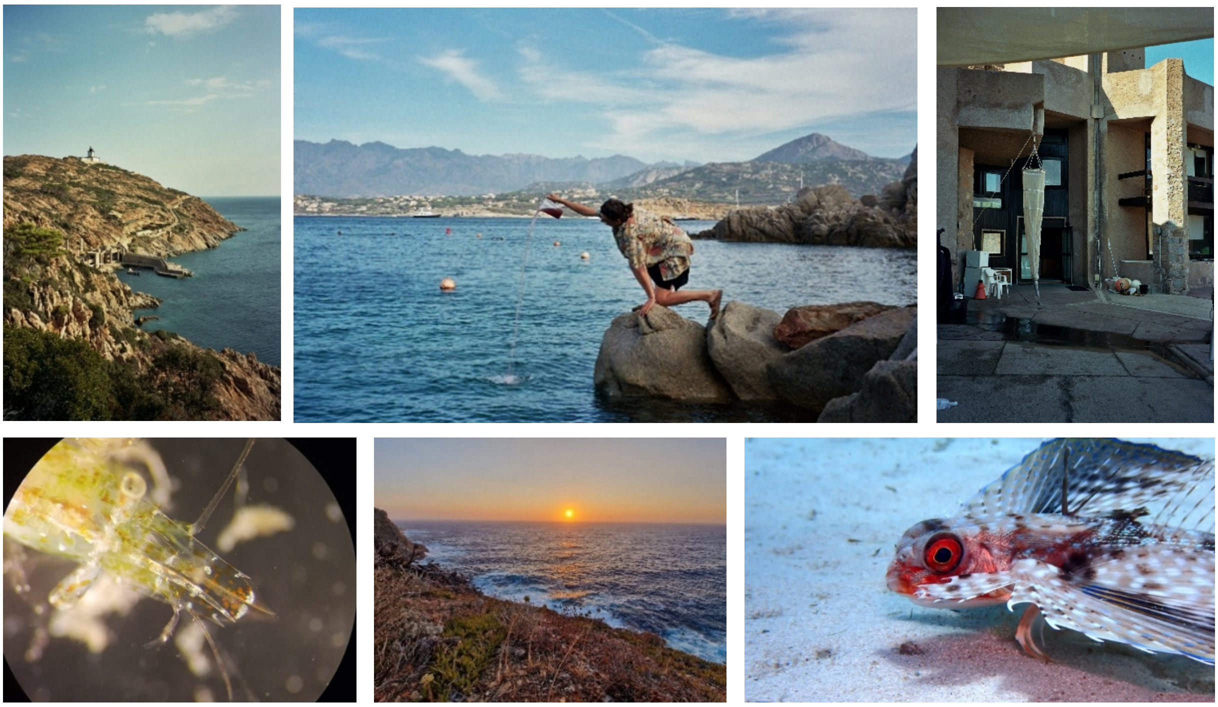

Marine Biological Excursion - Calvi 2023

Marine Biological Excursion

STARESO Station, Calvi, Corsica

July 30th - August 13th 2023

Photo: Claudia Eichbichler

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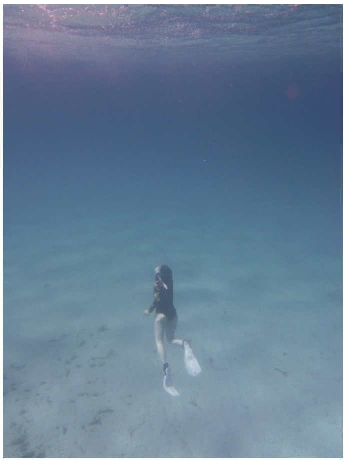

Daily reports

Boulder field

Anne Eilrich, Claudia Eichbichler, Sandra Schallhart, Smilla Todt

Introduction

Mediterranean coasts are mainly influenced by waves rather than tides, as there is a low tidal range of 40 cm. 50% of the Mediterranean coastline is built up by rocks and boulder fields (Hofrichter, 2020). Rock-associated habitats are widespread in Corsica too (Valette-Sansevin et al. 2019). Boulder fields are mainly formed by tidal erosion, waves, wind, and weather. When breakers collapse the wave energy is transmitted onshore and leads to the formation of boulders and the cliff (Ott, 1996). The waves carry away fine material and distribute the blocks according to their size. This creates a field full of rock blocks of different sizes, resulting in an enormous variety of diverse microhabitats that contribute to a high diversity of organisms. In this process, crevices and holes provide shelter for soft bodied organisms (vagile) and armoured organisms settle on the diverse surfaces (sessile) (Hofrichter, 2001). The transition from water to land is divided into three areas: Sublittoral (below the waterline), Eulittoral (intertidal zone) and Supralittoral (above the waterline, influenced by waves). Zoned colonization occurs due to various influencing variables, such as different light and water input, temperature fluctuations, salinity fluctuations, food sources, competition, predation, surf and the nature of the substrate (Tait, 1971). At the transition from water to land, the gradients are strongest and their variability greatest, so that the clearest zonation is found there. During the field course in August 2023, this diverse and varied habitat next to the STARESO research station was snorkelled for one and a half days to identify a wide range of sessile and vagile organisms living there.

Material & Methods

Sampling took place during the first week of August 2023 along the boulder fields in and nearby STARESO harbour and was carried out by snorkelling and free-diving. Organisms were collected from the sublittoral and eulittoral zones of the boulder field in a range from +0.5 to >5m meters depth, using bare hands and hand nets for catches. To discover organisms, the boulder field was closely examined by eye to find freely visible species. Additionally, larger boulders were turned around underwater to access more hidden species. Collected organisms were placed in water-filled plastic bags to be carried back to land by the snorkelers. Stones were turned back to their original position to ensure the least possible impact to the ecosystem. On land, samples were gathered in big plastic boxes filled with sea water for later analysis.

The water was regularly replaced, and the boxes were kept in a cool and shady place to ensure the well-being of the organisms.

Organisms were identified on the day of collection in the lab of STARESO by eye and by using stereo microscopes if necessary. As an identification key “Fauna und Flora des Mittelmeeres” (Riedl, 1983) was used, in case of insufficient or outdated information further literature was consolidated (Hofrichter 2001, Rodríguez-Prieto et. al. 2015, Poppe & Goto (1991 & 1993), Trainito 2011). Determination to species level was carried out wherever possible.

In comparison to the last years, more time was assigned during this year’s course to the collection and investigation of boulder field species. One day with two 1-2 hour snorkelling sessions was used to focus on the collection of macrobenthic, mostly mobile animals, and a second collection day with one 1-2 hour snorkelling session was assigned to gather smaller and more sessile organisms. Whenever additional boulder field associated species were found during the following days of the course, they were added to the list of gathered organisms. Fish and algae species as well as seagrass associated species are not considered in this protocol, as these are discussed in detail in their respective parts.

For later analysis, the pool of boulder field - associated species was split into ‘sessile organisms’ and ‘vagile organisms’. A list of identified species was created for each group (Table 1, 2) and the number of species per phylum as well as per class was calculated and is depicted in the following paragraphs. The number of species is used as a proxy for species diversity per phylum in this protocol.

Sessile Organisms

Results

During the marine biology course 43 sessile species were collected in the boulder fields around the STARESO station. Table 1 shows all collected and identified species, the phylum, and the class they belong to and common English and German names.

Table 1: Species list of sessile organisms found in the boulder field around the STARESO station during the marine biology excursion in 2023. Species names refer either to the World of Marine Species (WoRMS, 2023) or to Riedl (Riedl, 1983), which was used for the identification during the course.

Phylum	Class	Species	Common name (English/German)
Porifera	Demospongiae	*Antho sp.* (WoRMS,2023)	
Porifera	Demospongiae	*Chondrosia reniformis* (WoRMS,2023)	Leater-sponge/ Nierenschwamm
Porifera	Demospongiae	*Scalarispongia scalaris* (WoRMS,2023)	(Black) Leather-Sponge/ Schwarzer Schwamm
Porifera	Demospongiae	*Axinella damicornis* (Riedl,1983)	Yellow sponge/ Lamellen-Geweihschwamm

Porifera	Demospongiae	*Chondrilla nucula* (Riedl,1983)	Chicken liver sponge/ Glänzender Lederschwamm
Porifera	Demospongiae	*Crella rosea* (Riedl,1983)	
Porifera	Demospongiae	*Spirastrella cunctatrix* (Riedl,1983)	Encrusting orange sponge/ Orangefarbener Strahlenschwamm
Porifera	Demospongiae	*Hemimycale columella* (Riedl,1983)	Crater sponge
Porifera	Demospongiae	*Crambe crambe* (Riedl,1983)	Orangeroter Krustenschwamm
Porifera	Demospongiae	*Sarcotragus spinosulus* (WoRMS,2023)	Black leather sponge/ Schwarzer Lederschwamm
Porifera	Demospongiae	*Phorbas tenacior* (WoRMS,2023)	Bluish encrusting sponge/ Blauer Krustenschwamm
Cnidaria	Anthozoa	*Anemonia sulcata* (Riedl,1983)	Mediterranean snakelocks sea anemone/ Wachsrose
Cnidaria	Anthozoa	*Parazoanthus axinellae* (Riedl,1983)	Yellow cluster anemone/ Gelbe Krustenanemone
Cnidaria	Anthozoa	*Hormathia coronata* (WoRMS,2023)	
Cnidaria	Anthozoa	*Balanophyllia europaea* (Riedl,1983)	Pig-tooth coral/ Vielfarbige Solitärkoralle
Cnidaria	Anthozoa	*Actinia equina* (Riedl,1983)	Beadlet anemone/ Pferdeaktinie
Cnidaria	Anthozoa	*Balanophyllia regia* (WoRMS,2023)	Scarlet and gold star coral/ Goldene Sternkoralle
Cnidaria	Anthozoa	*Anemonia viridis* (WoRMS,2023)	snakelock anemone/ Wachsrose
Cnidaria	Anthozoa	*Leptosammia pruvoti* (Riedl,1983)	Sunset cup coral/ Kelchkoralle
Cnidaria	Anthozoa	*Cladocora caespitosa* (Riedl,1983)	Mediterranean pillow coral/ Rasenkoralle
Cnidaria	Anthozoa	*Cornularia cornucopiae* (WoRMS,2023)	Röhrenkoralle
Cnidaria	Hydrozoa	*Plumularia sp.* (Riedl,1983)	Glassy plume hydroid/ Flaumfederchen
Cnidaria	Hydrozoa	*Eudendrium racemosum* (WoRMS,2023)	Traubenförmiger Bäumchenpolyp
Foraminifera	Globothalamea	*Homotrema rubrum* (WoRMS,2023)	Red Foram/ Kammerling
Bryozoa	Gymnolaemata	*Myriapoda truncata* (Riedl,1983)	false coral
Bryozoa	Gymnolaemata	Suborder Ascophora (Riedl,1983)	
Bryozoa	Gymnolaemata	*Cellepora pumicosa* (Riedl,1983)	
Bryozoa	Gymnolaemata	*Schizobrachiella sanguinea* (Riedl,1983)	red encrusting bryozoan/ Rotbraunes Krustenmoostierchen
Bryozoa	Gymnolaemata	*Aetea anguina* (Riedl,1983)	
Bryozoa	Stenolaemata	*Lichenopora radiata* (Riedl,1983)	

Bryozoa	Stenolaemata	*Crisia sp.* (Riedl,1983)	
Bryozoa	Stenolaemata	*Patinella radiata* (WoRMS,2023)	coralline bryozoan/ Korallen-Moostierchen
Annelida	Polychaeta	*Spirorbis* sp. (Riedl,1983)	Spiral tubeworm/ Posthörnchenwurm
Annelida	Polychaeta	*Eupolymnia nebulosa* (Riedl,1983)	Spagetthi worm/ Erdbeerwurm
Annelida	Polychaeta	*Pomatotoceros triqueter* (WoRMS,2023)	Keelworm/ Dreikantröhrenwurm
Annelida	Polychaeta	*Portila tubularia* (WoRMS,2023)	smooth tubewormed-spotted horseshoe/ Glatter Kalkröhrenwurm
Mollusca	Gastropoda	*Petaloconchus glomeratus* (WoRMS,2023)	
Mollusca	Bivalvia	*Pinna rudis* (WoRMS,2023)	Rough pen shell/ Raue Schinkenmuschel
Chordata	Ascidiacea	*Halocynthia papillosa* (Riedl,1983)	Red sea squirt/ Rote Seescheide
Chordata	Ascidiacea	*Microcosmus sp.* (Riedl,1983)	order of sea squirts/ Gattung Seescheiden
Chordata	Ascidiacea	*Didemnum maculosum* (Riedl,1983)	Krustenseescheide
Chordata	Ascidiacea	*Ascidia mentula* (Riedl,1983)	Stumpen-Seescheide
Chordata	Ascidiacea	*Polysyncraton lacazei* (WoRMS,2023)	

The 43 sessile species that could be discovered in the boulder field belong to eight different phyla: Porifera, Cnidaria, Foraminifera, Bryozoa, Annelida, Mollusca and Chordata. Figure 1 shows the different phyla and the number of collected species in each phylum. Comparing the phyla, Cnidaria and Porifera occurred with the most species and include nearly 50% of all the detected species. Within the Bryozoa and the Annelida, eight and four species could be found. In all remaining phyla five or less than five species were identified.

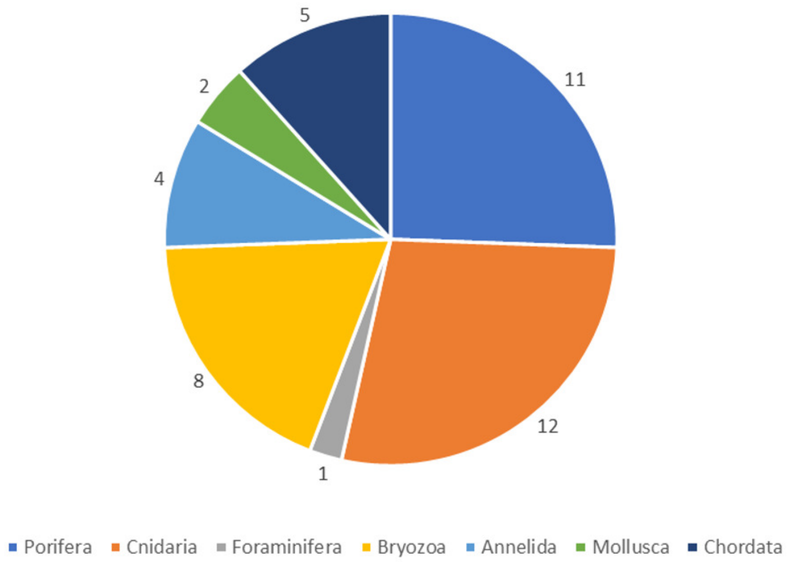

Figure 1: Graphical representation of the different phyla and the species per phylum, which were collected from the boulder fields around the STARESO station during the marine biology course in 2023.

Figure 2 shows a more detailed categorization of the identified sessile species into their class and their phylum, to gain a better understanding of the diversity in the boulder field. Within the Porifera only one class (Demospongiae) with eleven species was collected. In Cnidaria two classes: Anthozoa and Hydrozoa could be observed, in which Anthozoa includes the highest number of species. Besides, for the Foraminifera only one class (Globothalamea) with one species could be added to our list. With five species (Chordata) and eight species (Bryozoa) these two phyla show a high species diversity. The gathered Bryozoa divide into two classes, Gymnolaemata and Stenolaemata, and the Chordata contain four species belonging to the class Ascidiacea. For the sessile Molluscs only one species in the class Bivalvia and one in the class Gastropoda could be observed. Furthermore, four species of the class Polychaeta (Annelida) were determined during the course.

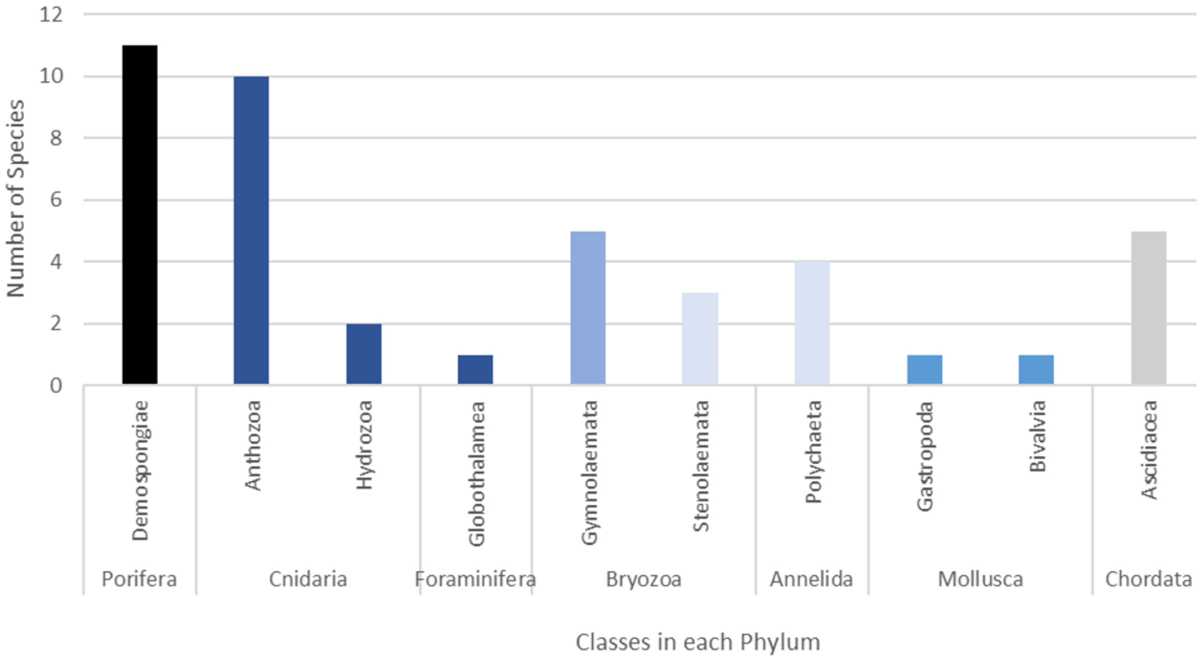

Figure 2: Graphical representation of the different species within their phyla and their classes, which were collected from the boulder fields around the STARESO station during the marine biology course in 2023.

A comparison of the sessile species from the course years 2012, 2021 and 2023 is shown in figure 3. The years 2012 and 2021 were used as a comparison because a comparable number of sessile species was found in these years, while in other years the focus was more on the mobile organisms. Considering the eight phyla that could be found in 2023, no individuals of the two phyla Bryozoa and Foraminifera were collected and identified in 2012 and 2021. Sessile organisms of Mollusca, Annelida and Cnidaria were observed in all three displayed years, though in the phyla Cnidaria and Annelida more than double the number of species could be identified in 2023. Five species of the phylum Porifera were gathered in 2012, 11 in 2023 but none in 2021. The phylum Chordata was only detected in 2021 with one species and in 2023 with five species.

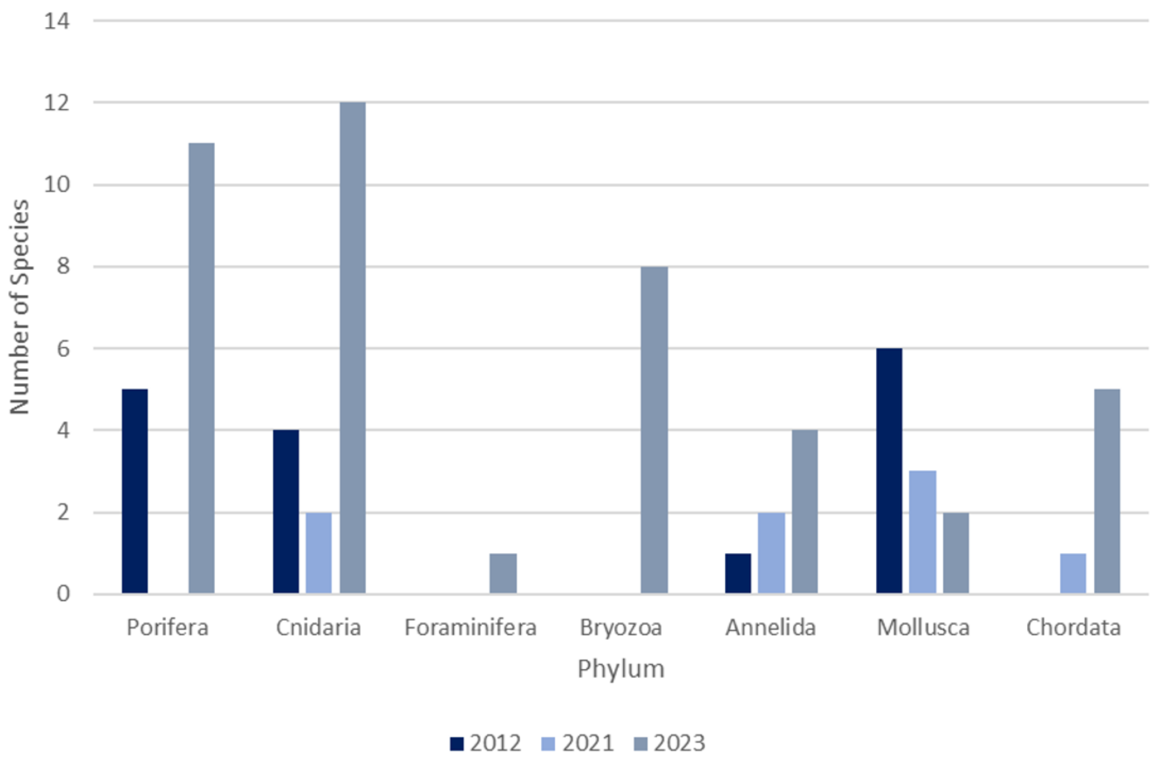

Figure 3: Comparison of the sessile species found in the boulder fields around the STARESO station in the years 2012, 2021 and 2023.

Discussion

According to the fact that during the course in 2023 an extra day was invested to collect only sessile organisms in the boulder fields around STARESO the data is not fully comparable to the sessile species found in the last courses. Most of last year's boulder field sampling focused on macrobenthic, mostly mobile animals that were easy to catch and recognize and are therefore mostly regarded in the ‘vagile organisms’ part of this protocol. For example, the most abundant phyla found in the boulder field sampling of all last years’ courses is Mollusca. In the ‘sessile organisms’ part of this year's protocol this phylum is only represented by two species. This is due to the fact that most molluscs are considered mobile and therefore discussed in part two of this protocol.

A comparable number of sessile species was only found in the years 2021 and 2012. However, in none of these two years any Bryozoa or Foraminifera species was determined, as well as any Porifera species in 2021 and any Chordata species in 2012. This different species composition is most likely explained by the different sampling and identification efforts of the different years. The boulder field sampling commonly takes place during the very first days of the course when snorkeling skills as well as identification skills are not yet as well trained in all course members as during the later part of the course. As Bryozoa, Foraminifera and

Porifera species are more difficult to determine on species level, it is hypothesized that these Phyla were not the focus of identification during last years' boulder field sampling.
As an extra day with special focus on sessile species was assigned in 2023, during the later days of the course more Bryozoa, Foraminifera and Porifera organisms could be sampled and determined. Additionally, the 2021 boulder field protocol states that crust-building animals like Bryozoa and sponges are disregarded in the boulder field protocol and are discussed separately in other parts. Three Bryozoa species are named in that year's Corraligène protocol, ten Bryozoa and three Foraminifera species in the sea gras protocol. These are discussed and compared in their respective 2023 protocols.

Vagile Organisms

Results

During the course, 43 vagile species were collected in the boulder fields located directly by the STARESO station. Table 2 shows all collected and identified species, the phylum, and the class they belong to and common English and German names.

Table 2: List of vagile species that were collected in the boulder field surrounding STARESO station in 2023.

Phylum	Class	Species (Riedl)	Species (WoRMS)	Common names
Cnidaria	Scyphozoa	*Pelagia noctiluca*	*Pelagia noctiluca*	Purple people eater Leuchtqualle
Mollusca	Gastropoda	*Columbella rustica*	*Columbella rustica*	-
	Gastropoda	*Haliotis lamellosa*	*Haliotis tuberculata*	Abalone Seeohr
	Gastropoda	*Gibbula varia*	*Steromphala varia*	-
	Gastropoda	*Monodonta turbinata*	*Phorcus turbinata*	Turbanschnecke
	Gastropoda	*Hexaplex trunculus*	*Hexaplex trunculus*	Banded dye-murex
	Gastropoda	*Thais haemostoma*	*Stramonita haemastoma*	Red-mouthed rock shell Rotmund-Leistenschnecke
	Gastropoda	*Gibbula umbilicalis*	*Stramonita haemastoma*	Flat top shell
	Gastropoda	*Murex alucaster*	*Cerithium alucastrum*	-
	Gastropoda	*Patella aspera*	*Patella aspera*	Azorean limpet
	Gastropoda	*Patella caerulea*	*Patella caerulea*	-
	Gastropoda	*Mitrella maldonadoi*	*Mitrella bruggeni*	-

	Gastropoda	*Clanculus cruciatus*	*Clanculus cruciatus*	-
	Gastropoda	*Thuridilla hopei*	*Thuridilla hopei*	Saftsauger
	Gastropoda	*Conus mediterraneus*	*Conus ventricosus*	Mediterranean cone Mittelmeerkegel
	Bivalvia	*Gourmya rupestris*	-	-
	Bivalvia	*Glycymeris insubrica*	*Glycymeris nummaria*	-
	Bivalvia	*Barbatia barbata*	*Barbatia barbata*	Bärtige Archenmuschel
	Bivalvia	*Arca noae*	*Arca noae*	Noah´s ark shell Arche Noah-Muschel
	Polyplacophora	*Chiton olivaceus*	*Rhyssoplax olivacea*	Green chiton Grüne Käferschnecke
	Polyplacophora	*Callochiton achatinus*	*Callochiton septemvalvis*	-
Annelida	Polychaeta	*Eulalia viridis*	*Eulalia viridis*	Grüner Blattwurm
	Polychaeta	*Typosyllis prolifera*	*Syllis prolifera*	-
	Sipunculida	*Physcosoma granulatum*	*Phascolosoma granulatum*	Peanut worm Spritzwurm
Nemertini	Pilidiophora	*Lineus geniculatus*	*Notospermus geniculatus*	-
Arthropoda	Malacostraca	*Clibanarius erythropus*	*Clibanarius erythropus*	Mediterranean intertidal hermit crab Mittelmeer-Felsküsteneinsiedlerkrebs
	Malacostraca	*Xantho poressa*	*Xantho poressa*	Jaguar round crab Jaguarkrabbe
	Malacostraca	*Eriphia verrucosa*	*Eriphia verrucosa*	Warty crab Italienischer Taschenkrebs
	Malacostraca	*Pseudoprotella phasma*	*Pseudoprotella phasma*	-
	Malacostraca	*Palaemon elegans*	*Palaemon elegans*	Rock pool shrimp Kleine Felsengarnele
	Malacostraca	*Pisa nodipes*	*Pisa nodipes*	-
	Malacostraca	*Maera inaequipes*	*Quadrimaera inaequipes*	-
	Malacostraca	*Pisa tetraodon*	*Pisa tetraodon*	-
	Malacostraca	*Nerocila bivittata*	*Nerocila bivittata*	Fischassel
Echinodermata	Echinoidea	*Paracentrotus lividus*	*Paracentrotus lividus*	Purple sea urchin Steinseeigel
	Echinoidea	*Sphaerechnius granularis*	*Sphaerechnius granularis*	Violet sea urchin Violetter Seeigel
	Echinoidea	*Arbacia lixula*	*Arbacia lixula*	Black sea urchin Schwarzer Seeigel
	Asteroidea	*Asterina gibbosa*	*Asterina gibbosa*	Starlet cushion star

	Asteroidea	*Echinaster sepositus*	*Echinaster sepositus*	Mediterranean red sea star Purpurstern
	Ophiuroidea	*Ophiomixa pentagona*	*Ophiomixa pentagona*	-
	Ophiuroidea	*Ophioderma longicauda*	*Ophioderma longicaudum*	Glatter Schlangenstern
	Holothuroidea	*Holothuria* cf. *tubulosa*	*Holothuria tubulosa*	Cotton spinner Röhrenseegurke
	Holothuroidea	*Holothuria* cf. *poli*	*Holothuria poli*	White spot cucumber Weißspitzen-Seegurke

The 43 vagile species found in the boulder field belong to six different phyla: Cnidaria, Mollusca, Annelida, Nemertini, Arthropoda and Echinodermata. Figure 4 shows the quantitative distribution of the different vagile phyla and the number of collected species in each phylum. Almost 50% of the vagile organisms found belong to Mollusca (20 species), followed by Arthropoda (9 species) and Echinodermata (9 species), which each represent about 20%. The remaining phyla are represented by only 1-3 species.

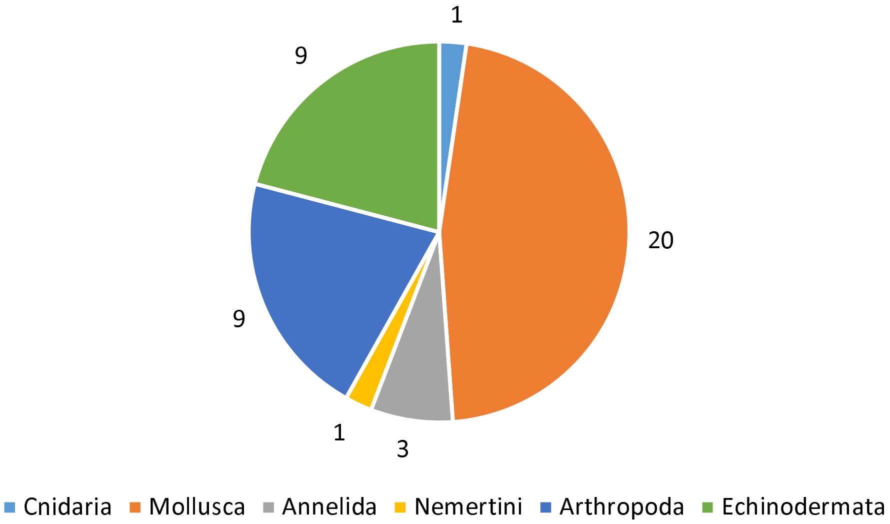

Figure 4: Graphical representation of the different vagile phyla and the species per phylum, which were collected from the boulder field around the STARESO station during the marine biology course in 2023.

Figure 5 gives a more detailed overview of the vagile organisms, listing all classes found within the phyla. Cnidaria are present with one class (Scyphozoa), Mollusca with three classes (Gastropoda, Bivalvia, Polyplacophora), and Gastropoda are by far the most represented. The Annelida are present with two classes (Polychaeta and Sipunculida). Nemertini (Pilidiophora) and Arthropoda (Malacostraca) are both represented by one class. Echinodermata with four classes (Echinodea, Asteroidea, Ophiuroidea, Holothuroidae) are the richest in classes, followed by Mollusca, where only 2-3 representatives from each class were found.

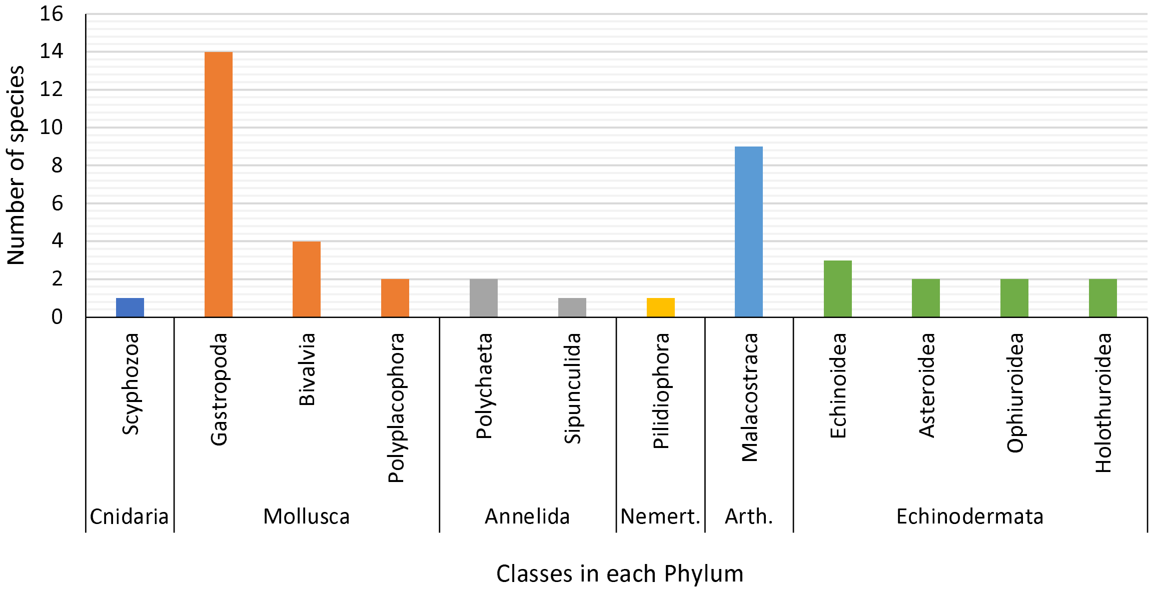

Figure 5: Graphical representation of the different vagile species within their phyla and their classes, which were collected from the boulder field around the STARESO station during the marine biology course in 2023.

Figure 6 shows a comparison between the results for vagile species in the boulder field of 2023 and the last two courses in 2018 and 2021. In all years, mostly Mollusca were found, followed by Arthropoda and Echinodermata. Less frequently were Cnidaria, Platyhelminthes, Annelida, and Nemertini. Important to note is that the same Cnidaria species was collected in 2021 and 2023 and the same Nemertini species in 2018 and 2023, while the Platyhelminthes species found in 2018 and 2021 differed among the years. Each year, different Annelida species were found. There is a bit more overlap of species (ca. 30%) in the more dominant phyla, however, there were also new species found each year.

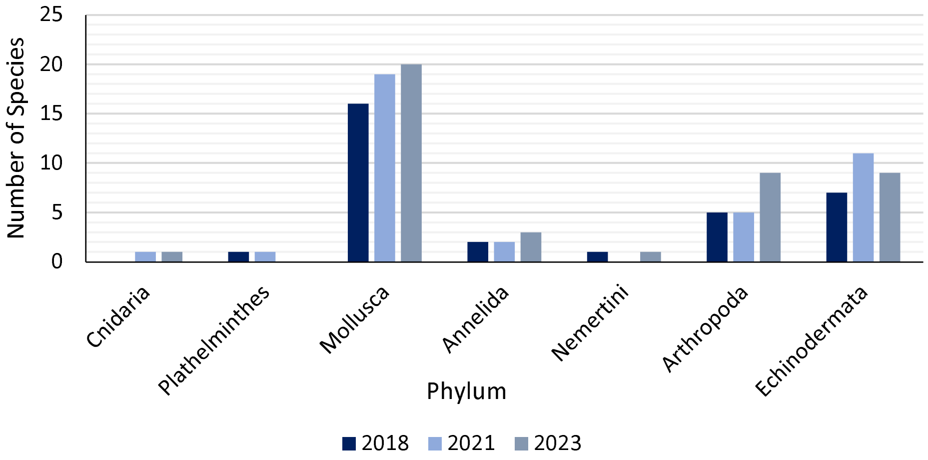

Figure 6: Graphical representation of the different vagile species per phyla compared between the last three courses at the STARESO station (2018, 2021, and 2023).

Discussion

The dominance of Mollusca (especially Gastropoda) can be explained by the microhabitats that a boulder field generates. Menconi et al. (1999) conducted a comprehensive study in a boulder field in the Northwestern Mediterranean and found that the availability of crevices as microhabitat positively influenced the abundance and vertical distribution of vagile invertebrates, particularly Gastropoda. The boulder field around the STARESO station in Corsica is characterized by many crevices, depressions, and rocks of various sizes that provide a variety of hiding places for the vagile organisms. This simultaneously explains the consistency across the years, as the habitat itself has not changed much. Additionally, the sampling technique and effort has been similar throughout the years with a focus on vagile species over sessile species. The boulder field has always been sampled in the first days with some more species added over the rest of the course period, which means that the snorkelling expertise and the ability to access hidden organisms in the habitat is comparable among the years. Still, new species have been found each year, which is especially true for the phyla with only few representative species. Annelida and Nemertini for example are generally smaller and move more quickly than Mollusca, Arthropoda, and Echinodermata. They are therefore more difficult to collect, which explains the small amount but high variability within these phyla compared across the past three courses. The Cnidaria species *Pelagia noctiluca* is not directly associated with the boulder field because it is a planktonic, pelagic species. It does not appear in some other protocols not because it has not been seen, but simply because it has not been collected for the boulder field protocols.

Conclusion

It can be concluded from the boulder field sampling and comparison with previous years that sessile species have been highly overlooked before. With 44 species we found even more sessile than vagile species (43), suggesting that the sessile species are just as important in the boulder field habitat as vagile species and should be considered in following protocols as well. While the overall phyla distribution within the habitat has not changed much throughout the years in case of the vagile organisms, it is important to note that not always the same species represent a phylum because new species have been found due to various reasons.

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Macroalgae occurrence and distribution in the Mediterranean Sea near Calvi, Corsica

Andrea Ourednik, Fenja-Marie Möller

Abstract

Macroalgae are characterized by their pigment composition in the cells, which define their appearance and occurrence in the depth and light environment and their important role in marine ecosystems. In the Mediterranean Sea, their species occurrence is highly diverse. Near STARESO, south of the Bay of Calvi, Corsica, sampling of macroalgae species was carried out at different depths and light environments. The results revealed Ochrophyta to be the most abundant, followed by Chlorophyta and Rhodophyta. Comparing the depth and light distribution, our results revealed a distribution preference for brown algae in intermediate depths and sun-exposed areas, while more red algae were found in deeper and shadow-exposed areas, as well as green algae. Aditionally, compared to previous sampling, one invasive species has disappeared in the bay. Overall, our sampling yielded important information to record and assess regional changes in the Mediterranean Sea over the years.

Introduction

The Mediterranean Sea, a biodiversity hotspot and one of the seas most affected by anthropogenic impacts (e.g. coastal and habitat changes, pollution, overfishing or increase of non-native species, etc.), is facing widespread loss of macroalgae forests and populations in different parts of the Mediterranean Sea (e.g., Coll et al., 2010, Bianchi et al., 2014, Thibaut et al., 2015, Žuljević et al., 2016, Blanfuné et al. 2019).

Macroalgae, macroscopically visible marine algae can be divided into three main taxonomic groups Chlorophyta (green algae), Ochrophyta (brown algae), and Rhodophyta (red algae). The names refer to their appearing color, which is based on the composition of protective pigments in their cells (Pereira, 2021). Green algae photosynthesize chlorophyll a and b in different concentrations depending on the species (Nakamura et al., 1976) giving them their green color. However the intensity of the color is liable on the breakdown of chlorophyll (Hortensteiner, 1999). In brown algae the algae specific xanthophyll fucoxanthin is prevalent (Din et al., 2022) and phycoerythrin, a red pigment embedded primarily in rhodophytes gives red algae their color (Tan et al., 2022).

More than 10 000 macroalgae species are known, in the Mediterranean Sea occur around 1000 species, however due to molecular research and introduced species these numbers undergo a constant change (Hofrichter, 2020). Macroalgae occur in a wide range of depths, although their appearance is linked to the refraction of light. Depending on their cellular mechanisms and pigment components some species can adapt to a deeper and darker environment compared to others. Most species occur to 30 m depth, some can go down to 60 m. However, light gets reduced with increasing depth and changes in wavelength from the littoral to the circalittoral. However, isolated macroalgae species, such as red algae and specialized green algae, can survive at depths to 200 m (Hofrichter, 2020).

Depending on the species, macroalgae vary greatly in anatomy and their ways of living, represented styles are attached to the bottom, benthic, or within sediments (Barsanti, 2014). They are able to grow to a length of several meters and, as primary producers, are an important part of the marine environment, serving as the basis of the food chain for many herbivorous organisms and providing shelter (Pereira, 2021). For example, brown algae are rich in health beneficial nutrients and therefore have potential as a food source for marine life as well as for humans (Din et al., 2022). Additionally, for marine life, macroalgae can be used for housing or nest building, as seen for the Ocellated wrasse *Symphodus ocellatus*, where males use algae during the reproductive period to build nests (Sinopoli et al., 2015). Furthermore, algae are also an important indicator for water quality, as their occurrence is linked to different water parameters like temperature, pH, phosphate, etc. (Ebrahimzadeh et al., 2021).

However, in the Mediterranean Sea, macroalgae ecosystems are facing several threats leading to biodiversity loss across the food web (e.g., Coll et al., 2010, Bianchi et al., 2014). Invasive species can become problematic like the Mediterranean, *Chrysophaeum taylorii* is one of the invasive algae that has been spreading rapidly in the last years and affects marine life in harmful ways with their produced mucilage (Caronni et al., 2017). These changes need to be investigated and recorded in order to understand and prevent them.

In a marine biological excursion, we sampled and identified various algae species of the Mediterranean Sea in different depths and light zones, in order to record their distribution at the bay of the STARESO Research Station on Corsica.

Material and Methods

Macroalgae sampling and identification took place on the 1st of August 2023 in two runs in the morning and the afternoon. The sampling area was restricted the rocky shore in direct vicinity to the harbor of the STARESO near Calvi, Corsica. By snorkeling, a group of eight person sampled macroalgae (Chlorophyta, Ochrophyta, and Rhodophyta) at different water depths (<1m, 1-3m and >3m) in both runs. In addition, the sampled algae were distinguished between shady and sun-exposed algae. After sampling, the algae were identified using the identification literature of Riedel (1983). After identification, the algae were returned to the sea. In our documentation of the algae species found, we have also added some algae species that were identified in the same environment but on a different sampling day that actually focused on sessile boulder field species.

Results

In total 20 different species of three different phyla were found near STARESO (Table 1). 42% of the algae were Ochrophyta (brown algae), 32% were Chlorophyta (green algae), and 26% were Rhodophyta (red algae) (Figure 1). The species found were distributed differently on the rocky shore habitat depending on depth and light exposure (Figure 2).

Regarding the species diversity, at least 4 species (sun <1 m; sun >3m) or more were found in all areas. The highest species diversity in shadow areas was found at a depth of more than 3 m with a total of 9 species, not considering the phyla. In sun-exposed areas, however, the diversity was found at a depth of 1 - 3 m, also with 9 species. Considering the different phyla, a total of 7 Chlorophyta species were found. In shadow areas, 3 species each were found in 1-3 m and deeper than 3m. Only one species was found near the surface at less than 1 m. In sun-exposed areas, most Chlorophyta were found at a depth of 1-3 m (3 species), while in the other areas only one species each was found. A total of 8 different species of Ochrophyta were found, most of them (4 species, *Cystoseira* sp., *Dictyota dichotoma*, *Halopteris scoparia*, *Padina pavonia*) occurred in sunny areas at a depth of 1 to 3 m. Two species each were found in the shallower and deeper areas of the sun-exposed areas

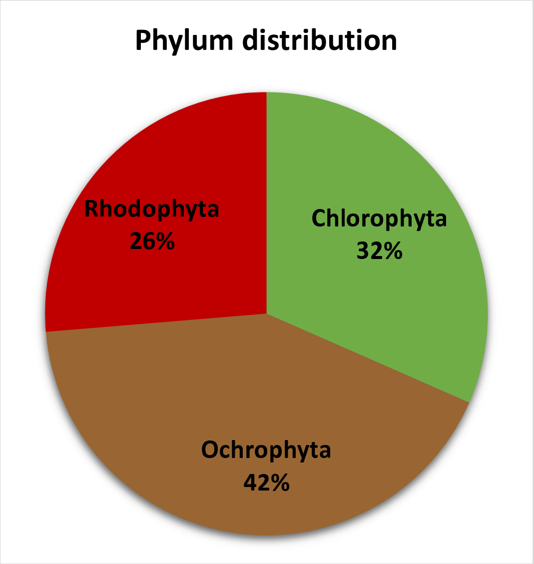

Figure 1: Distribution (%) of the different phyla found near STARESO.

Table 1: Identified algae using the identification key of Riedl (1983) and their distribution in water depth (m) and their light exposure (grey fields: shadow-exposed, yellow field: sun-exposed) in the STARESO bay. Phylum, Class and Genus/Species were recorded.

Phylum	**Class**	**Genus/Species**	**Depth (m)**		
			0-1	1-3	>3
Chlorophyta	Ulvophyceae	*Acetabularia acetabulum*		x	
		Anadyomene stellata		x x	
		Caulerpa racemosa		x	x
		Chaetomorpha aerea	x		
		Codium bursa		x	x
		Halimeda tuna			x
		Udotea petiolata	x	x	x
Ochrophyta	Phaeophyceae	*Cystoseira fimbriata*	x		
		Cystoseira sp.		x x	
		Dictyota dichotoma	x	x x	x
		Halopteris filicina	x		
		Halopteris scoparia		x	x
		Padina pavonia		x x	x x
		*Sphacelaria plumula**			x
		Taonia atomaria	x		
Rhodophyta	Florideophyceae	*Corallina mediterranea*	x	x	
		Liagora viscida	x	x	x
		Peyssonnelia squamaria	x	x	x
		Pseudolithophyllum expansum			x
		*Tricleocarpa fragilis**			x
Sessile Boulderfield					
Rhodophyta	Florideophyceae	*Lithothamnium lenormandi*	-		
		Pseudolithophyllum expansum	-		
		Amphiroa rigida	-		
		Hildenbrandia crouaniorum	-		

*AlgaeBase: Guiry and Guiry (2023)

and in the <1 m deep shadow areas. A total of 5 species of Rhodophyta were found and most of them in deep (>3m) shadow areas (3 species, *Peyssonnelia squamaria*, *Pseudolithophyllum expansum*, *Tricleocarpa fragilis*).

Additionally, the macroalgae of the sessile boulder field sampling were Rhodophyta, in total 4 species were found and identified (Table 1).

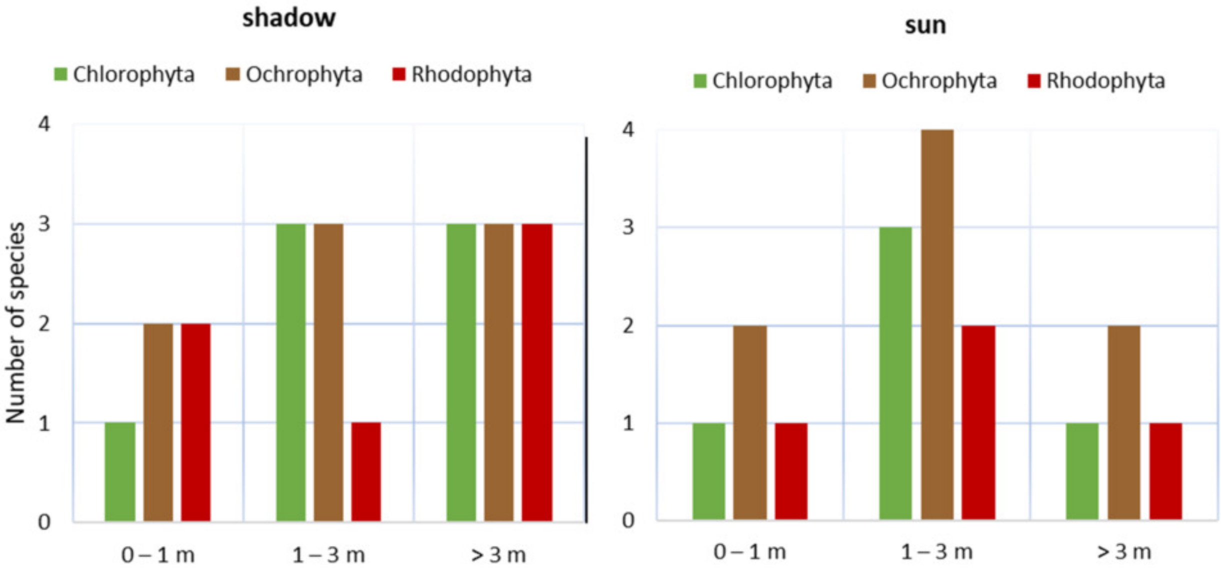

Figure 2: Distribution of algae species. The numbers of species are presented by light and depth exposition and the colors represent the different algae phyla.

Discussion

Our assessment provides a current status of the macroalgae species that were present in STARESO Bay in summer 2023. Brown algae dominated the algae diversity. Whereas in shadow-exposed areas they indicated no depth preference and were almost evenly distributed, in sun-exposed areas they seemed to favor the intermediate depths. In contrast, red algae have mechanisms adapted to deeper and darker environments (Voerman et al., 2022), we found them in all areas, but our results also indicate their preference for deeper, shadow-exposed sites. The characteristics of green algae could lead to the assumption that their living is restricted to shallow and sunny environments, however, in both light exposures each we found only one species of green algae at the surface (shadow: *Chaetomorpha aerea*; sun: *Udotea petiolata*) and most species in deeper, particularly shadowed sites. It shows that green algae are not only restricted to the surface. Green algae that live at different depths differ in their thallus appearance and their absorption spectrum, e.g. different *Codium* sp. are adapted to deeper waters due to their thicker thalli and numerous chloroplasts (Yokohohama, 1989, Doroftei et al., 2010). Thus, specialized green algae can even be up to 200 m deep;

with high chlorophyll contents, they can be better adapted to dark environments (Hofrichter, 2020).

Nevertheless, we should critically consider our results, we could only observe minor differences between the phyla distribution in terms of depth and light exposure, which is due to the fact that our assessment only took place on one day and the depth investigated was limited due to our snorkeling skills. However, macroalgae are able to live in much deeper areas (Hofrichter, 2020).

In comparison to the macroalgae occurrence two years ago in the same area, we did not find the invasive algae *Chrysophaeum taylorii* during our sampling. *C. taylorii* has been reported from tropical and subtropical Atlantic and Western Pacific coasts (Aktan and Topaloğlu, 2011) and has recently been expanding in the Mediterranean Sea, with strong spatial and temporal variation in occurrence (Caronni et al., 2014). As a species adapted to warm water temperature (Aktan and Topaloğlu, 2011), *C. taylorii* might need time to adjust to the Mediterranean temperature. Due to climate change and increasing temperature, it is likely to expand all over the Mediterranean in following years. In addition, high hydrodynamic stress, water flow and wind intensity may prevent the cells of *C. taylorii* from settling successfully on the substrate (Caronni et al., 2014), which could also explain the current change in the occurrence of this species at STARESO Bay.

Interestingly, we also observed that more *Cystoseira* sp. were spotted near STARESO than in previous years. The Mediterranean Sea is known for its unique macroalgae forests of brown algae of the genus *Cytoseira* with smaller lying, stretched or buoyant flexible canopies that drape the sea floor (Sala et al., 2012). *Cystoseira* species can be used as indicators of environmental quality because they are very sensitive to change and decline when pollution is high, but also recover when environmental quality is good (Ballesteros et al., 2007, Diez et al., 2009, Sales et al., 2011). It is also possible that the marine environment around STARESO Bay has experienced a change in water quality in recent years.

In general, compared to previous years, similar conditions were found locally near STARESO in the distribution of macroalgal phyla, with each phylum represented between 20 and 50 % over the years. In most years, between 20 and 25 species were collected, thus no significant change was observed in the number of species over time (see previous reports).

However, with rising temperatures in the Mediterranean Sea (Sakalli, 2017), an increase in growth rate of algae (Singh and Singh, 2015), or the ability of invasive species to survive in new locations (Aktan and Topaloğlu, 2011), could occur more frequently. Additionally, loss of the unique macroalgae forests changes whole habitat structures, from high diverse macroalgae rocky shore habitats to widespread, less complex barren grounds (Sala et al., 2012). The local extinction of macroalgae populations and their inhabitants is occurring more and more frequently, and their recovery represents a problem as their restoration is dependent on factors such as environmental pollution and climate change (Cebrian et al., 2021).

In conclusion, the Mediterranean Sea has already undergone major changes in recent decades, ongoing climate change and negative anthropogenic impacts leading to temperature increases, fluctuations in pH or available nutrients, etc. could cause alterations in macroalgae biomass and diversity and potentially negatively affect Mediterranean marine ecosystems in the future (e.g., Lejeusne et al., 2010, Coll et al., 2010, Bianchi et al., 2014, Blanfuné et al. 2019).

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Posidonia oceanica - a biodiversity hotspot for sessile and vagile organisms

Georgia Avgerinou & Moritz Stegner

Introduction

Posidonia oceanica, a species of seagrass endemic to the Mediterranean Sea, is the only member of the genus Posidonia to be found outside Australia. The plant consists of the leaves, which are arranged in bundles of 4 to 8 leaves and can reach up to 1.5 m in length, the rhizome and the roots. The rhizome, which is up to 2 cm thick, grows both horizontally and vertically, helping the plant to reproduce vegetatively. From the nodes of the horizontal rhizome, roots up to 40 cm long anchor the plant to the substrate.

P. oceanica can reproduce either sexually/generatively or asexually/vegetatively. Sexual reproduction is by flowers and fruits, with flowering from September to October. However, this period can be influenced by the depth of the corresponding Posidonia meadow. This period can be 2 months later if it occurs in deeper areas. Asexual propagation is by rhizome elongation or cuttings. The horizontal rhizome can produce stolons, which in turn anchor themselves with roots to the nodes or cuttings, which can be produced by mechanical damage and can travel further. The distribution of meadows can range from 1 to 35 meters deep, depending on the clarity of the water (Abadie et al., 2018). Throughout the Mediterranean, *P. oceanica* meadows cover about 3% of the total area, i.e. between 2.5 and 5 million hectares. Around Corsica, 62,000 hectares are covered (Pasqualini et al., 1998).

The vertical growth rate of a Posidonia leaf is about 1 cm per year and up to 10,000 leaves can fit in one square meter, resulting in an enormous biomass per plant and an extreme rate of carbon fixation through its photosynthetic activity. In addition, Posidonia can produce what are known as "matte structures". These structures are made up of dead rhizomes, roots and sediments, and do not decompose due to the hypoxia that occurs but instead create a few meters of carbon storage (similar to the highly effective carbon storage of terrestrial sphagnum peat bogs). Comparing the sequestration rates of a *P. oceanica* meadow and a similar sized Amazon rainforest, Posidonia can sequester 15 times more (Pergent-Martini et al., 2023).

Beyond this well-documented capacity for carbon sequestration, these seagrass meadows offer a remarkable 3D environment that supports a diverse array of marine life. The leaves of *P. oceanica* create a complex underwater habitat, providing shelter, food, and breeding grounds for numerous organisms. These meadows serve as a crucial nursery for various fish species, acting as a protective habitat for their young. It's estimated that in just one hectare of *P. oceanica*, 15 tons of animal biomass can thrive, underscoring the significance of these seagrass meadows in sustaining marine biodiversity and ecological balance in the Mediterranean Sea (Balata & Tola, 2018).

Material and Methods

A scientific investigation was conducted to assess species diversity associated with *Posidonia oceanica*. Sampling was performed at a specific meadow section, located 3-5 meters deep within the STARESO station harbour in Calvi, Corsica. The sampling of this protected species was carried out with careful consideration and consultation with the scientific team of STARESO, to minimize any potential negative impact on the meadow's growth and ecosystem.

The collection of seagrass pieces and rhizome segments was done by snorkelers, who carefully collected broken-off parts without causing harm to the meadow. Some plants were placed into plastic bags, while others were collected from the ground and then stored in collection bags. Additionally, other species associated with *P. oceanica* were sampled by gently sweeping the leaves with a fine meshed aquarium net. To avoid any loss of samples, the plastic bags were sealed underwater before surfacing.

The sampling was divided into two groups: a morning group focusing on leaf material and an afternoon group focusing on the rhizome part. Back onshore, the collected samples were placed in buckets and transported to the laboratory for further examination. The plant material was carefully analysed to identify both mobile and sessile organisms and categorized based on the age of leaves (young and old) and segments (basal and apical). The differentiation between basal and apical segments was determined by dividing the leaf length in half.

Additional categories were then made for species occurring on rhizome segments and also mobile species, sampled from the water or seen living in the meadow.

The plant parts as well as the collected surrounding water were then searched for organisms using a binocular (Wild-Heerbrugg M3C). Detected sessile or vagile species were taxonomically classified in phylum, class and species level using the determination key of Riedl (1983).

Additionally, to the investigation of the diversity habituated on *P. oceanica*, also the leaf structure was deeper looked into. Therefore, the structure of the apical bud, the middle section and the base section of one old l and one newly formed leaf were examined (Leitz Diaplan microscope).

Results

This year, emphasis was placed on the sessile organisms found in the leaves (Table 1) and on vagile organisms living in the rhizome (Table 2).

Table 1. Summary of the sessile organisms in Posidonia oceanica leaves.

		Phylum	Class	Order	Family	Species
New leaves	Basal	Retaria	Tubothalamea	Miliolida	Hauerinidae	*Massilina secans*
		Bacillariophyta	Bacillariophyceae	-	-	-
		Rhodophyta	Florideophycae	Corallinales	Corallinaceae	-
		Rhodophyta	Florideophycae	Ceramiales	Ceramiaceae	*Ceramium sp.*
		Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	*Electra posidoniae*
		Annelida	Polychaeta	-	-	-
	Apical	Retaria	Globothalamea	Rotaliida	Rosalinidae	*Rosalina sp.*
		Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	*Electra posidoniae*
		Annelida	Sedentaria	Sabellida	Serpulidae	*Janua sp.*
Old leaves	Basal	Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	*Electra posidoniae*
		Cnidaria	Hydrozoa	Leptothecata	Sertulariidae	*Sertularia perpusilla*
	Apical	Retaria	Globothalamea	Rotaliida	Rosalinidae	*Rosalina sp.*
		Rhodophyta	Florideophycae	Ceramiales	Ceramiaceae	*Ceramium sp.*
		Rhodophyta	Florideophycae	Corallinales	Corallinaceae	-
		Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	*Electra posidoniae*
		Cnidaria	Hydrozoa	Anthoathecata	Eudendriidae	*Eudendrium sp.*

Table 2. Summary of the organisms found in Posidonia oceanica rhizomes.

Phylum	Class	Order	Family	Species
Arthropoda	Malacostraca	Amphipoda	Caprellidae	*Caprella acanthifera*
		Amphipoda	Caprellidae	*Pseudoprotella phasma*
		Amphipoda	Hyalidae	*Hyale schmidtii*
		Isopoda	Gnathiidae	*Gnattia maxillaris*
		Decapoda	Hippolytidae	*Hippolyte inermis*
	Pycnogonida	Pantopoda	Nymphonidae	*Nymphon gracile*
Mollusca	Polyplacophora	Chitonida	Acanthochitonidae	*Acanthochitona communis*
		Chitonida	Chitonidae	*Chiton sp.*
	Gastropoda	Caenogastropoda	Cerithiidae	*Bittium reticulatum*
Annelida	Polychaeta	Sabellida	Serpulidae	*Pomatoceros triqueter**
Nematoda	-	-	-	-
Echinodermata	Ophiuroidea	Amphilepidida	Amphiuridae	*Amphipholis squamata*
Retaria	Globothalamea	Rotaliida	Homotrematidae	*Homotrema rubrum**

*sessile organism

This study provides a qualitative approach to examining epiphytic communities on *P. oceanica* and therefore the number of individuals belonging to the same species was not taken into consideration. A total of 29 different organisms were examined out of which 19 were successfully classified to genus or species level. The rest of them were classified within 4 taxa [Polychaetes, Nematodes, Coralline algae, Bacillariophyceae (Diatoms)]. The epiphytic community consists of 10 phyla (Fig. 1). The most abundant phylum is Arthropoda (21%), closely followed by Bryozoa (14%), Rhodophyta (14%) and Retaria (14%) (Fig. 2b).

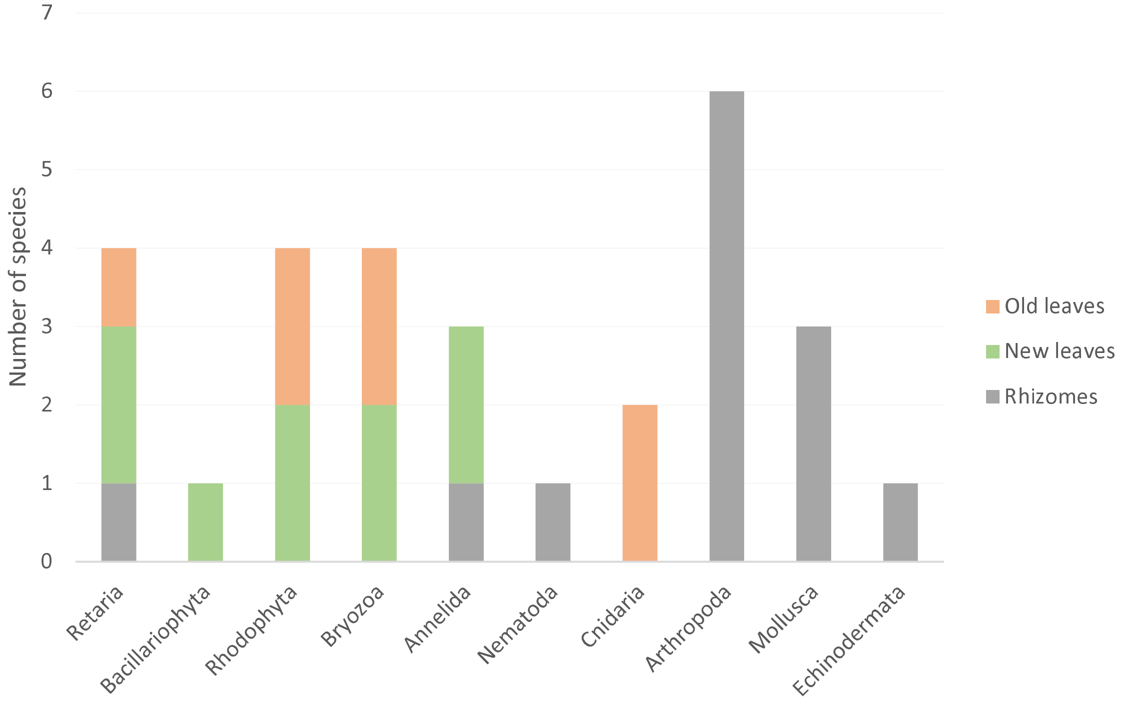

Figure 1. Number of species per phylum found in the different parts of *Posidonia oceanica*.

The distribution of phyla showed minimal variation between old and young leaves. Bacillariophyta and Annelida were found exclusively in new leaves while Cnidaria was found exclusively in older leaves (Fig. 1, Fig. 2a & Fig. 3). No distinct pattern could be observed between the apical and basal zone of the leaves (Fig. 3).

Rhizomes exhibited greater diversity of phyla compared to leaves. A different distribution of phyla could be observed between the leaves and the rhizomes. Arthropoda, Mollusca and Echinodermata were solely found in the rhizomes while Bacillariophyta, Rhodophyta, Bryozoa and Cnidaria were solely found in the leaves. Annelid worms represented by sessile polychaetes and Retaria represented by Foraminifera were found in both parts of the leaf (Fig. 1 & Fig. 2a).

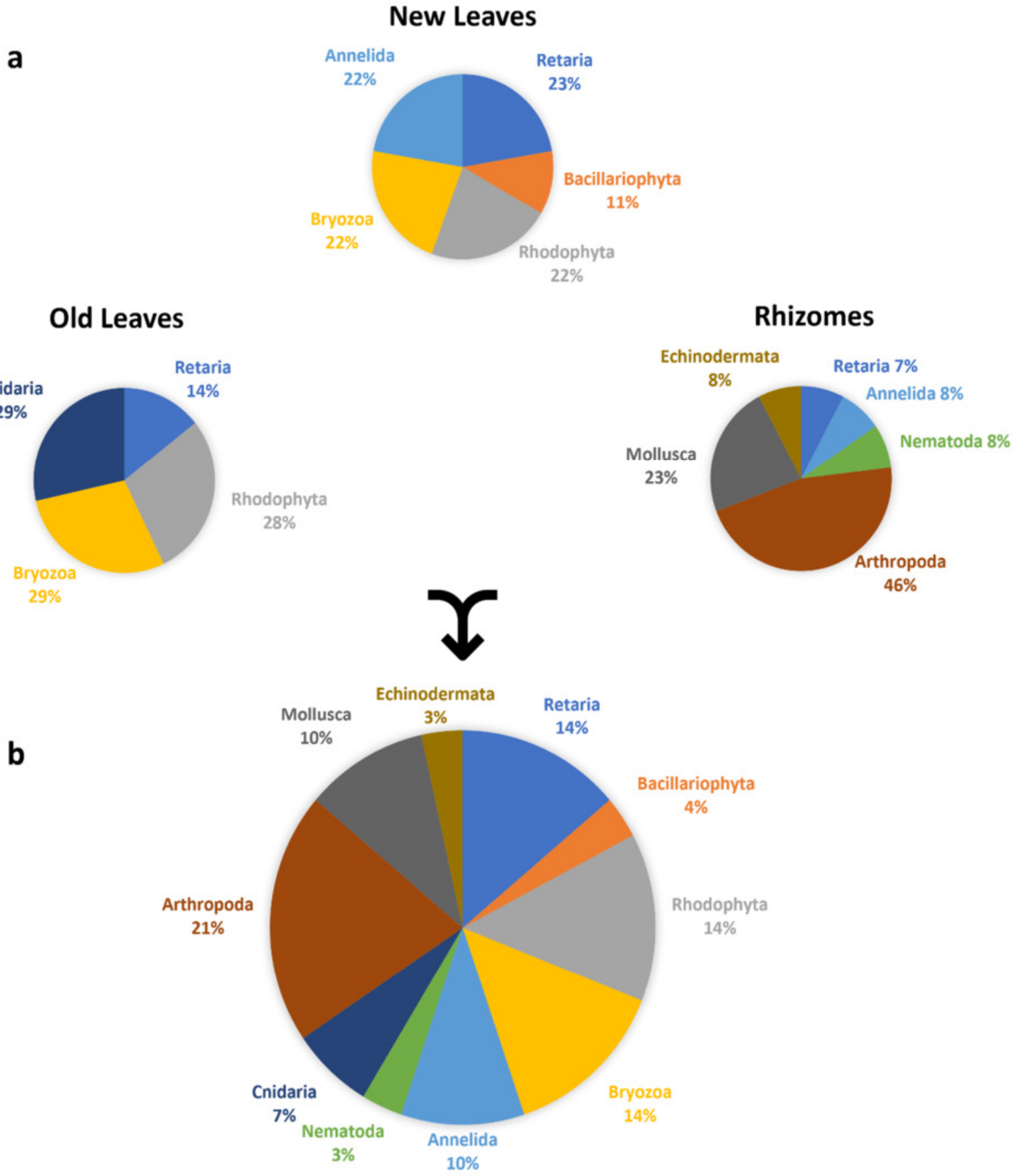

Figure 2. Relative distribution of phyla (a) in the different plant parts (Old leaves, Young Leaves and Rhizomes) and (b) in the entire *P. oceanica* shoot examined.

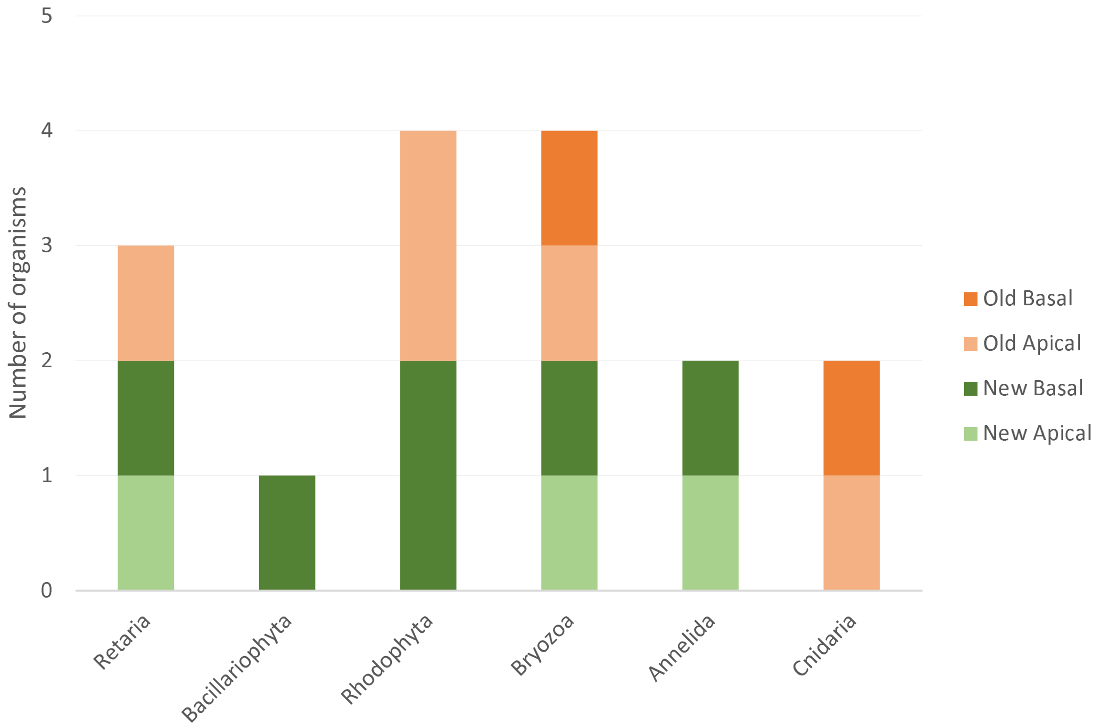

Figure 3. Number of organisms per phylum found in the leaves of different age (old and young) and different parts of the leaves (apical and basal) of *Posidonia oceanica*.

Discussion

Seagrasses leaves and rhizomes are characterized by high species diversity as they provide an ideal environment for the establishment of micro- and macrocolonizers that can be vagile or sessile (Mabrouk et al., 2014). This year, we identified organisms from 10 phyla, which is consistent with the results of previous protocols that examined seagrass in the same area (2014,2016: 10 phyla, 2021: 9 phyla). Although the number of phyla (around 10) and the main representatives (Arthropods, Bryozoa, Rhodophyta) remain relatively consistent over the years there is a slight variation in the relative contribution of these phyla. Also, it is interesting that some phyla are unique for each year. For example, in 2014 they identified individuals from Porifera and Nemertea and in 2016 from Platyhelminthes and Xenacoelomorpha, while we did not find any representatives of that phyla. However, this year we mention the presence of Bacillariophyta, represented by diatoms. Some reasons behind these minor differences between the results of the different excursions could be the different sampling approaches, the time spent on the investigation of the samples, the research focus and the nature of the organisms (larger, vagile organisms are easier to notice than smaller). Moreover, it should be noted that the composition of the epiphytic community is largely influenced by biotic factors such as plant's phenology, leaf age, and grazing pressure, as well as by abiotic factors such as light, temperature, depth, nutrients and hydrodynamics (Buia et al., 2004, Nesti et al., 2009) which may differ from time to time.

Difference in the vertical distribution of organisms in the plant is expected (Boero et al., 1985). There is an increase in species and abundance from the basal to the apical zone of the leaf, which is strongly affected by the light availability for the epiphytic flora and by the mesograzers on the epiphytic assemblages (Mazzella & Russo 1989, Gambi et al., 1992). Moreover, more epiphytic organisms are expected in older than in younger leaf blades as a result of accumulation over the years, mainly due to the conveyor belt growth. Additionally, the top surface of the leaf receives greater exposure to light, creating favourable conditions for the growth of photosynthetically active organisms (Casola et al., 1987). In our study, there is minimal variation between old and young leaves, as well as between the apical and the basal zone of the leaves. This is due to the fact that this year we did not sample directly in the meadow, but we collected parts of *P. oceanica* that were already detached and were floating in the water column or were laying on the seafloor. As a consequence, old and young leaves have similar phyla composition, and the expected patterns cannot not be observed. We noticed, however, a greater phyla abundance in the rhizome than the leaves. This is because *P. oceanica* rhizomes are a complex three-dimensional structure where both hard and soft substrates can be found. It acts as a shelter against predation, and it is less affected by the local hydrodynamic conditions. Therefore, diverse organism assemblages are sustained in the rhizomes (Albano & Sabelli, 2012).

Concerning future studies, since not any alive plant material can be removed from the water, alternative non-destructive methods for sampling organisms from the meadows could be applied, to the extent possible given the limited resources and time. Several methods have been tested already by Michel et al., 2010 near STARESO. Hand towed nets could provide a good alternative for collecting samples without collecting any plant material. This type of hand net has a narrow end that resembles the cod-end of a trawl net and therefore organisms can be captured more effectively in comparison to the aquaria hand nets we used. The other two alternatives proposed (air-lift for day sampling and light trap for night sampling) are more effective in sampling but require specific equipment and underwater effort. However, if this equipment is available, it would be worth trying. Another method with low impact that was also investigated near STARESO is cutting leaves above the ligule (Gobert et al. 2020). Of course, whatever the method used it should always be in consultation with the station's scientific manager and has to comply with local, regional or national regulations for protected species.

We acknowledge that limitations and challenges exist when examining seagrass fauna within a single day. Another recommendation would be that the examination of the seagrass during the excursion could be adjusted to exploring the overall seagrass meadow habitat. In this way students could gain insights into the structure of a meadow and its ecological role. This would provide a more comprehensive understanding of its ecological services, as well as the potential threats to this valuable ecosystem.

Supplementary material

Table S1. Summary of the organisms observed in *Posidonia oceanica* meadow while snorkelling.

	Class	**Order**	**Family**	**Species**
Chordata	Actinopterygii	Labriformes	Labridae	(juveniles)
		Labriformes	Labridae	*Symphodus tinca*
		Labriformes	Labridae	*Symphodus viridis*
		Labriformes	Labridae	*Symphodus roissali*
		Labriformes	Labridae	*Symphodus rostratus*
		Labriformes	Labridae	*Symphodus melanocercus*
		Labriformes	Labridae	*Serranus scriba*
		Eupercaria inc. sed.	Sparidae	*Sarpa salpa*
		Ovalentaria inc. sed.	Pomacentridae	*Chromis chromis*
		Anguilliformes	Muraenidae	*Muraena helena*
Mollusca	Cephalopoda	Sepiida	Sepiidae	*Sepia sp.* ☾
		Myopsida	Loliginidae	*Loligo sp.* ☾

☾ observed during night

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Plankton

Julie Hary & Laura Stephan

Introduction

The term of “plankton” was first introduced by Christian Andreas Victor Hensen in 1887. Following this definition, we mostly speak of small organisms with little or no movement on their own. Without their own locomotion, they are free-floating in the water (Hofrichter et al., 2020). In accordance with this, the "movement" takes only place through the water current (in contrast to nekton, which can swim against the current). In consequence, the function of their own mobility to maintain the body axis and to sink is only possible due to water current (Hays et al., 2005).

Due to the enormous diversity of plankton, it can be differentiated in various ways: Size, life cycle and systematics. In terms of size, plankton can be found from the micrometre range, whereby some representatives can reach up to several metres. Thus, viruses as well as medusae belong to plankton (***Tab. 1***).

Table 1 Classification of plankton by its size from Larink & Westheide, 2011

Term	Size range	Representatives
Ultraplankton	< 2 µm	Viruses, bacteria
Nanoplankton	2 – 20 µm	Flagellates
Microplankton	20 – 200 µm	Tinitinnids, ciliates, diatoms
Mesoplankton	0.2 – 20 mm	Copepods, larvae, hydromedusae
Makroplankton	2 -20 cm	Krill, arrow – worms
Mega- or Megaloplankton	0.2 – 2 m	Jelly fish, tunicates

Furthermore, plankton can also be classified according to life cycle. There is *holoplankton*, which spends its entire life as plankton, *meroplankton*, which only spends specific developmental stages as plankton (e.g. fish larvae) and *tychoplankton*, which occasionally becomes part of the plankton through accidental events (torn-off organisms).

Plankton in its integrity forms the basis of the marine food web, with a main distinction being made between phytoplankton and zooplankton. Phytoplankton consists mostly of plant plankton, which is mostly unicellular. Organisms belonging to phytoplankton are known as being primary producers. They are mainly found in the euphotic zone where they actively carry out photosynthesis (Hofrichter et al., 2020). It forms thereby the first category of the food web in the oceans. Because more than 70 % percent of the earth is covered by the ocean, plankton is found over a large area of the world and its amount of biomass is gigantic. Therefore, phytoplankton plays a major role in the entire climate of the earth, producing the largest amount of oxygen worldwide -even more than terrestrial plants. Thus, changes of phytoplankton can not only be problematic for marine life (as e.g. largest marine species such as the blue whale feed on plankton), but also for terrestrial ecosystems (Lumini and Nanni, 2019) .

The second element of the food web is the animal plankton (zooplankton), which also feeds on phytoplankton (Richardson, 2008). The occurrence of zooplankton is actually very seasonal: studies such as Gaudy and Champalbert (1998) show that for example in the Mediterranean sea, the highest diversity is found in spring. They were also able to show that the number of zooplankton depends strongly on the geographical latitude, i.e., highest peaks of plankton occurrence are found around spring in colder regions, while peaks in warmer regions remain more decent throughout the year. This can be inferred from the related reproduction cycles, resulting in optimal conditions in spring in temperate zones.

Our study consisted of observing the different components of the plankton. Since we used a net with a mesh width of 200 µm, as described in the Material & Methods section, we mainly dealt with meso-, macro- and megaplankton.

STARESO-Tour

We also had the chance to get a special insight into the work of STARESO being guided through the station by the marine biologist Aubin. He turned out to be a plankton specialist and showed us his studies. He was working with the ZooSCAN, which is a watertight scanner, where samples of water containing plankton can be placed. The scanner produces a digital image of organisms found in the sample. They then get identified by a machine learning algorithm and separately stored in a database. Therefore, the ZooSCAN is especially useful for large sample sets (Hydroptic, 2016). Aubin also showed us the stations plankton-bunker, where samples from decades are stored in small vials filled with formalin. STARESO has been doing long-term plankton surveys on a regular basis in the bay of Calvi for many years. At the end, Aubin played a video of his first night snorkelling right in front of the station. It turned out that snorkelling in darkness can be particularly interesting in springtime, where plankton-blooms can be expected in this area.

Material & Methods

Sampling was done on the 2nd of August 2023 in the bay of STARESO (*Station de recherches sous-marines et océanographiques*). A conical net with a metal ring at the entry side and a collecting container at the ending side was used (Fig. 1). The net had a mesh width of 200 µm and a length of about 4 to 5 meters. Leaving the bay by motorboat after about 100 meters from the coast, the net was put into the water and was then pulled very slowly with a speed of 2 to 3 km/h at a depth of several meters from the surface. Subsequently, it was pulled out after 12 minutes and the collected seawater containing the plankton was retained in a plastic container. The sample was then analyzed under binocular and transmitted-light microscopes.

Figure 1: Hensen net. Conical net with a metal ring at the entry side and a collecting container at the ending side. Julia (on the left), 172 cm and Laura (on the right), 166 cm as size reference.

Results

In our sample we found 32 different organisms. The most diverse phylum was the phylum of Arthopoda with 9 different organisms, followed by the phyla of Mollusca and Myzozoa with four determinations. Not all organisms were determined to species level as some stages of plankton are often not further identifiable. The most abundant organisms were Copepods. We did not find high abundances and diversity of primary producers in our sample.

Table 2. List of all species found while sampling plankton on 02.08.2023 near STARESO, Calvi, Corsica.

Phylum	Class	Order	Family	Species
Annelida	Polychaeta	Sipuncula		
Annelida	Polychaeta	Spionida	Spionidae	*Dipolydora sp.*
Arthropoda	Copepoda	Calanoida	Acartiidae	*Acartia clausi*
Arthropoda	Copepoda	Calanoida	Calanidae	*Calanus sp.*
Arthropoda	Copepoda	Calanoida	Centropagidae	*Centropages hamatus*
Arthropoda	Copepoda	Calanoida	Temoridae	*Temora sp.*

Arthropoda	Branchiopoda	Onychopoda	Podonidae	*Evadne nordmanni*
Arthropoda	Branchiopoda	Onychopoda	Podonidae	*Podon leuckartii*
Arthropoda	Malacostraca	Decapoda		
Arthropoda	Arachnida	Trombidiformes	Pontarachnidae	
Arthropoda	Oligohymenophorea	Sessilida	Zoothamniidae	*Zoothamnium sp.*
Bacillariophyta	Bacillariophyceae	Bacillariales	Bacillariaceae	*Nitzschia sp.*
Chaetognatha				
Ciliophora	Spirotrichea	Tintinnida		
Chordata	Teleostei			
Chordata	Appendicularia	Copelata	Oikopleuridae	*Oikopleura dioica*
Cnidaria	Hydrozoa			
Cnidaria	Scyphozoa	Semaeostomeae	Pelagiidae	*Pelagia noctiluca*
Myzozoa	Dinophyceae	Gonyaulacales	Ceratiaceae	*Ceratium horridum*
Myzozoa	Dinophyceae	Gonyaulacales	Ceratiaceae	*Ceratium massilense*
Myzozoa				*Ceratium sp.*
Myzozoa	Dinophyceae	Dinophysiales	Dinophysaceae	*Dinophysis sp.*
Echinodermata	Echinoidea	Echinoida		
Echinodermata	Holothuroidea			
Foraminifera	Globothalamea	Rotaliida	Globigerinidae	*Globigerinella siphonifera*
Haptophyta	Coccolithophyceae	Phaeocystales	Phaeocystaceae	*Phaeocystis globosa*
Mollusca	Bivalvia			
Mollusca	Gastropoda	Pteropoda	Creseidae	
Mollusca	Gastropoda	Pteropoda	Creseidae	*Creseis sp.*
Mollusca	Gastropoda	Pteropoda	Limacinidae	*Limacina sp.*
Radiozoa	Acantharia	Arthracanthida	Phyllostauridae	*Phyllostaurus siculus*
Radiozoa	Acantharia	Acantharia		*Amphilitium sp.*

Figure 2: Selection of plankton organisms found in the sample of the Calvi-excursion 2023. **A**: Auricularia larva (Holothuroidea). **B:** *Acartia clausi*. **C:** Fish larva (Teleostei). **D:** *Ceratium horridum*. **E:** *Phyllostaurus siculus*. **F:** *Phaeocystis globose.* **G:** Fish embryo (Teleostei). **H:** Veliger larva (Gastropoda). Pictures were taken using a transmitted-light microscope with 4x-zoom (B, C, G), 10x-zoom (A, D) and 25x-zoom (E, F, H)

Discussion

Compared to prior course protocols, plankton diversity was slightly higher in our sample: With 32 different organisms, we found more than students from 2018 (26) and 2021 (27). Small differences in sampling methods can cause big differences in the outcome of studies. This is especially true for plankton sampling. For example, the exact time and depth of sampling can have a strong impact on species composition, as planktonic organisms are known for their daily vertical migration. Plankton diversity and abundances fluctuate also over the year. Depending on the climate zone, there are blooms of phytoplankton alternating with blooms of zooplankton throughout the year (Hempel et al. 2017). This could explain differences in numbers of species in past protocols, as sampling wasn't done at the exact same date and time each year. Moreover, the quality of determination can be depending on time available, and number of people involved in identification. For our course, we were 16 students and had one day for determinations. This must be kept in mind when comparing past protocols as there were years where there was limited time and a different number of people for further investigations (see protocol 2018). We possibly found low abundance of primary producers in our sample due to the mesh size of our sampling net being too big for most phytoplankton. Since we used a net with a mesh width of 200 µm, we mainly dealt with meso-, macro- and mega-plankton. Compared to former protocols from 2018 and 2021, all protocols have high abundances of Copepods in common. Notably, we found two species of Chordata in our sample, whereas in 2018 there was only one, and in 2021 no specimen of Chordata was found. Moreover, we found two species of Echinodermata, with a Holothuroidea-larva being our highlight (Fig. 2A).

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Sandy beach

Pina Hentschel & Julia Ofer

Introduction

An estimated two-thirds of the world's ice-free coastline consists of sandy beaches (McLachlan and Brown, 2006). However, the distribution of rocky coast to sandy beach in the Mediterranean is 50% each (Furlani et al., 2014). This can also be assumed for the island of Corsica. Here, sandy beaches are found mainly on the east coast, while the north and west coasts are dominated by rocky shores.

Sandy beaches are characterized by a low slope of the subsoil. While the classification of specific zones is ambiguously described in literature, a general differentiation of nearshore littoral areas in three main layers is commonly accepted: the supralittoral, the medio- or eulittoral and the infra- or sublittoral (Figure 4). The littoral begins at the spray zone just above the high tide mark (supralittoral). From here it extends to the intertidal zone between the high and low water marks (medio- or eulittoral) and then out to the edge of the continental shelf (infra- or sublittoral) (Hofrichter, 2020).

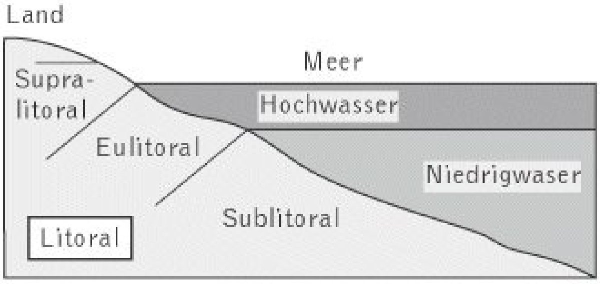

Figure 4: Zonation of the litoral adapted from Spektrum.de (2001).
Infra- or sublittoral zone, where data was collected, is highlighted in green.

In general, beaches are physically dynamic habitats that are formed by the interaction of sand, waves, and tides (McLachlan and Brown, 2006). Wave energy is absorbed on sandy beaches, resulting in substrate instability. Within the infra- or sublittoral zone, the zone we focused our observation on the sandy beach of Revellata, two distinct layers can be distinguished. From the border to the medio- or eulittoral down to a depth of 2 m, a high energy situation is found with an enormous substrate instability, high turbidity and large sand grain size, while deeper areas down to 10 m are less exposed. When the waves are calm there is little overturning and ripples form on the seabed with grain size decreasing at greater depths (Hofrichter, 2002). During storms, sand is transported from the shore to the open sea and returned to the coast during calm conditions. The instability of the sandy substrate causes a variety of adaptations in the species that inhibit it (McLachlan and Brown, 2006).

In the past, sandy beaches have often been regarded as “marine deserts”. Nowadays we know that the sandy beach ecosystem is populated by a variety of different species. In and on the sandy substrate is a spectrum of microscopic to macroscopic organisms such as clams, whelks, worms, sandhoppers, crabs, sea lice, sand dollars, and microorganisms such as protozoa, microscopic plants, and bacteria (McLachlan and Brown, 2006). Macrofauna refers to the animals larger than 1 mm while microfauna includes all animals passing a filter with a mesh size of 63 µm. All organisms with a size between 63 µm and 1 mm are considered as members of the meiofauna (Sommer, 2005). Depending on the preferred habitat of the organisms, a distinction is made between epifauna and infauna or endofauna (Figure 5) (Tardent, 2005). In addition, tidal beaches are influenced by a dynamic influx of species, with some species migrating onto the beach as the tide rises and others descending from the dunes as the tide recedes (McLachlan and Brown, 2006).

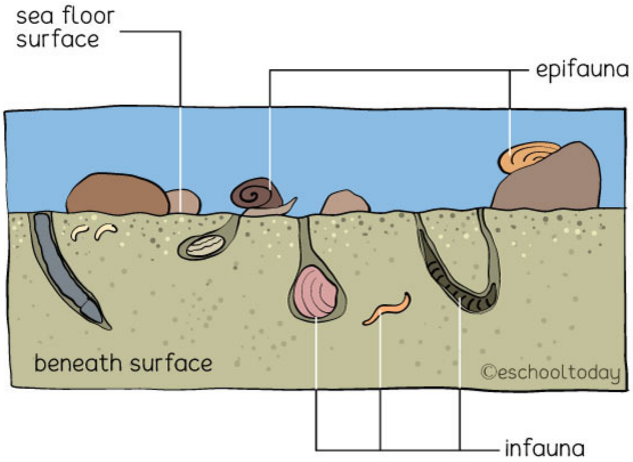

Figure 5: Differentiation of fauna on and in sandy beaches according to preferred environment from eschool today (2022).

All species inhabiting sandy beaches must be able to cope with rapidly changing environmental conditions. Animals in the water need to ensure that they are not swept out to sea or onto land. To do this, they need a high degree of mobility to maintain their position or regain it should they lose it (McLachlan and Brown, 2006). To protect themselves from incoming waves, animals must be able to burrow quickly into the soft substrate (McLachlan and Brown, 2006). Semiterrestrial animals cannot venture too far into the land, nor too far into the open water. Therefore, for their migrations, they evolved not only a high degree of mobility, but also a precise night-day and tidal rhythm (Naylor, 2010). Thus, there are no truly sessile animals on sandy beaches because of the unstable substrate (McLachlan and Brown, 2006).

Sandy beaches are highly vulnerable to human activity in many places. For example, highly touristy beaches are often cleared of macrophytes by raking and sieving the sand with heavy equipment (Dugan et al., 2003; Davenport and Davenport, 2006; Kinzelman et al., 2003; Fanini et al., 2005). This cleaning removes material, disturbs resident organisms, and roughens the sand, which means more surface area for erosion by wind (Defeo et al., 2008). Sea level rise and other effects of climate change are also expected to exacerbate other anthropogenic pressures (Defeo et al., 2008). Thus, appropriate conservation strategies must be found to preserve sandy beaches as unique and often highly productive ecosystems (McLachlan and Brown, 2006). However, for this to happen, the sandy beach system must be sufficiently researched and understood.

During a field trip we mainly studied the macrofauna of the Revellata sandy beach. The objective was to determine the species observed or collected in a sandy beach environment. The collected data will then be compared with the results of previous years to determine any trends or differences.

Materials and methods

All presented data was collected through snorkelling at the Revellata sandy beach near Calvi, Corsica (Figure 6). This took place at the 3th of August 2023. On this day, the weather conditions were good with bright sunshine and over 30°C, but with strong wind from the southeast. However, the swell was gentle and the visibility was good, even in shallow water. The whole data was collected in two snorkelling runs, each about 40 min with a 30 min break in between. For the data collection, two different methods were used:

1. **Observation**: Many animals were only identified by observing them in their habitat. This was the method of choice for most of the fishes and echinoderms.
2. **Collection**: Mussels and Gastropods were mainly identified by having a closer look on them outside the sea. For the collection either bare hands or hand-nets were used. All animals were taken from their habitat gently and put into a plastic box prefilled with fresh sea water. After identification, all animals were released.

All students had gathered some experience in species identification within the last days spent at the rocky shore. However, the different animal taxa were only added after literature check (Riedl 1983 and Ballesteros & Llobet 2020).

Unlike previous snorkelling trips to Revellata, all animals seen in this habitat have been considered and included in the species list.

Figure 6: Revellata beach near Calvi (Corsica, France).

Results

In total, 58 animal species belonging to 6 different phyla were identified at Revellata beach (Figure 7). Furthermore, 2 plant species, *Cymodocea nodosa* and *Posidonia oceanica*, were spotted.

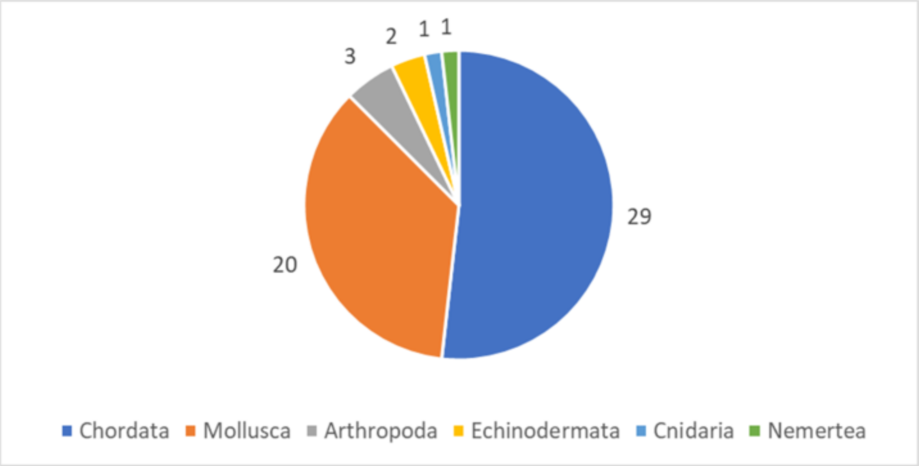

Figure 7: Dispersion of observed and collected animal phyla at Revellata in absolute numbers.

All species are listed in the table below.

Table 1. List of species found at Revellata.

phylum	class/ fish family	actual name	german name
Tracheophyta	Magnoliopsida	*Cymodocea nodosa*	Tanggras
Tracheophyta	Magnoliopsida	*Posidonia oceanica*	Neptunsgras
Cnidaria	Anthozoa	*Andresia partenopea*	
Nemertea	Holonemertea	*Amphiporidae sp.*	
Mollusca	Bivalivia	*Loripes lacteus*	
Mollusca	Bivalvia	*Ctena decussata*	
Mollusca	Bivalvia	*Arca noae*	Arche-Noah-Muschel
Mollusca	Bivalvia	*Paricardium scabrum*	
Mollusca	Bivalvia	*Venus verrucosa*	
Mollusca	Bivalvia	*Venerupis aurea*	
Mollusca	Bivalvia	*Angulus planatus*	
Mollusca	Bivalvia	*Mytilus galloprovincialis*	
Mollusca	Bivalvia	*Circomphalus casinus*	
Mollusca	Bivalvia	*Cardiomya costellata*	
Mollusca	Bivalvia	*Irus irus*	
Mollusca	Bivalvia	*Mactra corallina*	Bunte Trogmuschel
Mollusca	Bivalvia	*Spondylus gaederopus*	Stachelauster
Mollusca	Gastropoda	*Monodonta turbinata*	Turbanschnecke
Mollusca	Gastropoda	*Lunatia poliana*	
Mollusca	Gastropoda	*Gourmya vulgata*	
Mollusca	Gastropoda	*Patella caerulea*	Gewöhnliche oder Flache Napfschnecke
Mollusca	Gastropoda	*Haliotis tuberculata lamellosa*	Seeohr
Mollusca	Cephalopoda	*Octopus vulgaris*	
Mollusca	Cephalopoda	*Sepia elegans*	
Arthropoda	Malacostraca	*Pisa tetraodon*	
Arthropoda	Malacostraca	*Clibanarius erythropus*	
Arthropoda	Malacostraca	*Xantho poressa*	
Echinodermata	Echinoidea	*Paracentrotus lividus*	Steinseeigel
Echinodermata	Holothuroidea	*Parastichopus tremulus*	Rote Seegurke
Chordata	Muraenidae	*Murena helena*	Mittelmeer-Muräne
Chordata	Synodontidae	*Synodus saurus*	Eidechsenfisch
Chordata	Atherinidae	*Atherina boyeri*	Kleiner Ährenfisch
Chordata	Atherinidae	*Atherina hepsetus*	Ährenfisch
Chordata	Belonidae	*Belone belone*	Hornhecht
Chordata	Dactylopteridae	*Dactylopterus volitans*	Flughahn
Chordata	Moronidae	*Dicentrarchus labrax*	Europäischer Wolfsbarsch
Chordata	Serranidae	*Serranus scriba*	Schriftbarsch
Chordata	Sparidae	*Diplodus sargus*	Geißbrasse
Chordata	Sparidae	*Diplodus vulgaris*	Zweibindenbrasse

Chordata	Sparidae	*Oblada melanurus*	Brandbrasse
Chordata	Sparidae	*Sarpa salpa*	Goldstriemenbrasse
Chordata	Sparidae	*Sparus aurata*	Goldbrasse
Chordata	Sparidae	*Lithognathus mormyrus*	Marmorbrasse
Chordata	Sparidae	*Pagellus erythrinus*	Rotbrasse
Chordata	Sparidae	*Spondyliosoma cantharus*	Streifenbrasse
Chordata	Sparidae	*Spicara maena*	Laxierfisch
Chordata	Mugilidae	*Chelon auratus*	Goldmeeräsche
Chordata	Mullidae	*Mullus surmuletus*	Streifenbarbe
Chordata	Pomacentridae	*Chromis chromis*	Mönchsfisch
Chordata	Labridae	*Coris julis*	Meerjunker
Chordata	Labridae	*Labrus viridis*	Grüner-Lippfisch
Chordata	Labridae	*Labrus merula*	Amsellippfisch (Brauner Lippfisch)
Chordata	Labridae	*Symphodus cinereus*	Grauer-Lippfisch
Chordata	Labridae	*Symphodus roissali*	Fünffleck-Lippfisch
Chordata	Labridae	*Symphodus rostratus*	Schnauzen-Lippfisch
Chordata	Labridae	*Symphodus tincta*	Pfauen-Lippfisch
Chordata	Trachinidae	*Trachinus draco*	gewöhnliches Petermännchen
Chordata	Bothidae	*Bothus podas*	Weitaugen-Butt

Discussion

The **total number** of macrofauna species identified in 2023 was much higher compared to previous data from the Calvi courses. In 2016, a total of 32 species were reported, while in 2018 33 species were identified. In 2021, 37 species were recorded. This huge difference in the number of species can be explained by a change in the counting method. In contrast to previous years, not only new species not seen in the boulder field were counted, but all species. This means that the species list of 2023 includes all taxa that were either collected or observed at Revellata beach. In addition, the beach has been heavily used by tourists for several years. In connection with a beach bar, the beach was regularly cleaned of seagrass debris, raked and sieved. Following a ban on the beach bar, all buildings were deconstructed and the beach was no longer cleaned. This led to a recovery of the natural ecosystem and may be another explanation for the increased species numbers.

Regarding the **different phyla** of macrofauna found at Revellata, the list of 2023 lacks Porifera, Annelida and Platyhelminthes. Fewer filter feeders were found throughout the course than in previous years. This may be related to an increase in water quality and the associated reduction in nutrients. However, the dominance of fishes and molluscs is consistent with data from previous years.

Within the **fishes** most of the identified species in 2023 belong to the families Labridae and Sparidae. Also, in the last years, most of the fish could have been assigned to these families, the wrasses and the seabreams (Figure 8). However, in order to be sure that species are not present, or are present in smaller numbers, it would be necessary to have a uniform system for recognising or collecting species. This is difficult, if not impossible, to establish because of the new students on each course.

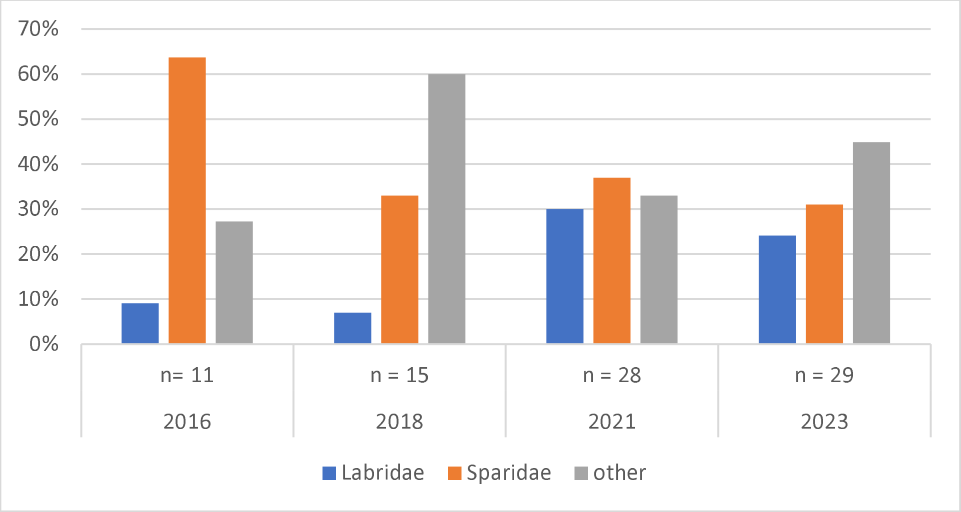

Figure 8: Comparison of fish family distribution at Revellata in different years.

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Macrozoobenthic communities in the Bay of Calvi

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Introduction

Coastal benthic communities are highly productive systems that provide numerous ecological functions. Those are for instance related to biogeochemical cycling and recycling of nutrients, biomass, and energy in the system, mediated by *benthic-pelagic coupling*. The latter refers to the processes linking benthic and pelagic environments (Marcus & Boero, 1998). Benthic systems consequently directly depend on the input of energy and nutrients from the overlaying water column (Zabala & Ballesteros, 1989). The functioning of marine benthic communities is furthermore closely linked to the ecological traits displayed by the contributing species (Bremner et al., 2003; Tilman et al., 1997). Hence, benthic systems are strongly influenced by processes that affect water properties and/or alter their diversity, including the global threats of pollution, eutrophication, habitat destruction, warming and bioinvasions (Bulleri et al., 2010; Casoli et al., 2021; Cappelletto et al., 2021).

Light availability is the abiotic key factor that determines the vertical zonation of benthic habitat formers like macrophytes or seagrass (Ballesteros, 2003). The waters in the Bay of Calvi (Corsica) are relatively unaffected by pollution and eutrophication, compared to the Adriatic Sea, the North Aegean Sea, and the South-East Mediterranean for instance (Katz et al., 2021; Cappelletto et al., 2021). Consequently, the waters are relatively clear and allow for macrophytes to form habitats at great depths. At around 60 meters depth however, light is so limited that large macrophytes like kelp no longer thrive. One would expect typical "Coralligène" assemblages instead, infra- or circalittoral communities that persist on secondary biogenic hard bottom substrates formed by encrusting, calcareous red algae (Rhodophyta, Corallinales; Ballesteros, 2003). These communities are however sensitive to physical destruction and other disturbances, such as bioinvasions and changes in water clarity or turbidity, as they grow and recover extremely slowly (Ballesteros, 2003).

Students of the field courses in previous years observed a decline in coralligenous red algae at a benthic dredging site near STARESO (Pittl & Wagner, 2018; Nowakowski & Vorleuter, 2021). This year, we used the same methodology to qualitatively assess the biodiversity of the same benthic habitat. We discuss and compare our findings in the context of the previous years' observations.

Material and Methods

The sampling took place on the 9th of August 2023, near the STARESO research station. To collect the benthic communities, the same dredge as in previous years was used (Figure 1).

Figure 9: Small dredge with two different mesh sizes

Dredge dimensions:

The heavy frame opening with jags, which is supposed to sink to the ground and scratch off the secondary hard substrate, is 20cm wide and 58,5cm long. Attached to this metal frame is a net with two different mesh sizes. For collecting zooplankton, the steert (cod end) mesh size is 0,5cm and for collecting Coralligène, other algae and bigger organisms, the outer door mesh size is 3cm.

Sampling:

After a 5-minute drive with the station's boat, the dredge was lowered into the water. At a depth of approximately 60 meters, the boat drove straight forward for about 20 meters at a constant, slow speed. When the dredge was pulled up from the sea, all the caught material was placed in a big plastic box filled with sea water and brought back to the station to sort out and identify the specimens.

Species determination:

Species were determined under stereo-microscopes using different literature (see references). Identified species were released back into the sea, while the invasive species *Caulerpa racemosa var. cylindracea* was discarded on land.

Results

In this year’s course, we were able to identify 20 different species (Table 1). In three cases only the genus of the specimen could be determined. Figure 2 shows the abundance of the different phyla within the sample. The phylum Arthropoda was the most abundant with 5 different species (25 %) followed by the phyla Echinodermata and Rhodophyta, both with 4 species (20%), the Chlorophyta with three species and Bryozoa, Ochrophyta, Mollusca and Chordata with only one species.

Table 2: Observed species. Species names are in accordance to Riedl (1983) If the current species names differ from Riedl (1983, the current species names are in brackets to facilitate comparisons with the protocols of previous field courses. Species marked with an asterisk (*) are not included in Riedl.

Phylum	Class	Species	Common name
Chlorophyta	Ulvophyceae	*Acetabularia acetabulum*	
Chlorophyta	Ulvophyceae	*Caulerpa racemosa var. cylindracea*	Trauben-Kriechsprossalge
Chlorophyta	Ulvophyceae	*Valonia sp.*	
Ochrophyta	Phaeophyceae	*Stilophora rhiziodes (Stilophora tenella)*	
Rhodophyta	Florideophyceae	*Amphiroa rubra (*)*	
Rhodophyta	Florideophyceae	*Osmundaria volubilis*	
Rhodophyta	Florideophyceae	*Phymatolithon calcareum (*)*	
Rhodophyta	Florideophyceae	*Rytiphlaea tinctoria*	
Mollusca	Gastropoda	*Calliostoma conulum (Calliostoma conulus)*	
Arthropoda	Malacostraca	*Inachus leptochirus*	
Arthropoda	Malacostraca	*Inachus dorsettensis*	
Arthropoda	Malacostraca	*Macropodia longirostris*	
Arthropoda	Malacostraca	*Pandalina brevirostris*	
Arthropoda	Malacostraca	*Pilumnus hirtellus*	bristly crab Europäische Borstenkrabbe
Bryozoa	Gymnolaemata	*Sertella sp. (Reteporella sp.)*	Neptunschleier
Echinodermata	Holothuroidea	*Holothuria sp.*	
Echinodermata	Echinoidea	*Echinus melo*	melon sea urchin Melonen-Seeigel
Echinodermata	Ophiuroidea	*Amphiura chiajei*	

Echinodermata	Ophiuroidea	*Ophiopsila aranea*	
Chordata	Actinopterygii	*Lepadogaster candollei (Lepadogaster candolii)*	Connemara-Schildbauch

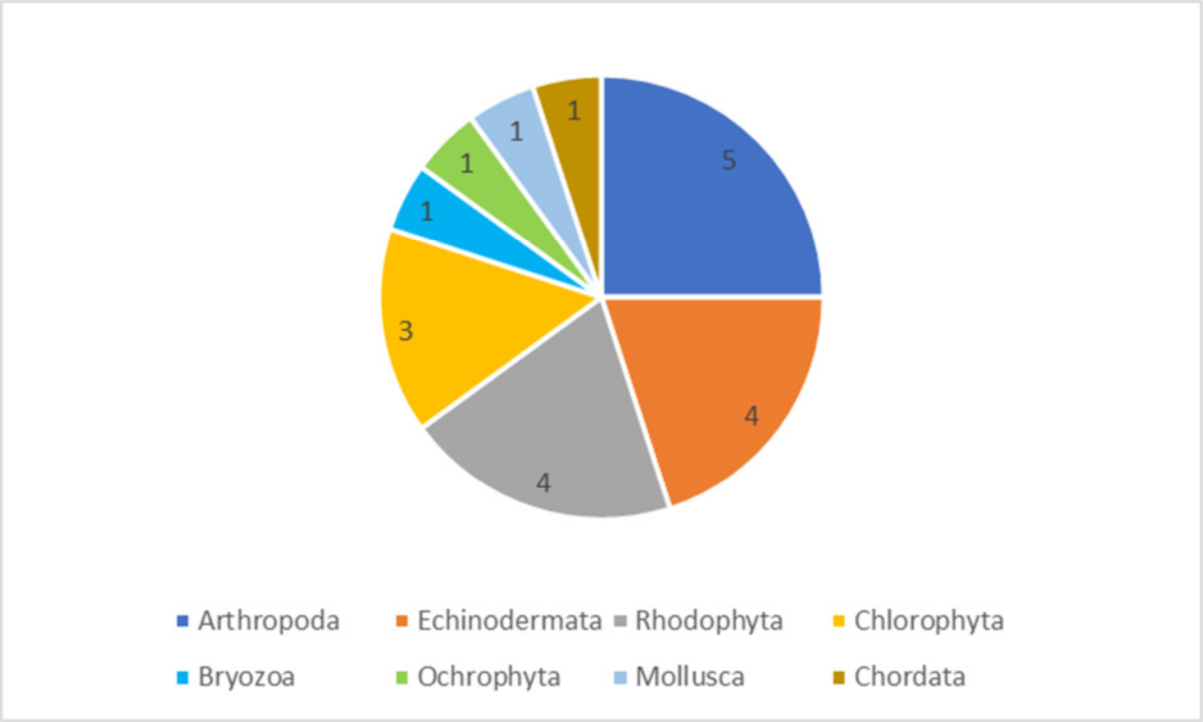

Figure 10: Distribution of phyla

Within the three different phyla of algae, the Rhodophyta were the most abundant with 4 different species (50%), followed by the chlorophyta with three different species and the Ochrophyta with one species (Figure 3).

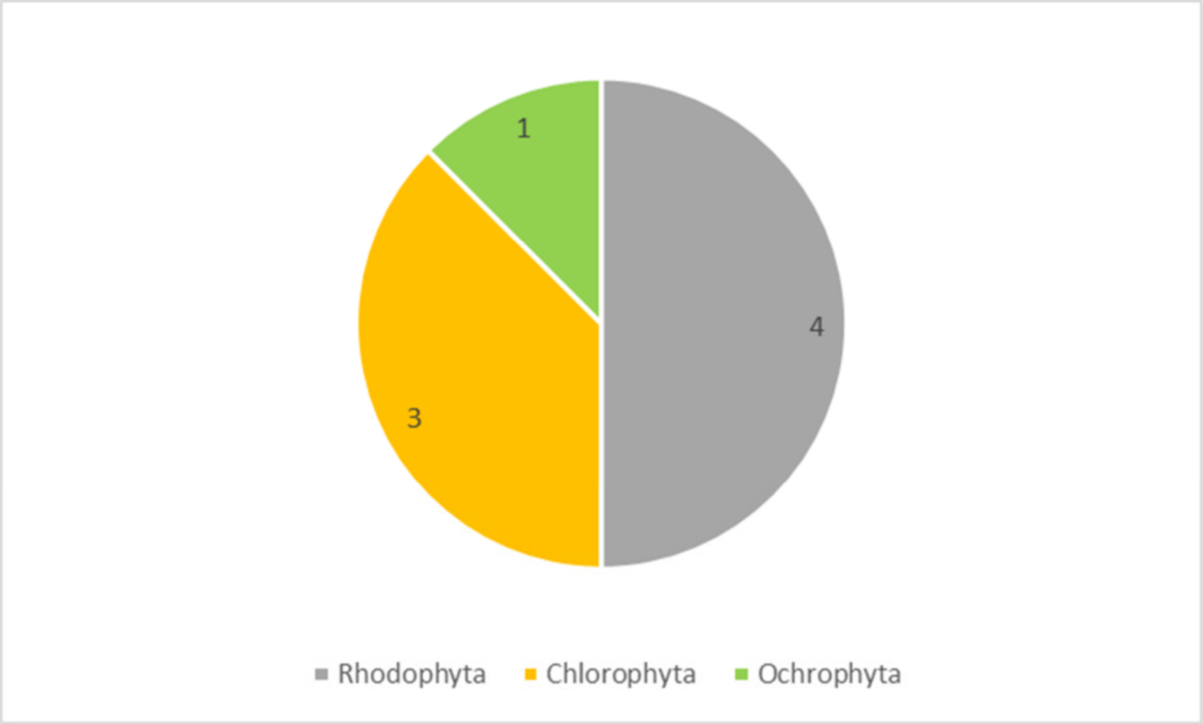

Figure 11: Distribution of algae

Figure 4 shows selected specimens from the Chlorophyta (including the invasive species *Caulerpa racemosa var. cylindracea*, 1B), and the Rhodophyta (including the coralligenous red alga *Phymatolithon calcareum*, 1C)

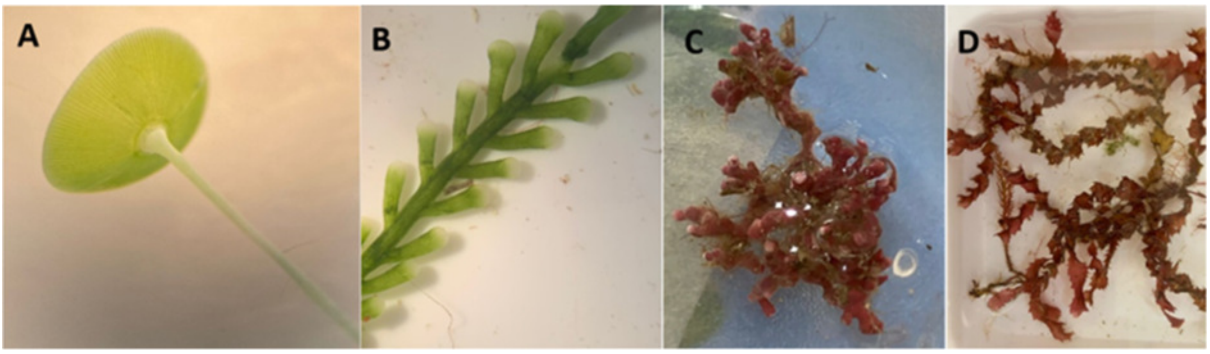

Figure 12: Selected algae; (A) *Acetabularia acetabulum*, (B) *Caulerpa racemosa var. cylindracea*, (C) *Phymatolithon calcareum*, (D) *Osmudaria volubilis*

Within the five phyla of animalia (Figure 5), the Arthropoda were the most abundant with 5 different species, followed by the Echinodermata with four different species. The Bryozoa, Mollusca and Chordata were each represented by one species.

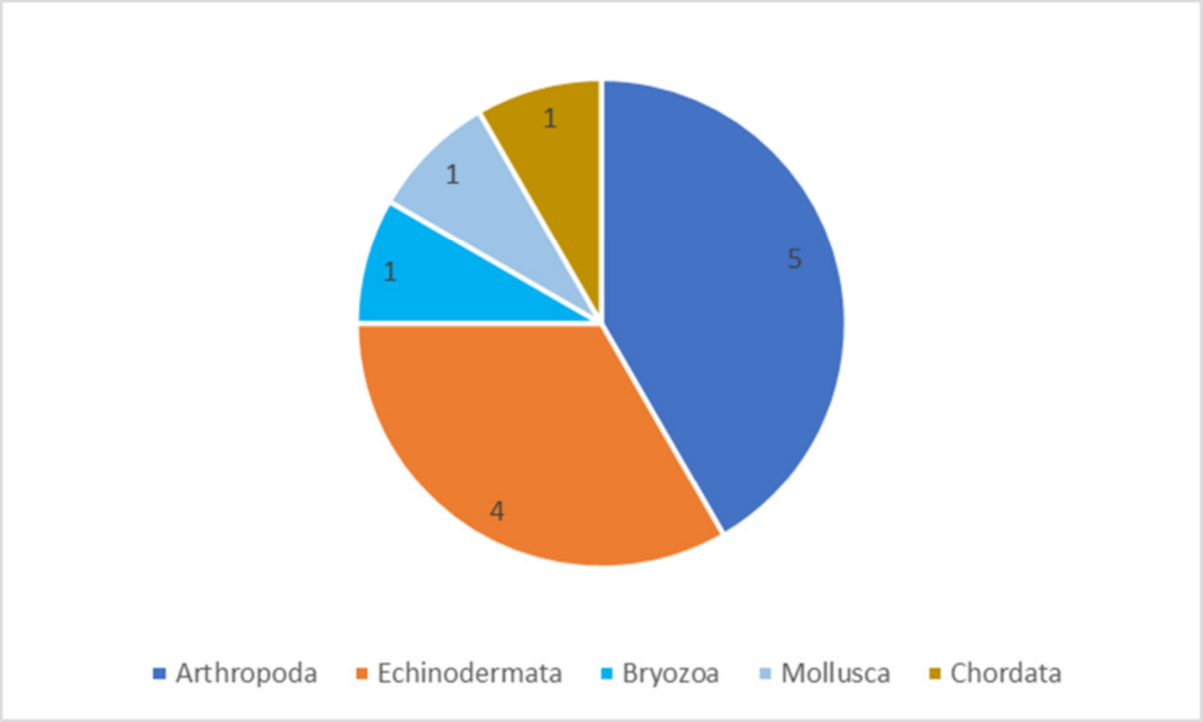

Figure 13: Distribution of animalia

Figure 6 shows selected species from Mollusca (2A), Arthropoda (2B and 2C), Echinodermata (2D) and Chordata (2E).

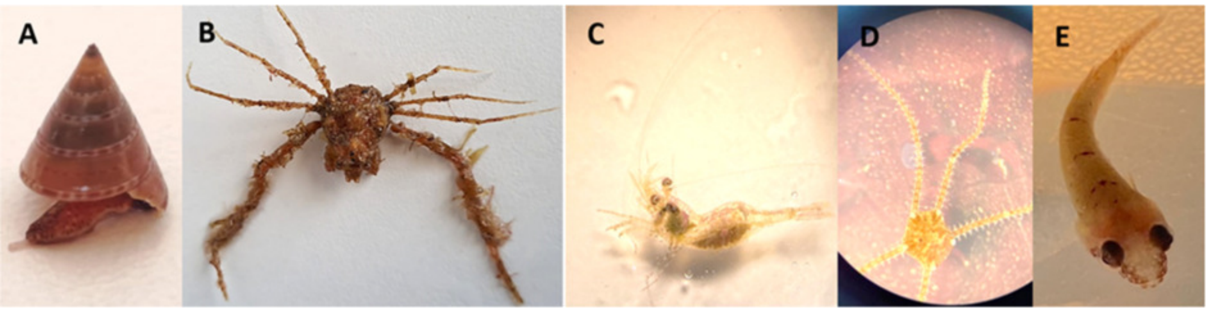

Figure 14: Selected animalia; (A) *Calliostoma conulum*, (B) *Inachus leptochirus*, (C) *Pandalina brevirostris*, (D) *Amphiura chiajei*, (E) *Lepadogaster candolii*

Discussion

We only found two coralligenous red algae in our dredging sample (*Phymatolithon calcareum* and *Amphiroa rubra*), and, apart from that, an assemblage of mainly red and green algae with mostly mobile epibionts (Arthropoda and Echinodermata dominate). Except from *Echinus melo*, no other bioeroders commonly present in the Coralligène were identified. Animal builders were also almost entirely lacking (typically Porifera, Polychaeta, Mollusca) with Bryozoa (*Reteporella sp.*) as sole representatives. Only 9 of the 20 identified species are known to be present in Coralligène assemblages (namely *Valonia sp., A. rubra, Osmudaria volubilis, P. calcareum, Pilumnus hirtellus, Holothuria sp., Echinus melo, Amphiura chiajei, Ophiopsila aranea*). We therefore conclude that our sample is not derived from a coralligenous but rather from a nonspecific benthic community with small red and green algae as habitat formers.

Comparison with previous years

The most prominent difference to the previous years (2018, 2021) is the much lower diversity in our sample. Around 60 species were identified in the scope of the last two field courses, while we only retrieved 20. In the second dredging in 2018 and in 2021, Mollusca (mainly Bivalvia and Gastropoda) and Arthropoda (Malacostraca) were the main contributors to biodiversity (¼ to ⅓ of diversity each). The first dredging in 2018 (40m depth) was dominated by Echinodermata (⅓) followed by molluscs and arthropods in third place. Arthropoda were the most diverse taxon in our sample as well (about ¼), but *Calliostoma conulum* (Gastropoda) was the only mollusc this year. Like in 2018 and 2021, Echinodermata were among the three most diverse taxa. Sponges, tunicates, nemerteans and fish larvae (only in 2018) were lacking entirely in our sample.

In both years, *P. calcareum* (also found in 2023) was the only coralligenous red alga in 2018 and 2021. Furthermore, Wagner and Pittl (2018) point out that the presence of asteroids and holothurians in their sample hints at a soft substrate. In conclusion, it appears that the last years' courses couldn't retrieve coralligenous assemblages either.

All three courses (2018, 2021, 2023) identified the invasive and highly competitive Australian chlorophyte *Caulerpa cylindracea* in their samples. In 2018, *C. cylindracea* made up 10% of the sampled biomass, while it was “highly abundant” in 2021.

With comparable diversities and taxonomic composition, the communities obtained by the dredging in 2018 and 2021 appear rather similar. Due to the much lower diversity and the lack of molluscs we observed, we conclude that our sample represents a benthic community clearly different to those observed in the courses before 2018.

What happened to the Coralligène?

In case our findings hint at a true decline in the Coralligène (which would also be in accordance with observations of STARESO employees), different factors could be involved.

A direct, anthropogenic habitat destruction appears unlikely, as no commercial fishing or dredging is allowed in the area and as the impact of recreational diving, if at all, is expected to be low in those depths.

Boudouresque et al. (1990) identify pollution and increased sedimentation as two of the main threats of the Coralligène. No data is at hand on changes in the water clarity or quality at our sampling site. However, it is presumably relatively unpolluted (Katz et al., 2021), so this appears unlikely.

The recording of the invasive *C. cylindracea* consequently remains as the most reasonable explanation for the lack of coralligenous algae in our sample. By reviewing multiple papers on the invasion of *C. cylindracea,* Klein and Verlaque (2008) conclude that total species numbers as well as the total macrophyte cover decrease in the presence of *C. cylindracea.* In case of a further spreading of the invasive chlorophyte in the last two years, this might explain the decreased total diversity in our dredging sample compared to the previous years. Klein and Verlaque (2009) also point out that colonization with *C. cylindracea* has a similar effect on macrophytes as increased sedimentation, which hints at detrimental effects of accumulating sediments originating from the *C. cylindracea* mat. This might account for the possible decrease of diversity and coralligenous red algae in the (small) macrophyte community we assessed. Bulleri et al. (2011, 2009) state that coralligenous algae even facilitate the attachment and establishment of *C. cylindracea* by providing more structural complexity in comparison to bare rock. However, Casoli et al. (2021) find that the Coralligène is least susceptible to *C. cylindracea* colonization compared to *Posidonia oceanica* meadows and *Phyllophora crispa* turfs. It therefore appears as if conflicting views exist on a possible facilitation of *C. cylindracea* settlement by coralligenous algae. Either way, a detrimental effect of *C. cylindracea* on Coralligène is evident in multiple studies, and the susceptibility of coralligenous assemblages to overgrowth by the invasive chlorophyte increases with previous habitat destruction.

Constraints and Outlook

The scope of the field course does not allow for final conclusions about dramatic, larger scale shifts in the benthic communities around STARESO as only one dredge was lowered blindly at *approximately* the same site as in the previous years. For detecting the extent of possibly remaining coralligenous assemblages, the general heterogeneity of the habitat, obvious physical destructions or overgrowth by invasive algae like *C. cylindracea,* Underwater Visual Census methods or a quantitative sampling approach with multiple dredgings and biomass assessments could be helpful. Due to the vulnerability of the Coralligène, less invasive methods should be favored though.

Further research could also focus on assessments of possible changes in the light regime at the seafloor or the nutrient composition of the seawater. Benthic photosynthesis in general relies on the properties of the overlaying water column but, as mentioned, coralligenous assemblages are particularly sensitive to pollution and higher sediment deposition (Boudouresque). To assess the potential of *C. cylindracea* as a habitat modifier, future monitoring projects could also focus on its impact on adjacent habitats such as seagrass meadows or habitats formed by larger macrophytes.

Conclusion

As in the last years, no real coralligenous assemblages could be retrieved with the dredge. In respect to biodiversity and taxonomic composition, our findings differ significantly from the years before 2018. More sophisticated assessments at a larger scale are required to confirm a genuine shift in the Bay of Calvi from the typical mediterranean Coralligène to a less diverse benthic community. However, our findings might hint at dramatic changes and a potentially detrimental effect of the invasive alga *C. cylindracea*. Due to the uniqueness and diversity of the Coralligène, future research should address those changes in much more detail.

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Fauna in the Gulf of Girolata and in the Fango river

Hanna Rudnick & Samantha Schmidt

Introduction

Girolata

The Gulf of Girolata is located on the north-western side of Corsica in the western Mediterranean Sea. Among others, Girolata, as well as the adjacent Gulf of Porto and the peninsula of Scandola, have formed the Corsican Regional Natural Park (Parc Naturel Régional de la Corse) since 1972 (Claudino-Sales, 2019). These areas have been declared a World Heritage site by the international union for conservation of nature (IUCN) in 1983 (WHC, 2013) (Fig. 1). The marine part of the protected area covers 4200 hectares (ha) (IUCN, 1983). The Gulf of Girolata provides an ecological buffer zone to the strictly protected Scandola Nature Reserve. Touristic and agricultural activities pose a threat to the natural heritage. By banning e.g., anchoring, sailing or fishing activities pressure should be taken off the marine flora and fauna and their restoration should be supported (PNRC, 2020). The marine coastal habitat in Girolata is defined by rocky shores and *Posidonia oceanica* meadows in the sublittoral zone (IUCN, 1983). Influenced by strong offshore currents, the north-western coastline of Corsica is visited by a wide range of pelagic fish typical for the western Mediterranean Sea including, various seabreams, e.g. the saddle bream *Oblada melanura* or the dentex bream *Dentex dentex.* Furthermore, the saupe *Sarpa salpa* or mullets like *Mullus surmuletus* were observed. In addition, Girolata offers a great diversity of marine invertebrates. For instance, the Mediterranean red coral, *Corallium rubrum* (Francour, 1991). Here, the Plage de Tuara, with approximately 300 meters (m) of coastline (Martin, 2011), served as the study site.

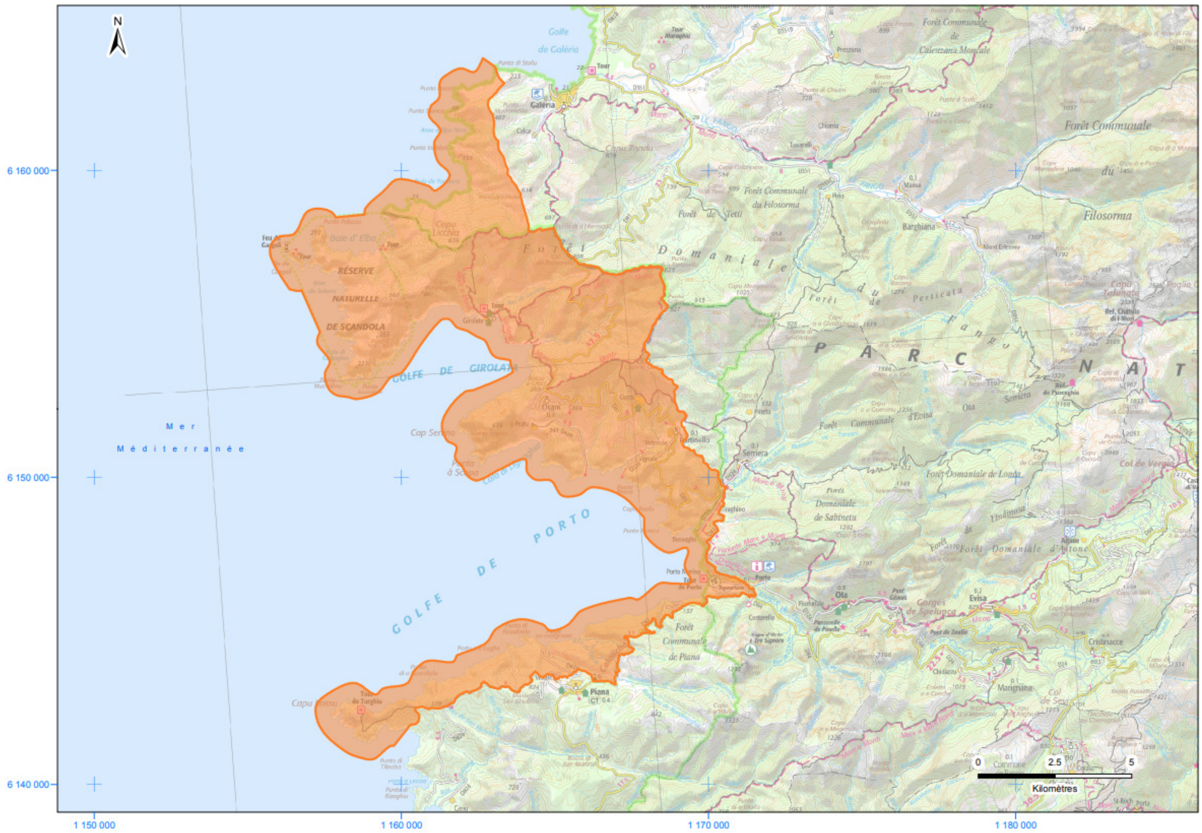

Figure 1: Delimitation of the World Heritage site, marked in orange, in the north-west of Corsica including the peninsula of Scandola, the Gulf of Girolata and the Gulf of Porto (from north to south). Cut from 'Atlas des biens francais inscrits sur la liste du patrimoine mondial' (Martin, 2011).

Fango

The Fango river is located in Galeria in the north-west of Corsica, France (Lengkeek & Didderen, 2006) (Fig. 2). The whole river valley belongs to the UNESCO Biosphere reserve, where it starts from an altitude of 2556 m and flows until the Delta in Galeria (*Fango Valley - Outstanding Biodiversity*, n.d.; Harris et al., 2019). The valley shows a high faunal abundance, being a habitat for example for 40 bird species. The area is characterized by wetland and marshland as well as dry, more typical Mediterranean vegetations, whereas the river bottom is covered by stones and boulders (*Fango Valley - Outstanding Biodiversity*, n.d.; Lengkeek & Didderen, 2006). Fango is considered to be a high energy river, receiving water from rain and in springtime from snowmelt and from flash flooding from storms (Lengkeek & Didderen, 2006; Neat et al., 2003). The mean width is about 43 m, the catchment area lies at 235 km^2, and the discharge area at 900 m^3/s (Gob et al., 2010).

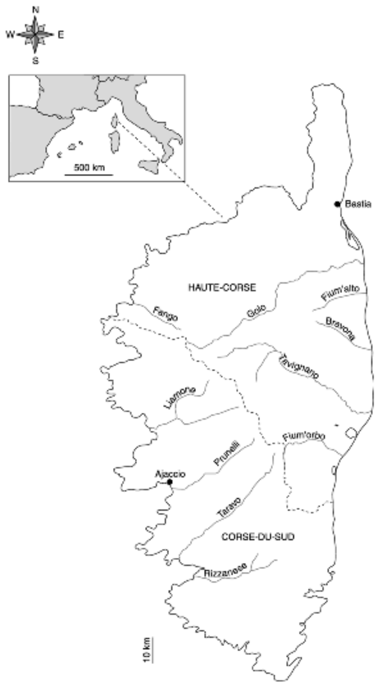

Figure 2: Overview of the rivers of Corsica. The Fango river is located in the north-west of the island. https://www.researchgate.net/profile/Bernard-Marchand-2/publication/26330888/figure/fig1/AS:601743930949639@1520478341859/Map-of-the-hydrographic-drainage-sampled-for-fish-in-Corsica-France.png

Materials and methods

Girolata

On the 4th of August of 2023 the Gulf of Girolata was reached by land via a hiking trail to the Plage de Tuara. The weather was windy and partly cloudy, which led to a choppy sea and therefore poor visibility in the water. In nearshore waters, the marine fauna was studied by an approximately one hour snorkelling-session. The investigation includes the identification and written documentation of all animal species sighted in the water. If direct determination of invertebrates was not possible, individuals were carefully collected by hand or with nets for a closer inspection on land. The determination key ‘Fauna und Flora des Mittelmeeres’, 3rd edtion, by Rupert Riedl (1983), was used. The animals were determined to species level as far as possible. Photographic documentation took place in the water, if possible, and otherwise on land. All live animals were appropriately returned to the sea.

Fango

The Fango river was reached via bus in the afternoon of the 4th of August in 2023. The organisms were directly investigated while going through the different bays of the Fango river and determined to species level as far as possible. No organisms were collected.

Results

Girolata

In total 25 species belonging to 5 different phyla, Chordata, Crustacea, Echinodermata, Mollusca, and Porifera were identified (Tab. 1). Twelve of these 24 species are Chordata. Four kinds of Labridae were observed including the Mediterranean rainbow wrasse, Coris julis, the Five-spotted wrasse, Symphodus roissali, the Pointed-snout wrasse, Symphodus rostratus and the Peacock wrasse, Symphodus tinca. The Red mullet, Mullus surmuletus, the Boxlip mullet, Oedalechilus labeo, the Mediterranean moray, Murena helena and the Pointed Comber, Serranus scriba, where each the only representatives of their taxonomic families. The Sparidae were represented by the Annular seabream, Dioplodus annularis, the Saddled seabream, Oblada melanura, the Salema porgy, Sarpa salpa and the Gilt-head seabream, Sparus aurata. Within the phylum of the Crustaceans only the Mediterranean intertidal hermit crab, Clibanarius erythropus, was seen. One starfish was found, which was identified as the Purple seastar, Echinaster sepositus. Five different Bivalvia were observed. The Noah's ark shell, Arca noae, the Warty venus, Venus verrucosa, Ctena decussata, the Milky white ark, Striarca lacteal, and the Thorny oyster, Spondylus gaederopus. The remains of one Common cuttlefish, Sepia officinalis, were found. Three different Gastropoda were found, which included Patella aspera and Astraea rugosa. The Muricidae found could only be determined to family level. Two sponges were seen in the Gulf of Girolata, which were indentified as the Chicken-liver sponge, Chondrilla nucula and the Black leather sponge, Sarcotragus spinosulus.

Table 1: List of the phylum, class, family, and species of all observed species in the Gulf of Girolata at the Plage de Tuara on the 4th of August of 2023. Empty cells could not be further determined.

Phylum	**Class**	**Family**	**Species**
Chordata	Actinoperygii	Labridae	*Coris julis*
Chordata	Actinoperygii	Labridae	*Symphodus roissali*
Chordata	Actinoperygii	Labridae	*Symphodus rostratus*
Chordata	Actinoperygii	Labridae	*Symphodus tinca*
Chordata	Actinoperygii	Mullidae	*Mullus surmuletus*
Chordata	Actinoperygii	Mugilidae	*Oedalechilus labeo*
Chordata	Actinoperygii	Muraenidae	*Muraena helena*
Chordata	Actinoperygii	Serranidae	*Serranus scriba*
Chordata	Actinoperygii	Sparidae	*Dioplodus annularis*
Chordata	Actinoperygii	Sparidae	*Oblada melanura*
Chordata	Actinoperygii	Sparidae	*Sarpa salpa*

Chordata	Actinoperygii	Sparidae	*Sparus aurata*
Crustacea	Malacostraca	Diogenidae	*Clibanarius erythropus*
Echinodermata	Asteroidea	Echinasteridae	*Echinaster sepositus*
Mollusca	Bivalvia	Arcidae	*Arca noae*
Mollusca	Bivalvia	Veneridae	*Venus verrucosa*
Mollusca	Bivalvia	Lucinidae	*Ctena decussata*
Mollusca	Bivalvia	Noetidae	*Striarca lactea*
Mollusca	Bivalvia	Spondylidae	*Spondylus gaederopus*
Mollusca	Cephalopoda	Sepiidae	*Sepia officinalis*
Mollusca	Gastropoda	Muricidae	
Mollusca	Gastropoda	Patellidae	*Patella aspera*
Mollusca	Gastropoda	Turbinidae	*Astraea rugosa*
Porifera	Demospongiae	Chondrillidae	*Chondrilla nucula*
Porifera	Demospongiae	Irciniidae	*Sarcotragus spinosulus*

Fango

5 species were found in total in the Fango river including 3 different phyla: Arthropoda, Chordata, and Plathelminthes. Only two organisms could be determined to species level (see Tab. 2). All organisms belonging to Arthropoda could either only be determined up to the order (Mayfly larvae, Ephemeroptera; and Caddis fly larvae, Trichoptera) or the family (Water bug, Gerridae).

Table 2: List of the phylum, class, family, and species of all observed species in the Fango river on the 4th of August of 2023. Empty cells could not be further determined.

Phylum	**Class**	**Order**	**Family**	**Species**
Arthropoda	Insecta	Ephemeroptera		
Arthropoda	Insecta	Hemiptera	Gerridae	
Arthropoda	Insecta	Trichoptera		
Chordata	Actinopterygii	Blenniiformes	Blenniidae	*Lipophrys fluviatilis*
Plathelminthes	Turbellaria	Tricladida	Dugesiidae	*Girardia tigrina*

Discussion

Girolata

In 2023 less species were observed in the Gulf of Girolata at the Plage de Tuara then in previous years. The main reason for this might be the poor visibility in the water due to strong winds and the resulting turbulences. Therefore, the decrease in diversity cannot be assessed negatively. A higher biodiversity was expected due to protective measures in the World Heritage site and the so far observed species-rich marine fauna (Claudino-Sales, 2019). Furthermore, there is the possibility of a spill-over effect from the strictly protected adjacent Scandola Nature Reserve. This would positively affect the biodiversity in the Gulf of Girolata. None of the observed species were unexpected, as they were seen during previous excursions. Especially *M. surmuletus, O. melanura* and *S. salpa* are listed as common fish in this area of the north-western Corsican coastline (IUCN, 2017). However, other exemplary fish, like the Dusky grouper, *Epinephelus marginatus and D. dentex* (IUCN, 2017) could not be found on our excursion day. For instance, *D. dentex* was seen in August of 2023 in nearshore waters of the peninsula La Revellata. Overall all identified invertebrates are common in the Gulf of Girolata and the western Mediterranean Sea (Francour, 1991).

Fango

The most abundant species we found was the freshwater blenny *Lipophrys fluviatilis. Girardia tigrina* was in a low abundance and mostly found under rocks. Compared to the protocol from 2021 (Peer, 2021) the European eel *Anguilla anguilla* was not seen this year which however was indicated in 2021 as special sighting and therefore was not to be expected in 2023.

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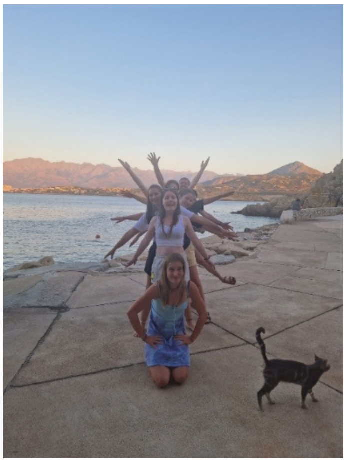

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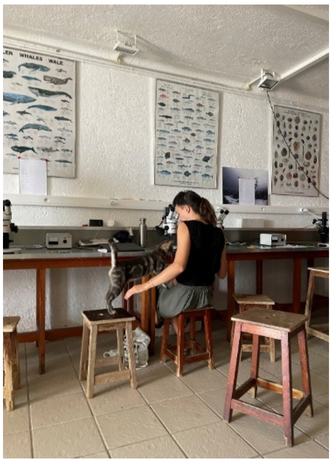

Impacts of natural and anthropogenic stressors on sea urchin development

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Laura Stephan, Moritz Stegner, Sandra Schallhart

Sandra Bracun, MSc

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Introduction

The Mediterranean Sea is exposed to extreme climate change events, leading to multiple environmental pressures due to sea water warming, increased heat waves, changes in salinity and pollution. By the year 2100, the water temperature of the Mediterranean Sea is expected to rise between 1.8 and 3.5°C (Lange, 2020). Additionally, extreme events like heat waves and anthropogenic pollution are predicted to increase in frequency, duration and intensity. Impacts like these have already caused large changes in the composition of species communities, species range shifts and an increase in mortality over the last decades. Therefore, it is important to study the reaction of organisms to such environmental stressors to gain a better understanding on how populations, communities and ecosystems will be impacted by continuing climate change (Santa, 2019).

Sea urchins are often used as model organisms in ecological studies due to several reasons. One important factor is the sensitivity of fertilization and development to environmental changes because of their interactive processes between eggs and sperm, blastomeres among themselves and all of these with the surrounding environment, where pollutants could disrupt the processes (Gambardella et al., 2021).

Arbacia lixula, also called the black sea urchin, is characterized by its deep black colour and the spherical shape. This species is distributed around the coastline of the Mediterranean Sea and a high density can be found and easily collected at Calvi Bay (GBIF, 2022). In several projects of the marine biology excursion over the last years, the embryonic development of *Arbacia lixula* was studied and a timeline of the exact developmental stages was established. Therefore, we decided to use the black sea urchin as our model organism and study the development of sea urchin embryos under different environmental stressors.

The idea of this project is to observe the development of sea urchin embryos under different environmental pressures through climate change. We decided to include changes in temperature, salinity and anthropogenic pollution through fertilizer, sunscreen and cigarettes.

Temperature

With ongoing climate change and global warming an increase in water temperature is one of the many consequences. Warm water temperatures can alter the reproduction of marine life. Different studies have shown that increased temperatures affect reproduction time and reproduction success i.e. in a soft coral (Liberman et al., 2021). Similar effects can be seen on the invasive sea star *Patiriella regularis*, where an increase of 4°C in water temperature can rise the mortality in the gastrulation stage up to 25% (Byrne et al., 2013). Different sea urchin species react in different ways. Tropical sea urchins such as *Echinometra lucunter* are better adapted to higher water temperature than species distributed in colder environment.

However, even in tropical species fertility reacts sensitive to temperature increase: at 26°C fertilization success is above 90%, whereas an increase of 2°C already leads to less than 90% of fertilization, and 30°C and above lead to only 80% fertilization success (Pereira et al., 2020). The increasing abundance of *Arbacia lixula* is said to be linked to the rising temperature in the Mediterranean, however studies show that temperature, only to a certain point, positively affects fertilization and larval survival rate. Fertilization success in temperatures at 27°C is lower than 1% and leads to mortality of larvae within 2 days (Gianguzza et al., 2014).

Salinity

An important and dynamic parameter of the Mediterranean Sea is the salinity of the water. The climate crisis is changing the global cycle of evaporation, precipitation and water circulation. The Mediterranean Sea will be more saline in the coming years. On the one hand, evaporation rates of the rivers flowing into the Mediterranean Sea will increase due to climate warming and thus less freshwater will reach the Mediterranean Sea. On the other hand, more water will also evaporate within the Mediterranean due to increased temperatures (Hofrichter, 2020). Existing studies focus mostly on the effects of lower salinities (= hypo-salinity) on echinoderm species, the impact of higher salinities (= hyper-salinity) remains understudied. So, we wanted to simulate this future change and therefore set the salinity to higher levels within a series of experiments to observe possible effects on the embryonic development of *Arbacia lixula* and furthermore combined treatments of hyper-salinity together with warmer temperatures in following runs.

Fertiliser

Fertiliser, a substance often associated with the prosperity and development of terrestrial plants, has a more complex relationship with marine ecosystems than one might initially think. The widespread use of fertilisers in agriculture and horticulture has unintentionally introduced these compounds into our waterways, ultimately reaching the sea. This run-off can contain various nutrients, such as nitrates and phosphates, which are vital elements for terrestrial plant growth. However, when these nutrients enter the marine environment, they can have unintended consequences. The excess nutrients can stimulate the overgrowth of algae, causing algal blooms that disrupt the balance of marine ecosystems. As these algae grow, they can block sunlight from reaching deeper waters, reducing the photosynthetic capacity of submerged plants. In addition, the decomposition of excess algae consumes oxygen, leading to hypoxic conditions that are detrimental to marine life (Sharp, 2001). Given the significant impact of excess nutrients on the aquatic environment, our research contributes to the understanding of the precise effects of fertiliser on sea urchin developmental stages and provides insight into a largely understudied aspect of this highly used marine stressor.

Cigarettes

Most people rarely consider the connection between marine life and cigarettes. Smoking couldn´t seem further from the oceanic world occupied by fish and marine mammals. However, the connected interplay of the Earth's water systems means that cigarette butts frequently end up in the ocean, even if they are not discarded directly from a boat or on the beach. Once these butts enter the water, the chemicals they release into the environment have an impact on the resident creatures. Since the first beach clean-ups started, cigarette butts are the most commonly found litter. For instance, in the year 2017 around 2.412.151 cigarette butts were collected within a worldwide beach clean-up. Some cigarette brands attract people by saying that the cigarette filters are biodegradable, but instead they are made from cellulose acetate, which degrades under UV light but still persists in the environment in smaller pieces.

Several studies have already analysed the toxicity of cigarette butts as it is dangerous for fish, water fleas and also marine bacteria and already one butt per litre has a highly dangerous impact on these organisms (Tobacco Free Life, 2023). Therefore, within our project, we want to evaluate the effect of cigarette butts on the development of sea urchin embryos.

Sunscreen

Ultraviolet (UV) radiation causes irreversible damage to the human body. Therefore, increased awareness of skin protection is essential to reduce the risk of skin cancer. However, wearing hats, shirts and other UV-protective clothing does not sound very appealing when spending a day at the beach and highly effective sunscreens are a welcome alternative. However, the chemical ingredients in sunscreens often disturb marine organisms. Studies of stressors on coral reefs have shown that chemicals such as oxybenzone and octinoxate, which are regarded as highly effective UV-filters, leach into the water when people swim with sunscreens. This leads to the expel of the symbiotic algae (zooxanthellae) living in the tissue of corals finally causing the feared coral bleaching, which is a clear indication that the coral is under stress and is in danger of dying. However, in 2018 oxybenzone (BP-3; benzophenone-3; 2-hydroxy-4-methoxyphenyl-phenylmethanone; 2-hydroxy-4-methoxybenzophenone; CAS No. 131-57-7) and octinoxate (OMC; ethylhexyl-4-methoxycinnamate; trans-octyl methoxycinnamate; ethylhexylmethoxycinnamate; CAS No. 5466-77-3) were the main ingredients in an estimated 70% - 80% of sunscreens and were widely used in personal-care products (Raffa et. al, 2018). To protect marine organisms some destinations like Hawaii and Palau ban sunscreens with such harmful ingredients (Zachos & Rosen, 2023). In response to the growing criticism on sunscreens, some companies started to use labels to guarantee that their products do not damage marine life. But ecosystems are fragile and the impacts of cosmetics on different marine organisms are not well understood. This is why we want to find out to what extent sunscreens affect the early development of sea urchins.

Material & Methods

Spawning

Several sea urchins, *Arbacia lixula*, were carefully detached from substrate in the bay of STARESO using a knife. Until induced spawning, they were kept in separate plastic bags filled with seawater. This step is important as stressed sea urchins spawn easily, which can induce a chain reaction of spawning in all sea urchins within the compartment.

In the lab, the sea urchins were then placed on a beaker with their mouths facing up. The beakers were filled to the brim with water so that the madreporic plate was always in the water (essential for sea urchin survival). To stimulate spawning, the sea urchins were placed in a stress situation. This was induced by injecting 1 mL of potassium chloride solution (0.5 M) between mouth and hard shell. This procedure is not lethal, and the sea urchins can recover completely from it. After a few seconds, spawning could be seen.

On the one hand, the sperm appeared white and mixed with the water while on the other hand the eggs appeared reddish-purple and settled on the bottom. After the sea urchins had finished spawning, they were immediately released back into water. Since the eggs had settled at the bottom, the supernatant could simply be decanted. After the sperm was again carefully mixed with the water, the sperm density and sperm mobility were observed under the microscope. Sufficient mobility was the key to good and successful fertilisation. Gonads from a single male were also stored in the refrigerator (removal by killing) to have a backup with good sperm. In the refrigerator the sperm can survive a few days without problems. A total of 5 females and 3 males were taken to spawn.

Embryo fertilisation

For fertilisation, a large Petri dish was filled with some seawater. The eggs and plenty of sperm were added. After about 3 minutes, the fertilisation efficiency was evaluated under the microscope. The eggs and sperm incubated then for another 7 minutes (10 minutes in total).

Experimental setup

For each experiment, 2 replicates were prepared, using two different females but the sperm of a single male.

Since we wanted to look at different anthropogenic and natural impacts on the embryonic development of *Arbacia lixula*, different so-called "treatments" were needed. These included different media and/or different environmental conditions. For each experiment, 50 mL of medium and 1 mL of "fertilisation solution" were equally placed in a Petri dish.

The medium was changed after 4 and 14 hours of incubation. For this purpose, the Petri dishes were slightly rotated so that the embryos accumulated in the centre. In this way 2/3 of the medium could be removed from the border of the Petri dish and changed without removing too many embryos.

Control

Fresh seawater was used as a medium for the control. The Petri dishes were kept at room temperature (24-25°C), as the water in the bay of the STARESO station had approximately the same water temperature.

Temperature

In total four different temperature treatments were done in two runs. In the first run, embryos were put in a 40°C incubator. In order to reduce the temperature to 37°C, the door was left open, and temperature was checked regularly to assure no major fluctuation in temperature. Overall, with that method temperature of 37°C with fluctuations of +/-1°C could be secured. The embryos were than incubated at 37°C for all time points. In the second run three different treatments were tested. Embryos were either kept at 37°C in the first two hours and then incubated at room temperature or constantly kept at 29°C and 32°C in two aquariums with immersion heaters to regulate constant temperature. Petri dishes were sealed with electrical tape to prevent water leak. Additionally, to reduce risk of bacterial growth with increased temperature, 50ml tubes with seawater were cooked in the microwave for one minute and then each incubated in needed temperature.

Salinity

Within the seasonal cycle, there are natural changes in salinity of 3.75 - 3.9 % in the Mediterranean Sea. It can therefore be assumed that the flora and fauna of the Mediterranean have adapted to smaller fluctuations in salinity. The maximum salinity of the eastern Mediterranean is 3.91%, that of the western Mediterranean is 3.87% and in lagoons salinities of up to 6.5% can be reached. Values of 4.0-7.0% salinity are already referred to as hyperhaline areas. Around Corsica, the average salinity of the sea is 3.85% (Hofrichter, 2020). To check the actual salinity of the bay at STARESO and producing the treatments, we used the SonTek CastAway-CTD salinity meter (https://www.ysi.com/castaway-ctd), which was provided to us by the station. Our first measurements of the salinity of STARESO-bay showed a value of 3.91%.

We conducted three runs of the experiment (S1-3), changing and testing new treatments each time: To choose a salinity gradient that is as high as possible but not unnaturally high, we chose a salinity of 5.0% for the treatment in the first test run (S1). The control group had the value of the measured 3.91%. For the second test run (S2), the salinity of STARESO-bay was measured again and taken as value for the control groups of S2 and S3 (3.885%). Then, three different salt concentrations between the values 3.885% (actual sea value) and 5.0% (salinity S1) were chosen to create a series of dilution: 4.1%, 4.4% and 4.7%.

To produce a salt solution that is as close to nature as possible, salt crystals and saturated salt solutions were collected from rock basins near the waterline at the harbor of STARESO. In this way, pure seawater taken from the bay was concentrated to the desired salt concentration. Using the salt-meter, the solution was measured again and again to achieve the desired values. The subsequently filtered liquids were then used as media for the sea urchin embryos in experimental series S1, S2 and S3. The following steps from fertilization of the egg to counting the embryos at specific time points were done in the same way as in the other treatments.

Combination temperature and salinity

In the last series of experiments (S3), the salinity of 4.1% was combined with a temperature increase to 29 °C to test for potential combined effects of treatments. Material and methods for temperature-treatments can be found in the chapter “Temperature” and for salinity-treatments in the chapter “Salinity”.

Fertiliser

For the liquid fertiliser stress treatment, 5 ml of a plant-based fertiliser containing 2.5% nitrogen, 6% potassium oxide and 37% glycine was dissolved in 1 litre of seawater. This dilution was then thoroughly mixed and used instead of seawater after the fertilised sea urchin eggs were added to the 200 mm plastic Petri dish. The change of liquid medium and the counting of embryonic developmental stages were carried out as described in section one of the experimental set-up.

Cigarettes

For the treatment with the human-induced stressor cigarettes a solution was prepared in advance. Therefore, one already smoked cigarette butt was diluted in 1l sea water. The solution was incubated in the sun for five hours and filtered afterwards. The filtered liquid was then used as a medium for fertilized sea urchin eggs in our first treatment round. The following steps of fertilization and counting of the embryos were performed in the same way as the other treatments.

Sunscreen

To find out whether sunscreen affects the early development of *Arbacia lixula*, two experiments were set up. Inspired by the growing trend for sunscreens to carry eco-labels, we decided to compare a “good” sunscreen with an eco-label and a “bad” sunscreen without eco-label. Furthermore, we took a look at the ingredients (Table 3) using the National Ocean Service website (2023). Any potentially harmful ingredients listed on the website were cross-checked with the ingredients in the sunscreens. The ”good” sunscreen did not contain any of the concerning ingredients, whereas the “bad” sunscreen contained two of them. Both sunscreens were bought in supermarkets, the “good” one directly in Calvi and the “bad” one in Austria, and both were in the same price range.

Table 3. Ingredient list of sunscreens used in experiments. *Highlighted ingredients are identified as ‘of concern’ by the National Ocean Service (red) (status October 2023, no guarantee of completeness).*

“good” sunscreen	**“bad” sunscreen**
Aqua, Glycerin, Bis-Ethylhexyloxyphenol, Methoxyphenyl Triazine, Isopropyl Palmitate, Ethylhexyl Salicylate, Pentylene, Glycol, Butyl Methoxydibenzoylmethane, Alcohol denat., Ethylhexyl Triazone, Zea Mays Starch, Potassium Cetyl Phosphate, Diisopropyl Sebacate, Oryza Sativa Cera, Tocopherol, Stearic Acid, PEG-100 Stearate, Triethanolamine, Trisodium Ethylenediamine Disuccinate, Palmitic Acid, Xanthan Gum, Caprylyl Glycol, Acrylates/ C10-30 Alkyl Acrylate Crosspolymer, Myristic Citric Acid, Glyceryl Stearate, Parfum.	Aqua, Homosalate, Ethylhexyl Salicylate, Octocrylene, C12-15 Alkyl Benzonate, Diisopropyl Adipate, Butyl Methoxydibenzoylmethane, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Butylene Glycol, Cetearyl Alcohol, Acrylates/C12-22 Alkyl Methacrylate Copolymer, Phenoxyethanol, Isohexadecane, Caprylyl Glycol, Benzyl Alcohol, Parfum, Chlorphenesin, Ceteth-10 Phosphate, Dicetyl Phosphate, Polysorbate 60, Xanthan Gum, Coco-Glucoside, Disodium EDTA, Aminomethyl Propanol, Mica, Linalool, Tocopheryl Acetate, Titanium Dioxide, Panthenol, Limonene, Aloe Barbadensis Leaf Juice, Silk Amino Acids, Sodium Ascorbyl Phosphate, Psidium Guajava Fruit Extract, Plumeria Axutifolia Flower Extract, Passiflora Incamata Fruit Extract, Magifera Indica Fruit Extract, Carica Papaya Fruit Extract, CI 77492.

For the two experiments we prepared two well shaken 1:1000 dilutions (1ml of sunscreen in 1l of fresh seawater), which was used as a medium for the fertilised eggs. The petri dishes were kept at room temperature (24-25°C), which is more or less the temperature measured in the bay of the research station. Furthermore, they were stored protected for direct sunlight but not in complete darkness. The evaluation of the different embryonic stages was performed in the same way as for the other treatments.

Analysis

To ensure that the counting process was as similar as possible each time, the Petri dishes were slightly rotated so that the embryos accumulated in the centre. Using a pipette, embryos were then taken from the edge of this concentration, from bottom to top, and viewed under the binoculars. For each sample, 100 embryos were counted, and the stage determined. A distinction was made between deformed, less than 2 cell stage, 2 cell stage, 4 cell stage, 8 cell stage, more than 8 cell stage, blastula stage, gastrula stage, prisma stage and pluteus larva. Each experiment was evaluated at 5 time points: after 2, 4, 14, 17 and 21 hours of incubation. The experiment was terminated after the last evaluation.

As we used two replicates for each experiment in each experimental series, as specified in the experimental setup, the arithmetic averages were used for results and discussion.

Results & Discussion

Control

Under controlled conditions (fresh seawater, room temperature), in the first two experimental series (S1 and S2), most embryos reached the eight-cell stage after two hours, the Blastula stage after four hours, the Gastrula stage after 14 hours, the Prisma stage after 17 hours and the Pluteus larvae stage after 21 hours (**Figure 15A-B**). The control for the third experimental series showed a slightly slower development, especially at the checkpoint after 2 hours and 4 hours (**Figure 15C**).

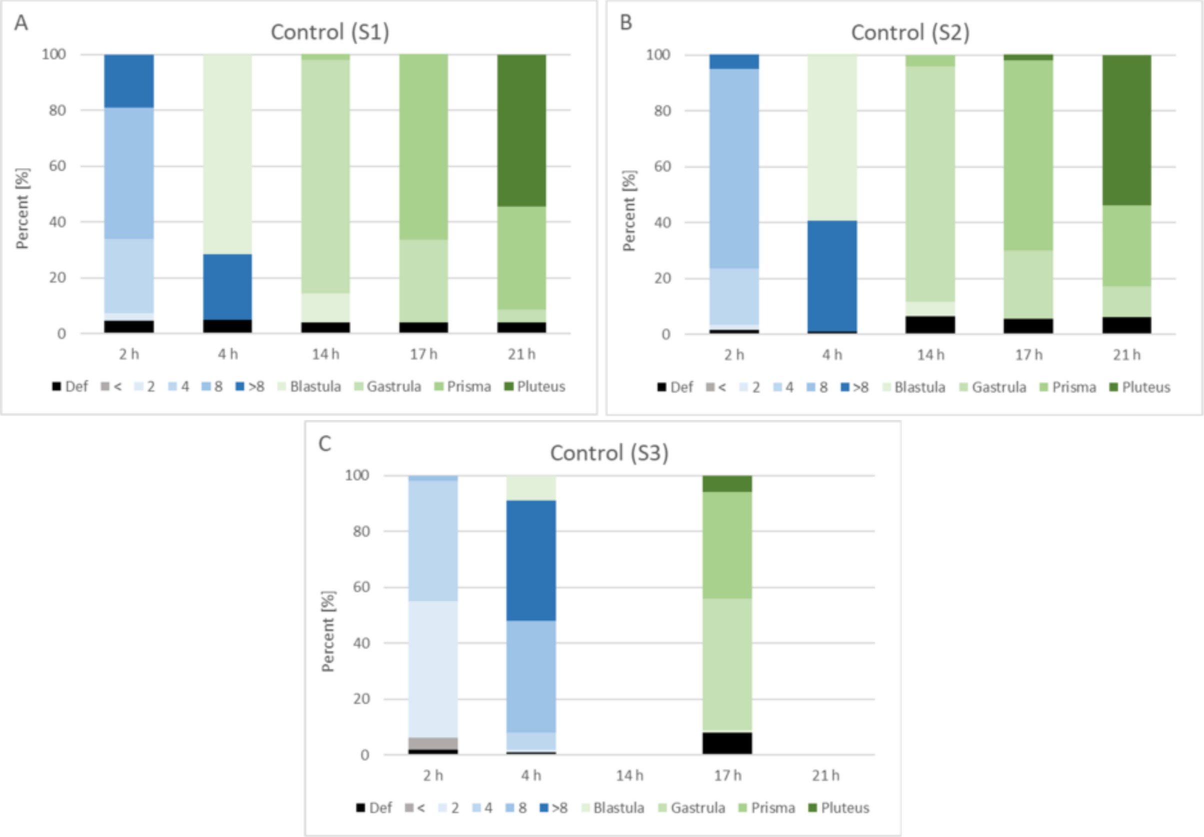

Figure 15. Embryonic development under controlled conditions**. A** Achieved embryonic stages at different checkpoints under controlled conditions for the first experimental series; **B** for the second experimental series, **C** for the third experimental series.

As taken from the literature (for example Gilbert, 2000), controls of series S1 and S2 highly correspond to a "normal" development for *Arbacia lixula*. Furthermore, the mortality and the deformation rate were extremely low for these two experimental series. At none of the checkpoints, the deformation rate passed 5%. For the third experimental series it should be noted that the development of the control was a bit slower than for the first two. In addition, the deformation rate was 8%. Nevertheless, also the control of series S3 was used for comparison.

Besides the development and deformation rate, our embryos appeared fully intact at first sight under the microscope, whereby **Figure 16** shows all embryonic stages of the development of *Arbacia lixula*.

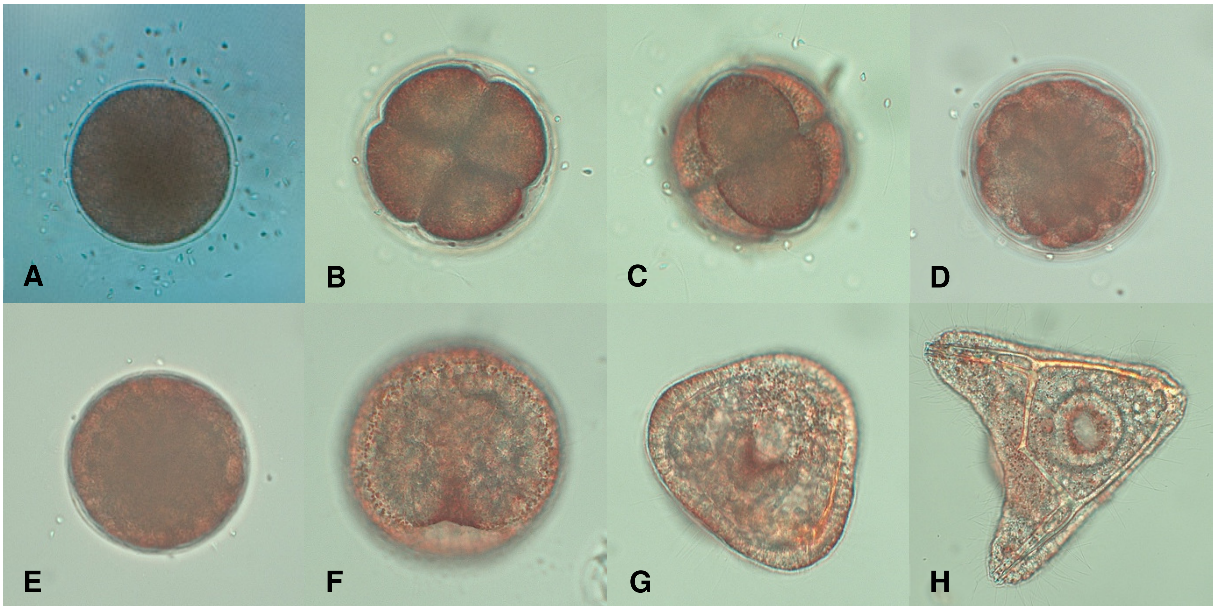

Figure 16. Developmental stages of Arbacia lixula under controlled conditions**. A** fertilized egg with prominent fertilization membrane; **B** four-cell stage; **C** eight-cell stage; **D** over eight-cell stage; **E** Blastula; **F** Gastrula; **G** Prisma; **H** Pluteus larvae (Magnification: x25)

Deformation

As shown in **Figure 17**, the deformation rate of all control groups remained under 4%. In contrast, different results were obtained after the treatments. With 12.5% deformed embryos, the treatment containing the cigarette but showed the least deformation rate. In contrast, for all other treatments, significantly higher rates of deformation were observed. The highest rates were seen for 5% salinity and 37 degree temperature. The changes in the deformation rate of each treatment are further studied in the following chapters.

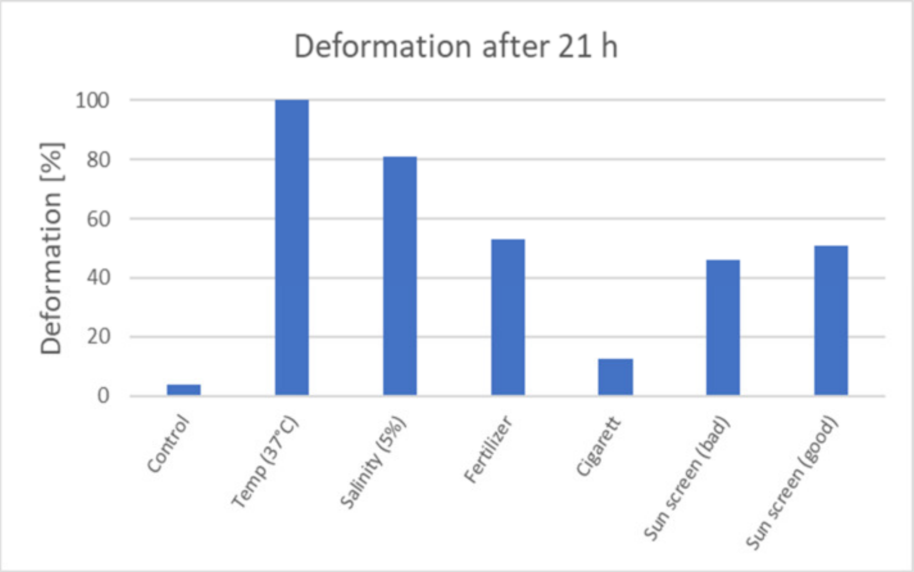

Figure 17: Deformation rate of different treatments after 21h.

Moreover, depending on the treatment, different types of deformation have been observed, which are characterised in **Figure 18**.

If the cells deformed due to a division defect and therefore showed an odd number of cells, they were characterised as irregular cleavage (**Figure 18A *(left)***). Since intact blastomers show a round shape, those that showed an abnormal shape were classified as irregular zygotic shape (**Figure 18A *(right)***) or irregular embryonic shape (**Figure 18F**), depending on their stage of development. Another abnormal shape of cells was seen when a bubble formed in the cytoplasm. Such cells, as also shown in **Figure 18B**, were termed as cytoplasmic bladder formation. Likewise, some deformed cells showed different types of disintegration: cells disintegrating anywhere outside the fertilization membrane (**Figure 18C**) were termed external disintegration, whereas cells disintegrating inside the fertilization membrane were termed internal disintegration (**Figure 18D**). In addition, external but local disintegration was also observed, meaning cells that disintegrated only at a specific spot outside the fertilisation membrane (**Figure 18E**). Cells that had already started to die but still had an intact fertilisation membrane, **Figure 18G**, were characterised as degeneration within fertilisation membrane. As shown in **Figure 18H**, cells that deformed due to a gastrulation defect were appropriately characterised as a gastrulation defect. Besides these characterised types, one could also observe clusters of cells, as in **Figure 18I**, which are not normal. Also, depending on the pigmentation, the degree of degeneration could be approximately determined (**Figure 18J**). The more advanced the degeneration, the less pigmented the damaged cell appears.

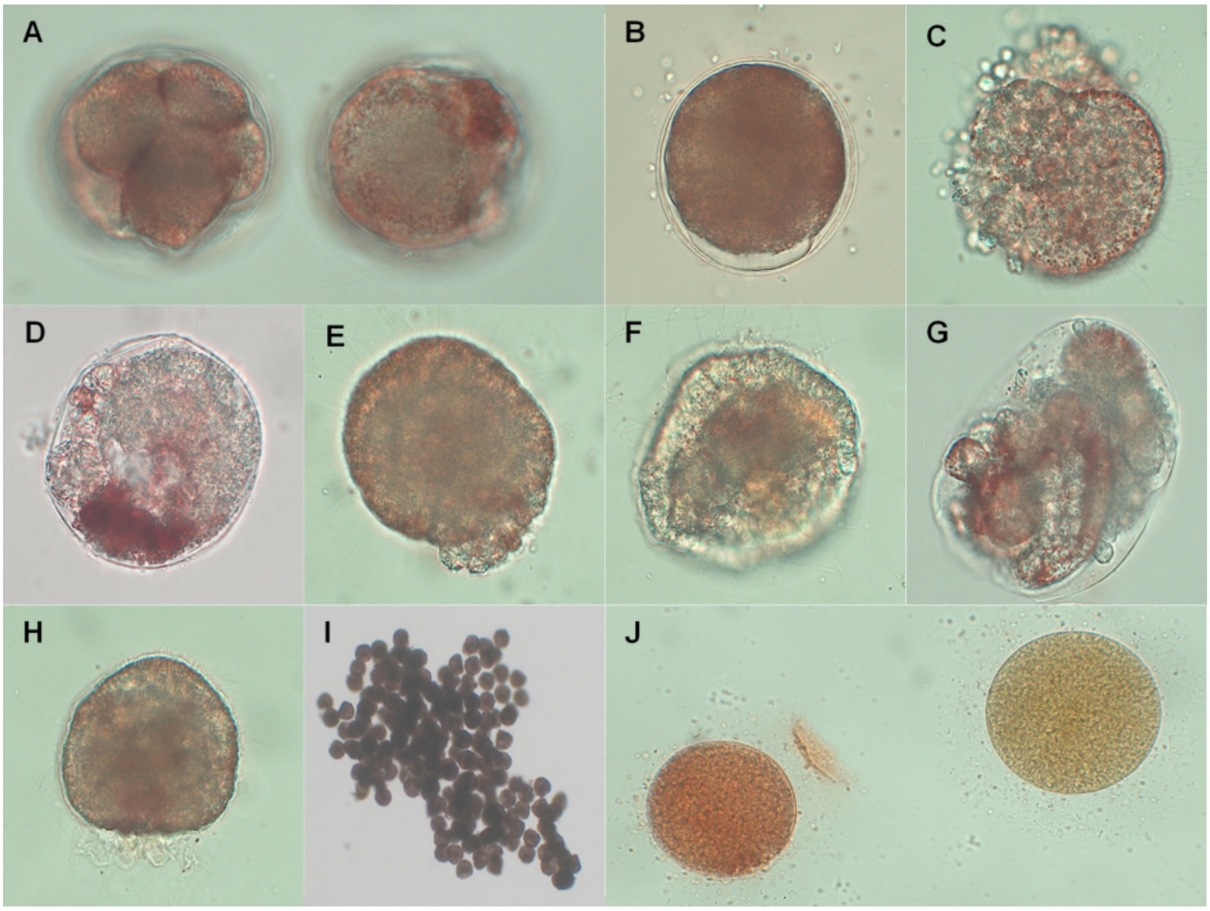

Figure 18.: Characterisation of deformed cells. **A** Irregular cleavage (left) & irregular zygotic shape (right); **B** Cytoplasmic bladder formation; **C** External disintegration; **D** Internal disintegration; **E** External but local disintegration; **F** Irregular embryonic shape; **G** Degeneration within fertilisation membrane; **H** Gastrulation defect; **I** Cluster formation; **J** Different degeneration stages.

Temperature

Mortality and deformation were high with all 3 temperature treatments (**Figure 19**). Compared to the control (**Figure 19A**), 29°C seems to positively affect development in the first hours (**Figure 19B**), on the 4 hours' time mark 58% have reached >8 cell stage, compared to control at this time only 40% have reached that stage. However, afterwards development seems to be slowing down and after 21 hours only a third has reached the pluteus stage, and another third has started to deform. The treatment with 32°C has a deformation rate of 50% after 2h and increases up to 100% after 17h (**Figure 19C**). The 37°C treatment, where embryos were incubated at 37°C in the first 2 hours, showed the highest deformation rate. In both runs there was 100% deformation at all time points (**Figure 19D**). It seems like embryos never get over the one cell stage, most embryos started to form a cytoplasmic bladder (**Figure 18B**) and slowly dissolve with ongoing exposure to temperature.

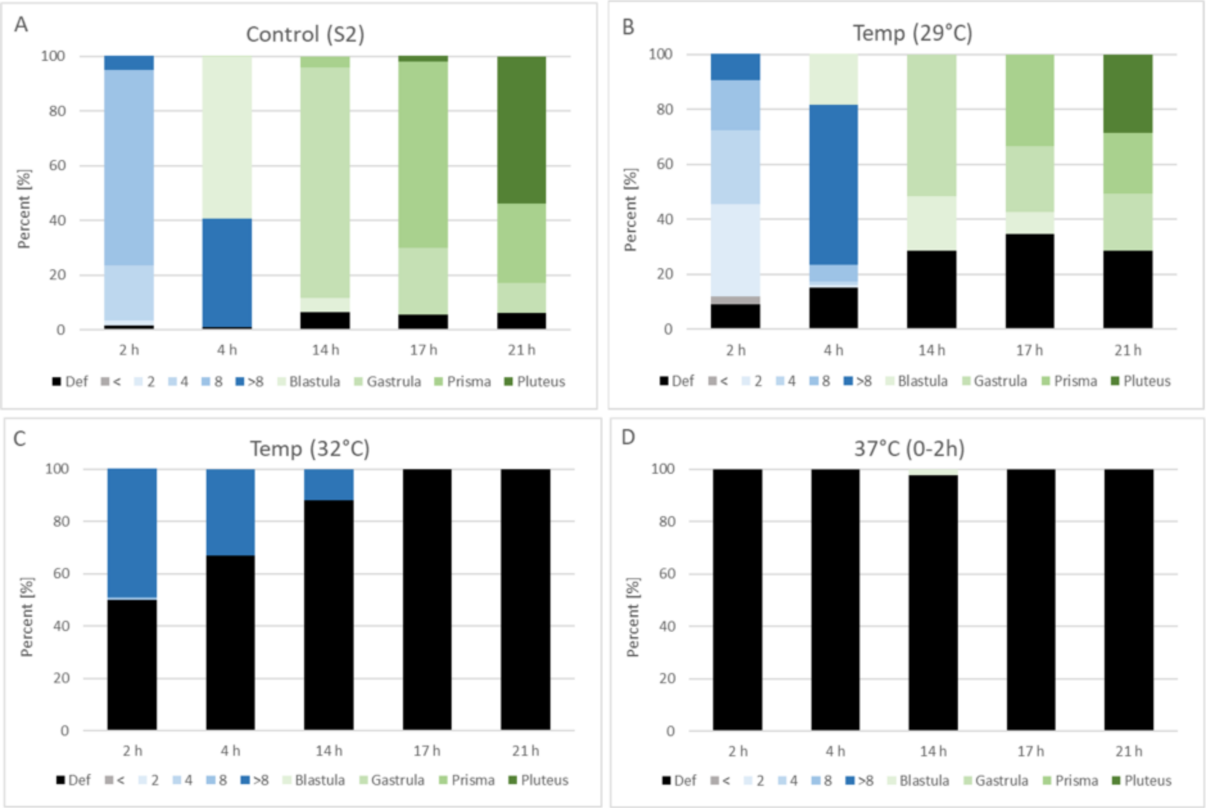

Figure 19. Developmental stages of the second run of temperature treatments**. A** control of the second experimental series**; B** 29°C treatment showed faster development**; C** 32°C treatment showed significantly more deformation during development**, D** 37°C incubation during first 2 hours showed 100% deformation during development**.**

Embryos of *Arbacia lixula* are without a doubt sensitive to higher temperature. *Arbacia lixula* has a peak in reproduction in May-July (Wangensteen et al., 2013), therefore embryos are usually exposed to lower temperature and not used to the temperature maxima occurring from late July to early September. Despite different sea urchins being able to synthesize heat shock proteins (Roccheri et al., 1986), 37°C seems to be too high to synthesize such proteins, additionally since embryos exposed to 37°C do not get over the one-cell stage, it seems like all other developmental processes are affected by that temperature too. This hypothesis is strengthened by the second run, where embryos were only exposed to 37°C in the first two hours after fertilization, even then embryos do not recover from this heat shock, as this treatment lead to the same results of degeneration. A critical point seems to be the 29°C mark, where embryos can tolerate the heat shock, however only up to 4 hours, afterwards, development slows down, one reason could be, that synthesis of said heat shock proteins parallel to embryonic development affects the speed of developmental processes. Moreover, a slow rise of temperature after fertilization might increase survival rate, thus molecular processes might adapt to slowly rising temperature better than quick and ongoing changes of temperature. Additionally, with rising temperatures there should be a check for pH, even though different pH values have been tested to not influence development (Byrne et al., 2009), it could have been, that due to cooking the seawater to prevent bacteria growth, might have altered the pH at such a high level that it added another stressor negatively influencing development.

Salinity

Like temperature, salinity proved to have a dramatic impact on the early development of *Arbacia lixula* (**Figure 20**). In the first run, the embryos were placed in a treatment with 5% salt water. Compared to the control group (**Figure 20A**), in which about 50% of the embryos have reached the pluteus stage after 21 hours and the deformation rate is approximately 5% over the entire period, the salt treatment showed a high rate of deformation (**Figure 20B**). Already after two hours, more than 20% of the embryos were deformed; after 21h more than 80%.

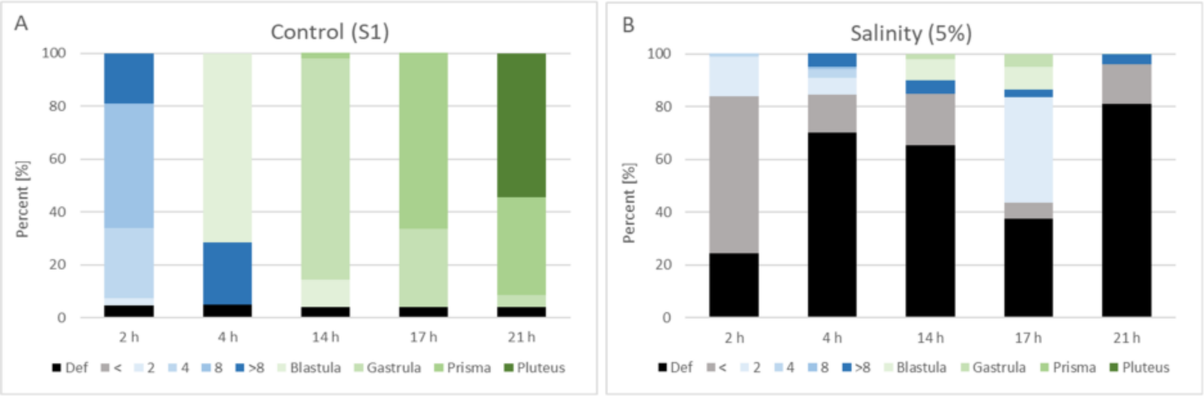

Figure 20. Developmental stages of the first run of salinity treatments. **A** control of the first experimental series; **B** treatment with 5% salted sea water showing high deformation.

The speed of development is also influenced by a higher salt content. In the control group, about 50% of the embryos reached the 8-cell stage already after 2h, after four hours more than 70% were in the blastula stage, after 14 hours more than 80% reached the gastrula stage and after 21 hours about 50% were in the pluteus stage. In comparison, the 5% salt treatment showed that after two hours approximately 60% of the embryos remained below the two-cell stage, after four hours only a few reached the >8-cell stage and after 17 hours the blastula stage was being reached as the furthest developmental stage for this treatment with only 5% of the embryos. After 21 hours, 5% of the embryos were found in the >8-cell stage, the rest were deformed or showed no development.

Regarding deformation and compared to the other treatments from the first run, the deformation rate of the 5% salt treatment is the second highest with 80%. Only the temperature treatment of 37°C led to 100% deformed embryos (**Figure 18**).

For the second run of the experiment (S2), the aim was to produce a higher resolution of salinity impact. The series of dilution showed increasing effects with increasing concentrations of salt (**Figure 21**). Regarding the control group, at the checkpoint after 21 hours, 54% of the developing sea urchins were in the pluteus stage (**Figure 21A**). Under the Salinity of 4.1% (**Figure 21B**), still 27,5% of the embryos reached the pluteus stage during time of observation, whereas highest developed embryos of the 4,4%-treatment reached only the prisma stage (18,5%) (**Figure 21C**). For the salinity of 4.7%, embryos only made it to the gastrula stage (10%) after 21 hours of observation (**Figure 21D**).

At first, it seemed that all three salinity treatments promoted sea urchin embryonic development up until four hours after fertilization, with 61% and 47,5% of sea urchins in the >8-cell stage within treatments of 4.1% and 4.7% salinity compared to the 39,5% in >8-cell stage of the control group. From here, embryonic development then clearly slowed down and deformation processes increased. After 21 hours, 38.5-48% of embryos of the salinity treatments were noted as deformed, while the control group showed 6% of deformation.

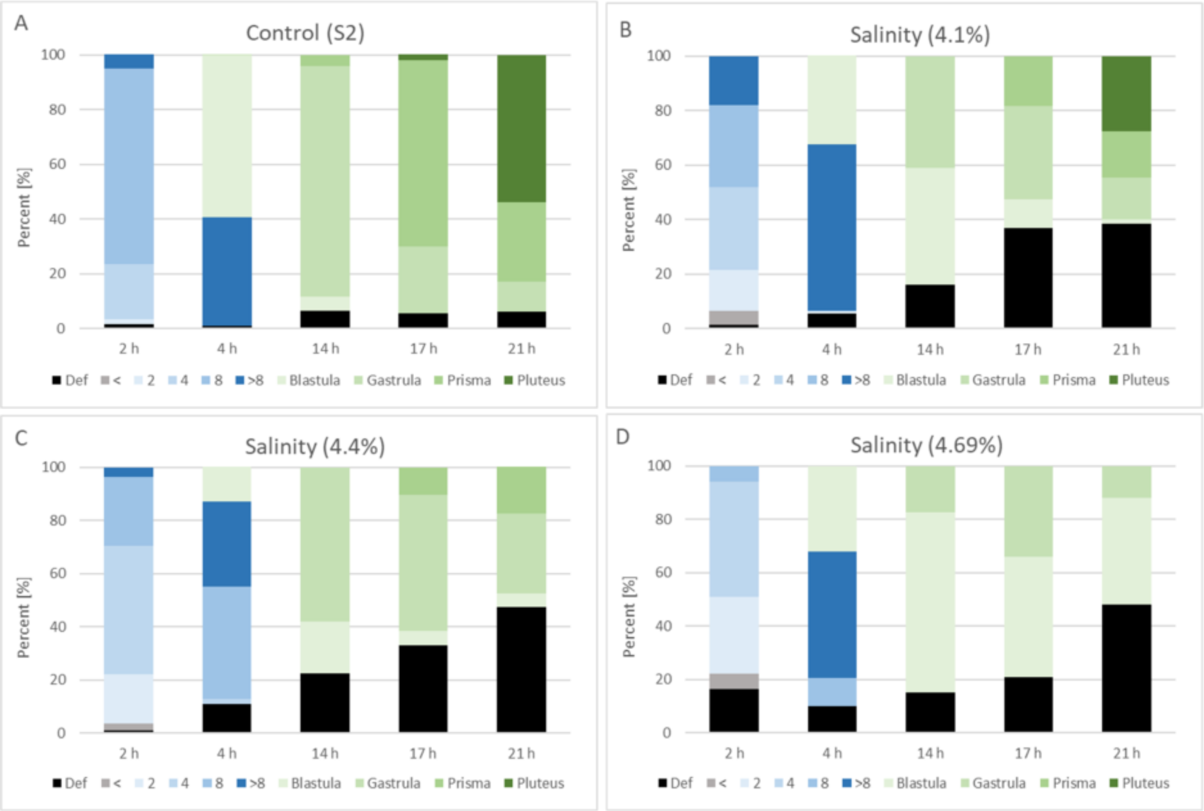

Figure 21. Developmental stages of the second run of salinity treatments. **A** control of the second experimental series; **B** treatment with 4.1%; **C** treatment with 4.4% and **D** treatment with 4.7 % salted sea water.

Echinoderms are marine animals – they are exclusively found in salty waters. This is why they are considered as stenohaline, which means they are not able to withstand wide fluctuations in the salinity of their surroundings (Roller & Stickle, 1993; Castellano et al., 2016). Our study proofed that theory in terms of the sea urchins *Arbacia lixula* early development in higher salinities. However, producing the different salt solutions for our treatments was not as simple as one could think. Firstly, we did not simply use NaCl to reach higher salinities because we aimed for more natural circumstances, as seawater consists naturally of mostly six ions: chloride (Cl–), sodium (Na+), sulfate (SO24–), magnesium (Mg2+), calcium (Ca2+), and potassium (K+) (Duxbury et al., n.d.). Secondly, handling the salt-meter was not the easiest, as it is not designed to test distinct smaller volumes of water. Moreover, it was discussed that the measurement-device possibly did not work properly as values of salinity of STARESO bay seemed quite high (3.91%) compared to the 3.85% described in Hofrichter (2020). To test the function of the device, various measurements were carried out. The measurement of fresh water directly from the station resulted in a value of 0, so it could be assumed that the device was working properly. Additionally, more measurements were taken inside and about 30 meters outside the harbor at different depths (surface to four meters depth). In total, the salinity was measured at seven different points. Higher salt concentrations (3.91% & 3.89%) were measured at the surface than at deeper points (3.87%, 3.88%). Despite all discussions, we still have relative effects resulting from our studies. Therefore, we recommend to not overfocus on absolute values.

As previous studies (e. g. Carballeira et al., 2011) showed, embryo-larval development of *A. lixula* is significantly impacted by salt concentrations and an optimum range of development between is defined from 2.9 to 3.55% salinity. Furthermore, Carballeira et al. (2011) started incubating gametes of *A. lixula* prior to fertilization. Results showed that higher salinities (maximum salinity tested was 4.05%) inhibited fertilization already heavily and therefore had a significant impact on sperm and egg cells. For our first run of the experiment, we tested a salt content of 5% which can be declared as extremely high compared to natural occurring salinities. We chose this value because we wanted to find out if there is an effect of salinity on embryonic development at all. After the first results being quite drastic, we decided for lower values to be tested in the following experiments. Our findings then showed that there is a turning point where the impact starts to increase rapidly for concentrations between 4.7 % - 5.0%. It could be possible, that the effect of increase in salinity on the development of sea urchin embryos is somewhat logistical. However, there is still much to find out. For future studies we would suggest trying to find a more precise point from which on increased salinity starts to impact drastically and defining a range of salinity tolerance. It could be an aim to even test for lower salinities as there is a multitude of literature to compare and evaluate differences in methods. However, for our third series, we decided to have a closer to the future reality of the Mediterranean Sea. Therefore, a moderate increase in salinity was combined with a moderate increase in water temperature in the last run of our experiment.

Combination temperature and salinity

For round three of the sea urchin experiment (S3), we tested for effects of combined treatments of an increased temperature plus increased salinity. As mentioned before, results showed that both single treatments, temperature- (**Figure 19**) and salinity-increase (**Figure 20** and **Figure 21**), had a negative effect on the development of *Arbacia lixula* embryos.

When treatments were combined (4.1% salinity and Temp 29°C), the development of sea urchin embryos was even more inhibited (**Figure 22**). After 17 hours, 6% of the embryos of the control group made it to the pluteus stage (**Figure 22A**), whereas none of the embryos of the combined treatment reached that stage (**Figure 22B**). Also 9% of the individuals of the combined treatment were still in the >8-cell stage after 17 hours of observation. Moreover, deformation processes were promoted by the combined treatment. After 17 hours, 43% of the sea urchin embryos were categorized as deformed, while the control group showed 8% of deformed embryos for that timestep.

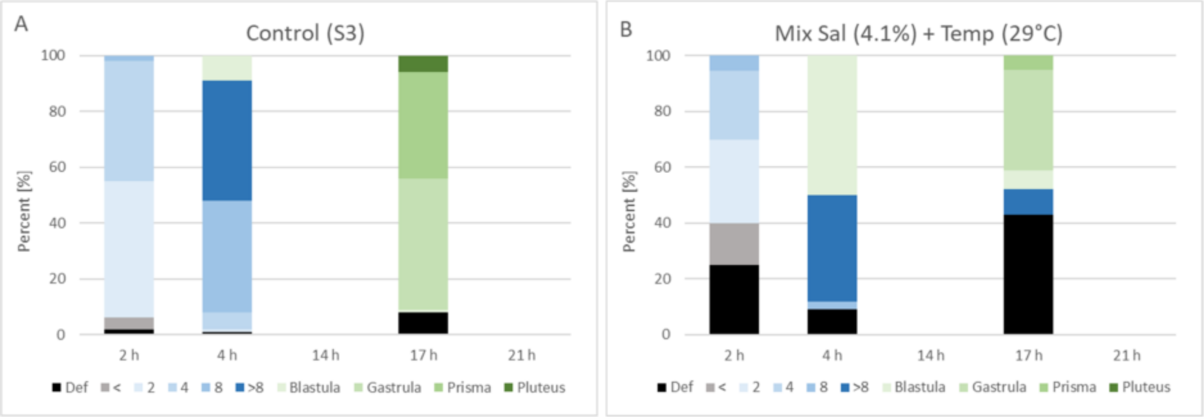

Figure 22. Developmental stages of the third run of combined treatment of salinity and temperature. **A** control of the third experimental series; **B** combined treatment with 4.1% salinity and 29°C temperature.

For the combined treatment, a moderate increase in salinity combined with a moderate increase in water temperature, we expected an amplification of the single treatments resulting in higher percentages of deformed and low-developed individuals. Our findings fulfilled our expectations but did not turn out as drastic as we expected them to be, as literature suggests an optimum development at 20°C (Carballeira et al., 2011).

To put this to conclusion,” salinity is the elephant in the room” (Röthig et al., 2023). As the treatments of temperature and salinity showed, the greatest impact on sea urchins early development compared to the other treatments at our first run of experiments (S1), we decided to concentrate on these two parameters in the following runs (S2 & S3). But there is still more to explore. In future studies we would propose to try out more runs of combined temperature- and salinity-treatments, ideally combining treatments of higher impacts together. In addition, another series of dilution between 4.7% and 5.0% could be designed. Another possibility for next experiments would be to start treatments of higher salt concentrations already before fertilization, as this is an essential point in reproduction and could already be a very critical time when gametes are exposed to different surroundings. This was not manageable for our experiment, as we already had many different treatments and doing even more would have become a logistical problem. It would also be interesting to replicate our experiment using other species of sea urchins, for example *Paracentrotus lividus* or *Sphaerechinus granularis*, as they are common around STARESO as well, and results could look different from one species to another. Last but not least, future experiments should be conducted using a different measurement device to test for salinity. In respect to our study and to facilitate comparison, it would be great to calibrate the SonTek CastAway-CTD salinity meter (https://www.ysi.com/castaway-ctd) to the new device, if possible.

Fertilizer

The effect of nutrient enriched seawater showed two notable differences from the development under controlled conditions (**Figure 23**). Compared to the control (**Figure 23A**), the development of the fertilizer treated animals (**Figure 23B**) was noticeably slowed down, as shown by the pronounced abundance of 2 and 4 cell stages at the 2-hour time point. In addition, the composition of sea urchin stages after 17 and 21 hours of exposure to the stressor showed significant deviations from the control group, in particular with a significant reduction in the number of prism stages after 17 hours and a complete absence of pluteus stages after 21 hours. Secondly, the number of malformations showed a significant increase with exposure to fertiliser, with over 50% of all cells counted showing deformations after 21 hours.

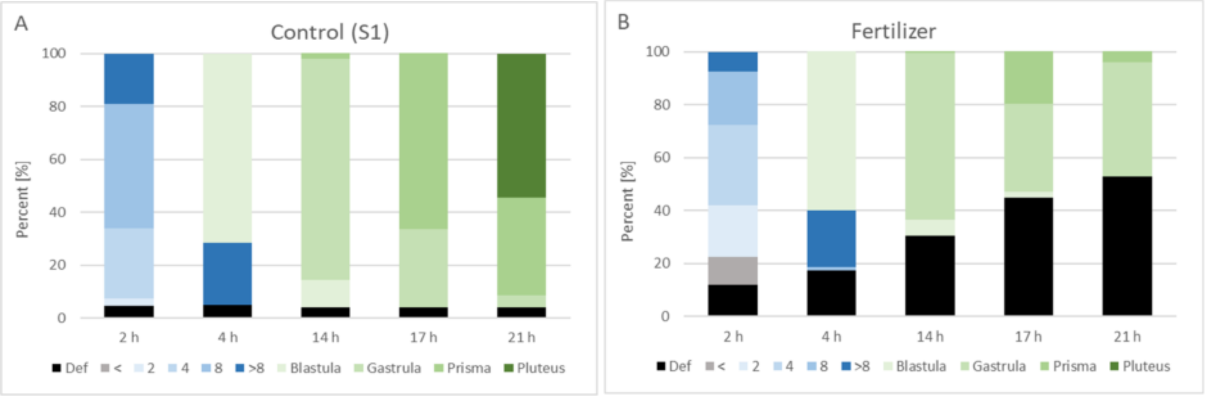

Figure 23. Developmental stages of the treatment with contaminated sea water through fertilizer**. A** control of the first experimental series; **B** treatment with 5 ml fertilizer per l sea water.

In the context of the observed effects of highly fertilised seawater on the embryonic development of *Arbacia lixula*, it is evident that the introduction of fertiliser as an environmental stressor has significant implications. The slowing of development, as evidenced by a notable abundance of 2- and 4-cell stages at the 2-hour time point, indicates a direct effect on the early stages of embryonic growth. This observed slowdown may be due to perturbations in the intricate processes of fertilisation and early blastomere formation, which are highly sensitive to environmental changes. In a broader ecological context, the pronounced eutrophication-induced stressors, such as macroalgal blooms and altered biogeochemical processes, may pose a significant threat to *Arbacia lixula*. The potential consequences, including disrupted benthic habitats and detrimental shifts in the phytoplankton community, could lead to increased mortality due to hypoxia, affecting the reproductive success of the species and altering population dynamics (Dorgham, 2014, Pardal et al., 2000).

Cigarettes

Embryos raised in sea water contaminated by cigarette butts showed altered development, especially during later embryonic stages (**Figure 24**). With regard to the chart of the control group (**Figure 24A**), the development of the contaminated sea water treated animals was similar in the early development stages (**Figure 24B**). After 2h most of the embryos reached the 4-cell or 8-cell stage. The next counting (4h) showed that the majority of embryos arrived at the blastula stage and around 30% were in the more than 8-cell stage. After 14h, the cigarette butt treated animals showed similar results as the control group, and most embryos developed to the gastrula phase. However, a difference can be observed in the last two phases of embryonic development, where after 17h the majority still remained in gastrula and did not develop to the prism stage. The same variation is shown after 21h, there around 60% persisted in gastrula and less than 30% developed to prism or even pluteus stages.

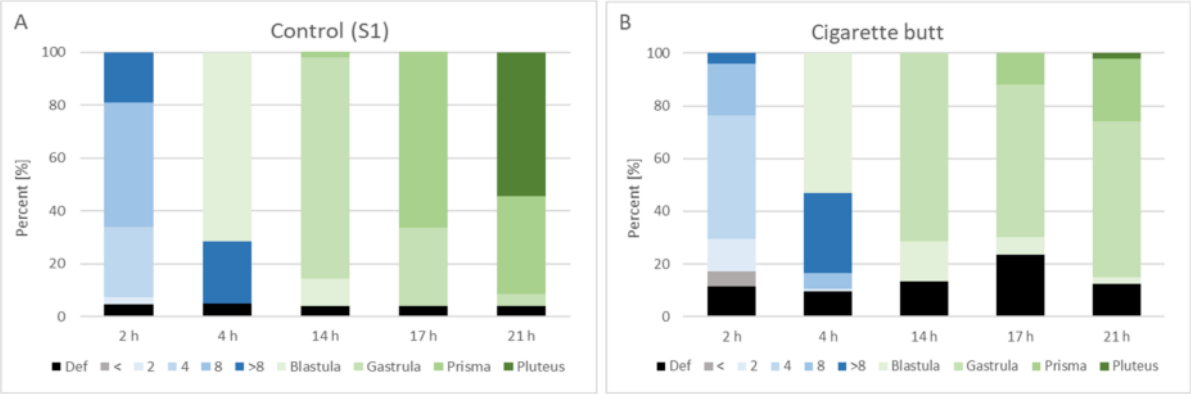

Figure 24. Developmental stages of the treatment with contaminated sea water through a cigarette butt. **A** control of the first experimental series; **B** treatment with 1 cigarette butt per l sea water.

Among all the treatments in the first round of experiments, the one with cigarette butts showed the least deformed embryos (**Figure 17**). After 21h of development only 12% of the embryos showed a deformation. Compared to the control group the deformation was just slightly higher for the embryos, which developed in the contaminated sea water.

Overall, the treatment with cigarette butts showed a small slowdown in the development especially in the phases of gastrula, prism and pluteus. But no remarkable number of deformed embryos could be counted. In the course of 2021, there was a similar experiment made with a comparison of the influence of smoked and unsmoked cigarette butts to embryonic development. There they used 5 cigarettes in 1l but they diluted it higher than in our experiment. Still the results were similar and the overall development was slowed down. Most of the embryos in their experiments stayed in the cleavage phase and less than 15% were deformed after 23h (Sodja, 2021). In a different paper it was shown that nicotine induces polyspermy in sea urchin eggs of *Paracentrotus lividus*. But this we could not observe due to the fast process of fertilization and the lack of equipment (Limatola et al., 2020).

To observe a more pronounced impact on the development of *Arbacia lixula,* a higher concentration of cigarettes would be needed, but it would probably be unrealistic to find such high concentrations in the oceans nowadays.

Sunscreen

Within our two experiments focusing on sunscreens, we wanted to study the effect of two different products on early developmental stages of *Arbacia lixula*. As read in the course protocol 2021, no significant effect was reported for the treatment with 1:250 of sunscreen diluted in seawater. We therefore initially tried to increase the concentration and diluted 1:100 in seawater, but then switched to a 1:1000 dilution because of the difficulty in counting due to the milkiness of the solution. Based on the data from 2021, we did not expect any significant effect on the sea urchin embryogenesis at this dilution. Surprisingly, our data does not support the results of the last course. In fact, the results show a completely different trend. The distribution of the developmental stages at determined counting is shown in (**Figure 25**).

The comparison with the control (**Figure 25A**) shows that both, the “good” and “bad” sunscreen, had a significant effect on the embryogenesis of the sea urchins. This can be seen by the speed of the embryonic development as well as in the rate of embryos showing any kind of deformation.

For the “good” sunscreen (**Figure 25B**), the results in terms of the speed of development seem to be quite normal at 2h after fertilization. However, counting after 4h even showed an increased speed. While in the control group only about one quarter of the embryos had reached the stadium with more than 8 cells, in the treatment with “good” sunscreen 50% of the embryos were found at this stage. At 14h after fertilization, three quarters of the embryos were in gastrula, almost the same as in the control group. However, this trend clearly turned around after 17h. At this point of time, all embryos in the control group that looked healthy were either in gastrula (29.5%) or in pluteus stages (68%), whereas most of the embryos treated with “good” sunscreen were still found in gastrula (40%) and blastula stages (4%). After 21h, more than 50% of the embryos of the control group reached the pluteus stage, while only 3% of the embryos treated with “good” sunscreen were observed to be in this stage.

Regarding developmental speed, the “bad” sunscreen (**Figure 25C**) seems to have an even more dramatic impact on embryogenesis than the “good” sunscreen. After only 2h treated with “bad” sunscreen, the embryos showed a significant slowdown in their development. Only 1% made it to a stadium with more than 8 cells, while in the control group this stage was reached from around 20% of the embryos. Furthermore, it should be noted that 11% didn’t even undergo the first cleavage to reach the 2-cell stadium. The trend continues at 4h where only half of the embryos reached the Blastula stadium, whereas in the control group, 70% were found at this stage. At 14h after fertilization, the majority (58.5%) of the “bad” sunscreen treatment had reached the gastrula stage. Also in the control, most of the embryos were in this stage of development (83.5%). However, the counts after 17h and 21h indicate that the peak of the development had already been reached for most of the animals. At the final count only 3% made it to the prism stage, 47.5 % were still either in blastula or gastrula stages. None of the embryos reached the pluteus stage, while in the control group, more than 50% of the embryos reached this stage.

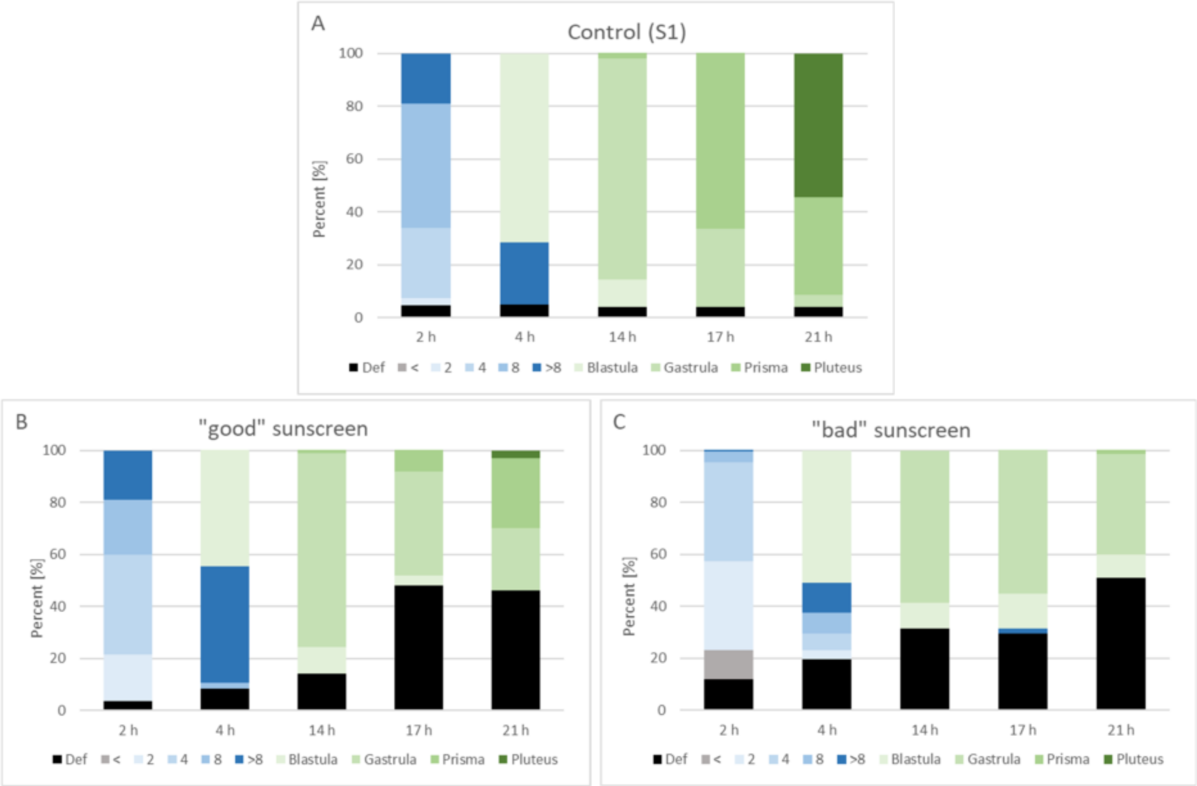

Figure 25. Developmental stages of treatments with sunscreens**. A** control of the first experimental series; **B** “good” sunscreen; **C** “bad” sunscreen.

With respect to the deformation rates of the embryos treated with sunscreen, for both, “good” and “bad” sunscreen, a huge effect of the treatment on the embryonic development can be reported (**Figure 17** and **Figure 25**). Treatment with “good” sunscreen led to a huge increase in deformed animals after 4h. At this point of time, the rate of deformed embryos was twice as high as in the controls. This trend continued after 14h, reaching around 50% after 17h and 21h. In contrast, the percentage of deformed embryos in the control remained stable at around 5%. Regarding the rate of the embryos treated with “bad” sunscreen, a gradual increase occurred from 12% at the first count at 2h to 51% at the last count after 21h.

Summing this up, the results of the two sunscreen-treatments indicate that the presence of sunscreen has a huge impact on early development of *Arbacia lixula*. In both experiments, we noticed a slowdown in the speed of development. Furthermore, in both experiments, about 50% of the embryos showed some kind of deformation. Similar results were also reported by Corinaldesi et al. (2017) in their study on *Paracentrotus lividus*. Based on our results, we draw the conclusion that the “good” sunscreen does not really keep what the eco-label ‘ocean conservancy’ promises. Furthermore, a closer look at the ingredients also showed that even the “good” sunscreen contains substances that are potentially harmful to the environment and to humans (**Table 4**). Our findings underline the importance of developing environmentally friendly sunscreens and emphasize the need not to blindly trust eco-labels.

Table 4. Ingredient list of sunscreens used in experiments**.** Highlighted ingredients are identified as ‘of concern’ by the National Ocean Service (red) and ‘critical’ according to the INCI list and codecheck.info (orange) (status October 2023, no guarantee of completeness).

“good” sunscreen	**“bad” sunscreen**
Aqua, Glycerin, Bis-Ethylhexyloxyphenol, Methoxyphenyl Triazine, Isopropyl Palmitate, Ethylhexyl Salicylate, Pentylene, Glycol, Butyl Methoxydibenzoylmethane, Alcohol denat., Ethylhexyl Triazone, Zea Mays Starch, Potassium Cetyl Phosphate, Diisopropyl Sebacate, Oryza Sativa Cera, Tocopherol, Stearic Acid, PEG-100 Stearate, Triethanolamine, Trisodium Ethylenediamine Disuccinate, Palmitic Acid, Xanthan Gum, Caprylyl Glycol, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Myristic Citric Acid, Glyceryl Stearate, Parfum.	Aqua, Homosalate, Ethylhexyl Salicylate, Octocrylene, C12-15 Alkyl Benzonate, Diisopropyl Adipate, Butyl Methoxydibenzoylmethane, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Butylene Glycol, Cetearyl Alcohol, Acrylates/C12-22 Alkyl Methacrylate Copolymer, Phenoxyethanol, Isohexadecane, Caprylyl Glycol, Benzyl Alcohol, Parfum, Chlorphenesin, Ceteth-10 Phosphate, Dicetyl Phosphate, Polysorbate 60, Xanthan Gum, Coco-Glucoside, Disodium EDTA, Aminomethyl Propanol, Mica, Linalool, Tocopheryl Acetate, Titanium Dioxide, Panthenol, Limonene, Aloe Barbadensis Leaf Juice, Silk Amino Acids, Sodium Ascorbyl Phosphate, Psidium Guajava Fruit Extract, Plumeria Axutifolia Flower Extract, Passiflora Incamata Fruit Extract, Magifera Indica Fruit Extract, Carica Papaya Fruit Extract, CI 77492.

Conclusion

Our comprehensive study of the embryonic development of *Arbacia lixula* in response to various environmental stressors provides valuable insights into the intricate balance of marine ecosystems. Under control conditions, sea urchin embryos exhibited a normal developmental pattern and served as a reference for our experiments. Elevated temperatures, particularly at 37°C, induced substantial deformations and developmental delays, highlighting the sensitivity of the species to temperature fluctuations beyond its typical habitat range. Salinity fluctuations, especially salinity at 5%, resulted in a high rate of deformations, highlighting the importance of maintaining optimal salinity levels for successful embryogenesis.

Unexpectedly, exposure to fertiliser, cigarette butt pollution and sunscreen showed clear effects. Fertiliser exposure resulted in slower development and increased deformations, suggesting a potential threat to marine life from nutrient run-off. Cigarette butt pollution showed a modest slowing of development but surprisingly few deformations, challenging conventional assumptions. Sunscreens, both eco-friendly and conventional, showed alarming developmental effects and a high incidence of deformations, underlining the need for environmentally friendly sunscreen formulations.

In conclusion, our results demonstrate the vulnerability of *Arbacia lixula* embryos to both natural and anthropogenic stressors. This study highlights the importance of understanding and mitigating the multiple environmental challenges faced by marine organisms and provides a basis for informed conservation efforts in Corsican coastal ecosystems.

In the context of climate change, understanding how marine organisms respond to different stressors is crucial for predicting and mitigating the impacts of environmental change on coastal ecosystems in the future.

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Fish surveys

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Fish Species List and Fish Transect

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Introduction

The Mediterranean is a semi-enclosed ocean, connected to the Atlantic Ocean by the Strait of Gibraltar in the west and to the Red Sea and the Indian Ocean by the Suez Canal in the east (Pastor & Francour, 2010). Being a key reservoir for biodiversity, a total of 664 fish species can be found in the Mediterranean, of which 127 are exotic (Ben Rais Lasram & Mouillot, 2009 (no current updates of these numbers available); Vanalderweireldt et al., 2022). The Mediterranean is undergoing dramatic changes due to both natural and anthropogenic causes (Pastor & Francour, 2010; Thiriet et al., 2016a). In particular, global warming has allowed certain species to expand their home range (Pastor & Francour, 2010). Additional anthropogenic stressors such as fishing, pollution and the introduction of invasive species have led to habitat degradation, also seen in the typical Mediterranean macrophyte-formed habitats such as *Posidonia oceanica* meadows (Di Iorio et al., 2018; Marengo et al., 2021; Thiriet et al., 2016b). Habitat degradation strongly threatens marine ecosystems. These changes can, amongst others, negatively impact ecosystem resistance and functioning and lead to a shift in community structure and organism distribution (Thiriet et al., 2016b). Fish provides an important ecological function. Especially coastal fishes are crucial as a food resource and for the transfer of nutrients in the marine food chain in general. Worryingly, the International Union for the Conservation of Nature (IUCN) announced that 8 % of the fish species native to the Mediterranean Sea are threatened (Marengo et al., 2021). Consequently, the European Union is taking measures to promote the protection of the marine ecosystem (Vanalderweireldt et al., 2022). To have an accurate overview for management, which requires an understanding of the whole community, it is important to obtain accurate data on fish abundance and composition by stock assessment (Marengo et al., 2021; Vanalderweireldt et al., 2022). Catch-based data is often limited due to its gear-specificity, which can lead to sampling biases (Caldwell et al., 2016). Therefore, to better assess the diversity of fish, several underwater visual census (UVC) techniques can be used. Examples are fixed point observations, rotating remote systems, remotely operated vehicles (ROV), baited video, traps, gillnets, or video acquisition by SCUBA divers (Caldwell et al., 2016; Marengo et al., 2021; Prato et al., 2017).

During our field course, we wanted to assess the fish abundance, diversity, and community structure around STARESO to better understand their role in the ecosystem. Furthermore, we will compare our observations with the ones from previous field courses and thereby aim to highlight potential shifts in the community and abundance, which might result from the above-mentioned challenges the Mediterranean ecosystem is facing. To address those questions, we assessed the fish abundance, diversity, biomass, and diurnal activity patterns using transect swimming.

Material and Methods

Fish Species List

All fish species confirmedly observed during the field course were recorded and added and compared to the table containing the observed fish species of the previous years (Table 3).

Transect

Figure 26: Transect around STARESO

We defined a transect around the harbor of STARESO and chose the strip transect method because it is commonly used and trusted and less time consuming than for instance video methods (Marengo et al., 2021; Prato et al., 2017; Fig. 1). The transect had a length of 177 meters and six meters width and was laid out around the harbor wall of STARESO using a rope that was weighed down with stones. Additional stones were marked and arranged on the seafloor three meters left and right of the rope for visual orientation. The transition of the four different habitat types represented in the transect - namely small boulders (40 m length), larger boulders (50 m length), seagrass (36 m length) and the harbor entrance (small boulders/soft bottom; 51 m length) - was marked with underwater buoys.
A depth profile of the transect was generated using a handheld echosounder (Fig. 2).

Since it is generally difficult to observe crypto-benthic fish in a transect, they were not considered here (Caldwell et al., 2016; Prato et al., 2017; Thiriet et al., 2016b).

Visual Census

Every two hours, a pair of snorkelers slowly followed the transect while swimming closely together. Fish species were recorded individually for each habitat using a writing board. Their abundances were estimated and assigned to four different categories (A-D, Table 2). The observed species are listed in Table 1. The time needed for each section of the transect was noted to assure comparable swimming speeds among different observers. Diving torches (one for each pair of snorkelers) were used during nighttime.

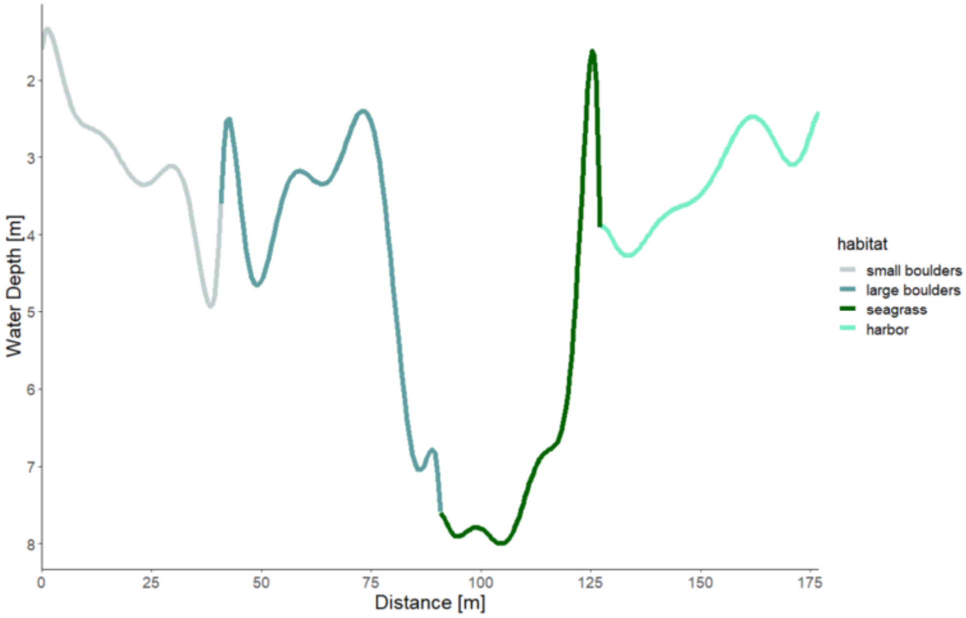

Figure 27: Depth profile of transect

Table 1: Fish species observed in the transect

Family	**Scientific name**	**Common name**	**German common name**
Sparidae	*Oblada melanura*	Saddled seabream	B(r)andbrasse
Sparidae	*Diplodus sargus*	White seabream	Gaisbrasse
Sparidae	*Diplodus annularis*	Annular seabream	Ringelbrasse
Sparidae	*Diplodus puntazzo*	Sharpsnout seabream	Spitzkopfbrasse
Sparidae	*Diplodus vulgaris*	Common two-banded seabream	Zweibindenbrasse
Sparidae	*Sarpa salpa*	Salema	Goldstriemenbrasse

Sparidae	*Spondyliosoma cantharus*	Black seabream	Streifenbrasse
Sparidae	*Boops boops*	Bogue	Gelbstriemenbrasse
Labridae	*Coris julis*	Rainbow wrasse	Meerjunker
Labridae	*Thalassoma pavo*	Ornate wrasse	Meerpfau
Labridae	*Labrus merula*	Brown wrasse	Amsellippfisch
Labridae	*Labrus viridis*	Green wrasse	Grüner Lippfisch
Labridae	*Symphodus tinca*	Peacock wrasse	Pfauen-Lippfisch
Labridae	*Symphodus melanocercus*	Mediterranean cleaner wrasse	Mittelmeer-Putzerlippfisch
Labridae	*Symphodus ocellatus*	Ocellated wrasse	Augenfleck-Lippfisch
Labridae	*Symphodus mediterraneus*	Axillary wrasse	Mittelmeer-Lippfisch
Labridae	*Symphodus roissali*	Five-spotted wrasse	Fünffleckiger Lippfisch
Labridae	*Symphodus rostratus*	Pointed-snout wrasse	Schnauzen-Lippfisch
Mugilidae	*Mugilidae (surface)*	mullet	Meeräsche
Mugilidae	*Oedalechilus labeo*	Boxlip mullet	Kastenmaul-Meeräsche
Mugilidae	*Atherina boyeri*	Big-scale sand smelt	Kleiner Ährenfisch
Mugilidae	*Atherina hepsetus*	Mediterranean sand smelt	Großer Ährenfisch
Serranidae	*Serranus scriba*	Painted comber	Schriftbarsch
Apogonidae	*Apogon imberbis*	Mediterranean cardinalfish	Meerbarbenkönig

Mullidae	*Mullus surmuletus*	Striped red mullet	Streifenbarbe
Pomacentridae	*Chromis chromis*	Mediterranean damselfish	Mönchsfisch
Muraenidae	*Muraena helena*	Mediterranean moray	Mittelmeer-Muräne
Scorpaenidae	*Scorpaena porcus*	Scorpionfish	Drachenkopf
Sciaenidae	*Sciaena umbra*	Brown meagre	Meerrabe
Synodontidae	*Synodus saurus*	Atlantic lizardfish	Atlantischer Eidechsenfisch
Congridae	*Conger conger*	European conger	Meeraal

Data Analysis

Fish Abundance and fish density

For estimating the fish abundance in the transect, we used the values in Table 2 for the different abundance categories (A-D). The abundances were then summed up for each fish family across the whole transect or for each habitat individually, and the total abundance across all fish families was summed up for each individual habitat.

Fish density was calculated by dividing the abundance of the individual fish family by the respective habitat area, and by dividing the total fish abundance within the individual habitat by the habitat area (Formula 1). We then calculated the fish abundance of the individual fish families for each time point to visualize the fish abundance over the course of the day.

Table 2: Categories of fish abundance

Category	Number of observed individuals	Conversion factor used for calculations
A	1	1
B	2-5	3
C	6-30	20
D	>30	60

Fish Biomass

For each fish species, the size of small and big individuals and the ratio between those among the observed fish along the transects was estimated (Table 3). The function "species-specific length-weight-relationships" provided by FishBase was used for estimating the weight of the differently sized fish. The weight of small and big individuals was then multiplied by their ratio and the results were summed for each species (see Formula 2). This average weight was multiplied by the abundance (see Formula 3). Eventually, the total fish biomass for each family was calculated to visualize the contribution of the different fish families to the total fish biomass. The calculation was repeated without considering the ratio between small and big species by just using the common length found at FishBase for comparing two different approaches (see Formula 4).

We also calculated the total fish biomass per square meter for the different habitats by dividing the family biomass within the habitat by the habitat area (see Formula 5). We used the result to compare how the biomass per square meter differs between the four habitat types.

Formula 1: Fish Density

$$\frac{Family\ abundance}{area\ in\ m^2}$$

Formula 2: Average observed weight

$$(Small\ fish\ weight * Small\ fish\ percentage) + (Big\ fish\ weight * Big\ fish\ percentage)$$

Formula 3: Biomass ratio

$$Average\ observed\ weight * Abundance$$

Formula 4: Biomass common length

$$Common\ length\ weight * Abundance$$

Formula 5: Biomass in g/m²

$$\frac{Family\ biomass}{area\ in\ m^2}$$

Table 3: 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 /GEOMAR Kiel in 2023

Observed species	**small individuals' length [cm]**	**big individuals' length [cm]**	**common length Fishbase [cm]**	**Ratio small/big individuals**
Oblada melanura	2,5	14	20	7:3
Diplodus sargus	10	25	22	3:7
Diplodus annularis	11		13	1:0
Diplodus puntazzo	11	17	30	1:4
Diplodus vulgaris	10	20	22	7:3
Sarpa salpa	6	23	30	1:1
Spondyliosoma cantharus	14		30	1:0
Boops boops	11		20	1:0
Coris julis	9	16	20	3:2
Thalassoma pavo	8	17	20	9:1
Labrus merula	17		40	1:0
Labrus viridis	20		37,5	1:0
Symphodus tinca	7	20	25	4:1
Symphodus melanocercus	9		11	1:0
Symphodus mediterraneus	6		12	1:0
Symphodus roissali	8		12	1:0

Symphodus rostratus	8		10	1:0
Oedalechilus labeo	8	24	25	7:3
Atherina boyeri	4		15	1:0
Atherina hepsetus	5		15	1:0
Serranus scriba	7	17	25	3:2
Apogon imberbis	8		10	1:0
Mullus surmuletus	10	17	25	9:1
Chromis chromis	1	6	13	7:3
Muraena helena	100		80	1:0
Scorpaena sp.	13		30	1:0
Sciaena umbra	9	20	28	1:1
Synodus saurus	18		20	1:0
Conger conger	100		100	1:0

Results

Fish Species List

Since the first Calvi course in 1992, 110 fish species were identified in total, which are listed in Table 4. In this year's course, we were able to identify more than half of them with 63 fish species. Two fish species were newly added to the list: *Tripterygion delaisi, Tripterygion melanurus*. In return, compared to 2021, 6 species were not sighted in 2023. The small red scorpionfish, *Scorpaena notata,* was not observed while being observed in the last 4 field courses on Corsica. In addition, the comber *Serranus cabrilla* was absent, but was sighted in all the last field courses. The 4 other species not sighted are cryptobenthic fishes, either blennies or gobies (*Coryphoblennius galerita, Parablennius rouxi, Gobius cobitis*, and *Gobius fallax*). This decline of cryptobenthic fishes was in general very obvious in this year's course.

Table 4: 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 /GEOMAR Kiel since 1996; species marked red haven't been found though consistently present throughout the last courses; species marked green were documented for the first time

Species	2023	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	x				
Pteroplatytrygon violacea									x					
Myliobatis aquila									x	x	x			
Muraena helena	x	x	x	x	x	x	x	x	x	x	x	x	x	
Conger conger	x	x			x			x		x		x	x	
Anguilla anguilla		x	x						x	x		x	x	
Engraulis encrasicolus			x		x				x					
Synodus saurus	x	x			x		x							
Phycis phycis	x		x	x	x				x		x			
Gaidropsarus mediterraneus		x												
Oedalechilus labeo	x	x	x	x	x	x	x	x	x	x	x			

Liza aurata	x	x	x			x	x	x	x	x	x	x	x	
Atherina boyeri	x	x	x	x	x	x	x	x	x	x	x	x	x	
Atherina hepsetus	x	x	x	x	x	x	x	x	x	x	-	x		
Belone belone	x	x	x	x	x	x	x	x	x		x			
Hippocampus guttulatus													x	
Syngnathus typhle											x			
Dactylopterus volitans	x	x					x							
Scorpaena porcus	x	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		x	
Chelidonichthys sp.										x				
Dicentrarchus labrax	x	x		x			x			x				
Epinephelus marginatus	x	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	x	
Anthias anthias						x					x			
Apogon imberbis	x	x	x	x	x	x	x	x	x	x	x	x	x	
Seriola dumerili	x		x	x	x				x	x				
Trachinotus ovatus	x		x						x					
Trachurus mediterraneus					x					x				
Pomadasys incisus							x							
Boops boops	x	x	x	x	x	x	x	x	x	x	x			
Dentex dentex	x	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	x
Diplodus puntazzo	x	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	x
Diplodus vulgaris	x	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		x	
Oblada melanura	x	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		x	
Pagrus pagrus											x			
Sarpa salpa	x	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	x
Spondyliosoma cantharus	x	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		x							
Sciaena umbra	x	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	x	
Chromis chromis	x	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	x
Labrus viridis	x	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	x
Symphodus cinereus	x	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	x		
Symphodus melanocercus	x	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	x

Symphodus roissali	x	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	x
Symphodus tinca	x	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	x	
Xyrichthys novacula	x		x	x	x									
Gymnammodytes cicerelus				x										
Trachinus araneus					x					x			x	
Trachinus draco	x	x	x	x	x	x	x	x	x					x
Trachinus radiatus					x									
Uranoscopus scaber			x						x					
Tripterygion delaisi	x													
Tripterygion melanurus	x													
Tripterygion tripteronotus	x	x	x	x	x	x	x	x	x	x	x		x	
Adiablennius sphinx	x	x	x	x	x	x	x	x	x		x		x	
Coryphoblennius galerita			x	x	x		x	x						
Lipophrys trigloides	x			x					x	x				
Microlipophrys dalmatinus								x						
Microlipophrys nigriceps					x		x	x	x	x				
Parablennius gattorugine	x	x	x	x	x				x	x	x		x	
Parablennius incognitus	x	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		x	
Parablennius zvonimiri	x	x	x	x	x	x	x	x	-	x		x	x	
Salaria fluviatilis	x	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		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		x	
Lepadogaster lepadogaster		x		x			x							
Opeatogenys gracilis						x			x	x				
Callionymus pusillus	x	x	x	x										
Sphyraena sphyraena		x												
Sphyraena viridensis	x	x	x	x	x	x	x	x	x	x				
Scomber colias			x											

Bothus podas	x	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: 110	63	66	62	68	64	49	61	60	66	65	53	35	46	18

Fish Transect

Fish Abundance and Density

In total, 30 fish species within 13 families were observed during the 30 hours of observation. Figure 3 shows the abundance of the different fish families within the transect. Sparidae are the most abundant family with more than 3000 individuals contributing 31,56 % to the total fish abundance. They are followed by Atherinidae with almost 2500 individuals (25,18 %) and Pomacentridae with around 2000 individuals (21,84 %).

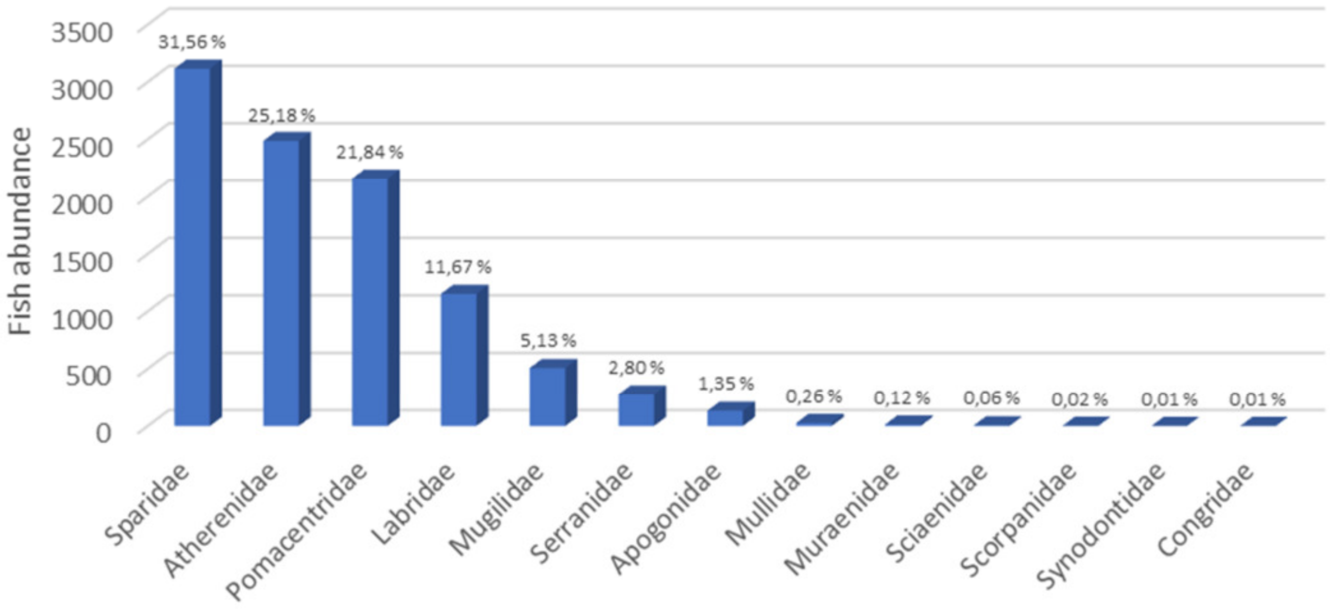

Figure 3: Total fish abundance of the observed fish families

All the families except for the Pomacentridae were most abundant in the last section, the harbor with the small boulders and soft sediment in between (Fig. 4). The Pomacentridae were most abundant in the harbor wall section. All other families were least abundant in the seagrass section as well, while the Pomacentridae were least abundant in the first section, the shallow small boulder field (Fig. 4). Regarding fish abundance in all habitats (Fig. 5) it is obvious that the habitat with most counted fish is the harbor with more than 3500 individuals (36% of all fish), followed by the harbor wall with 3046 individuals (31%), the small boulder field with around 2100 individuals (21%) and the seagrass habitat with the fewest fishes (1145 individuals, around 12%).

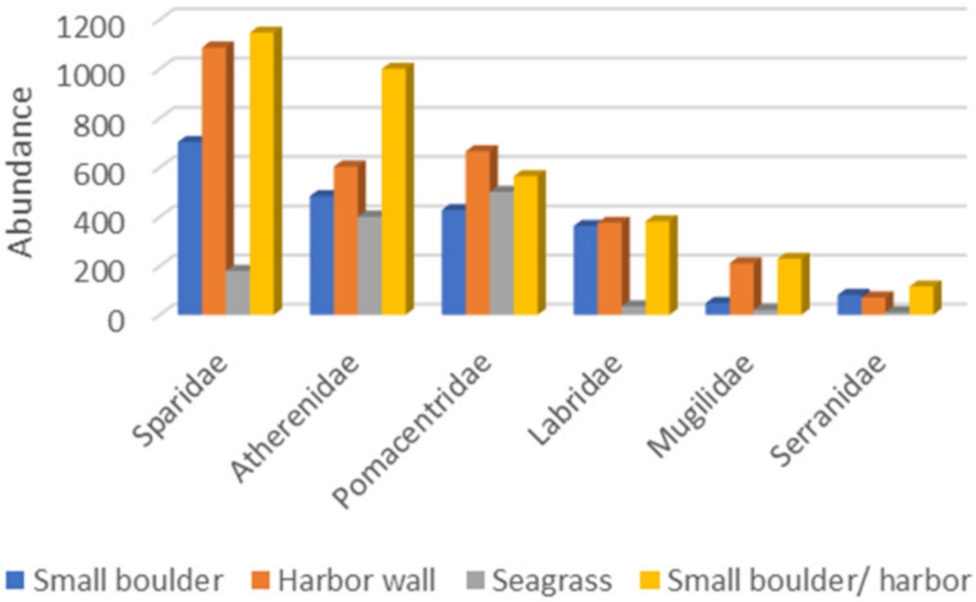

Figure 4: Fish abundance of the most abundant fish families by habitat

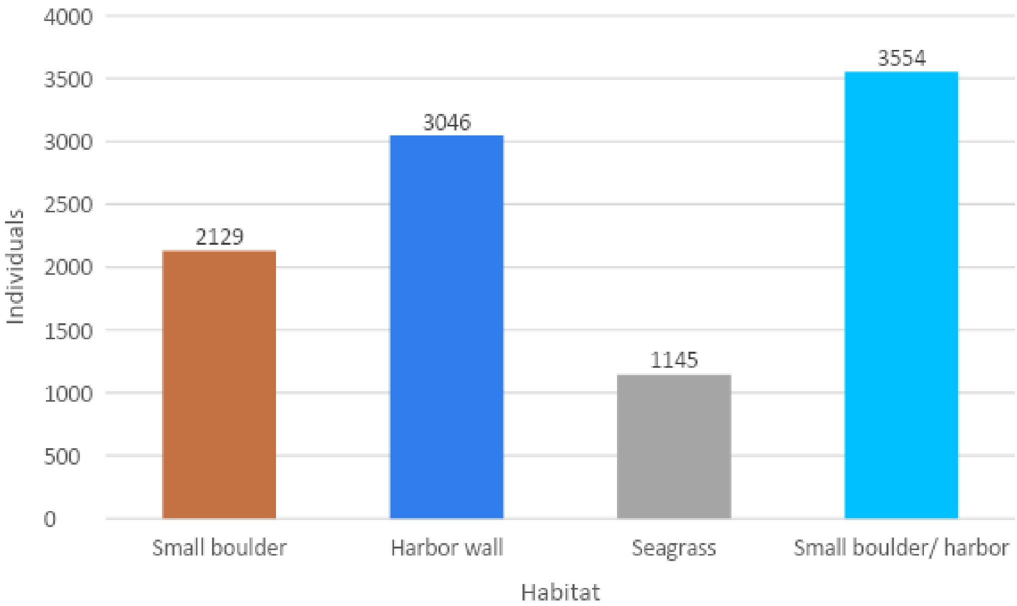

Figure 5: Fish abundance across all families within the different habitats

When assessing fish density – the number of individuals per square meter (Fig. 6, compare with Fig. 4) -- the following pattern emerges: Sparidae have the highest density at the harbor wall with around 4 individuals per square meter, while their highest abundance was observed at the harbor entrance.

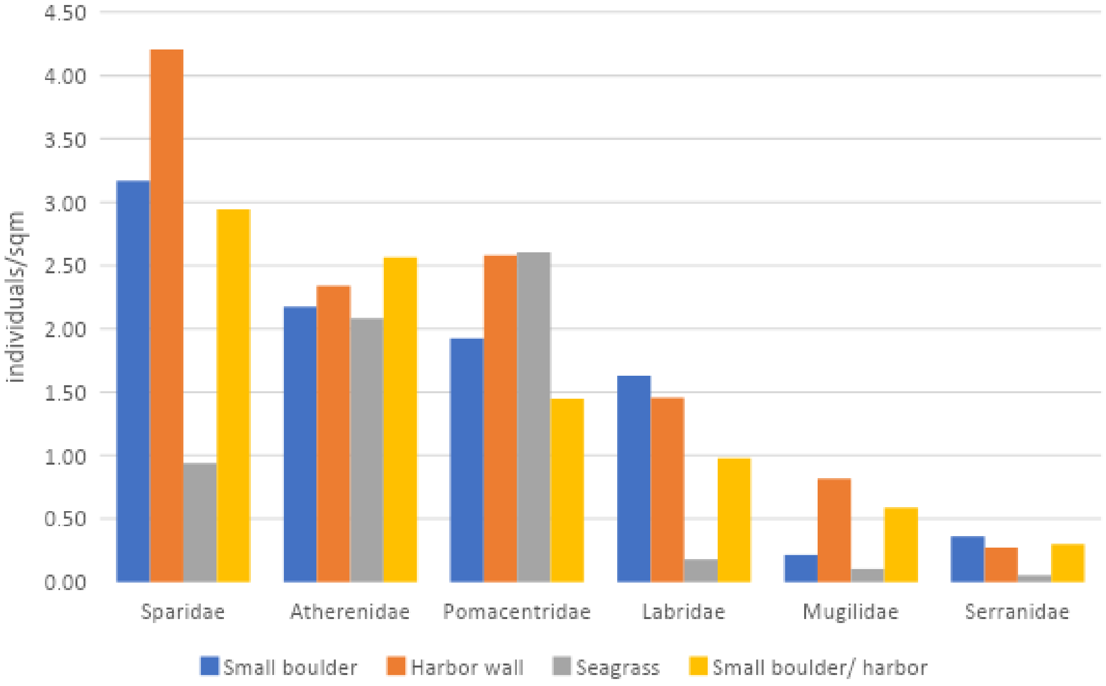

Figure 6: Density of most abundant fish families by habitat

Like the abundance, the density of the Atherinidae is highest at the harbor entrance with around 2.5 individuals per square meter. However, in contrast to their abundance, the density of the Atherinidae barely differs between the habitats.

The Pomacentridae (*Chromis chromis*) show the highest density in the seagrass and the lowest density in the harbor entrance with small boulders (2.5 vs. 1.5 individuals per square meters), while those two are the habitats with intermediate abundances of Pomacentridae.

When examining the fish density by habitat (Fig. 7), you can see that the harbor wall is the habitat most densely populated by fish with 12 individuals per square meter, followed by the small boulder field (almost 10 individuals per square meter) and the harbor entrance with small boulders (9 individuals per square meter) and the seagrass with 6 individuals per square meter, while the first two habitats were swapped for fish abundance (compare with Fig. 4).

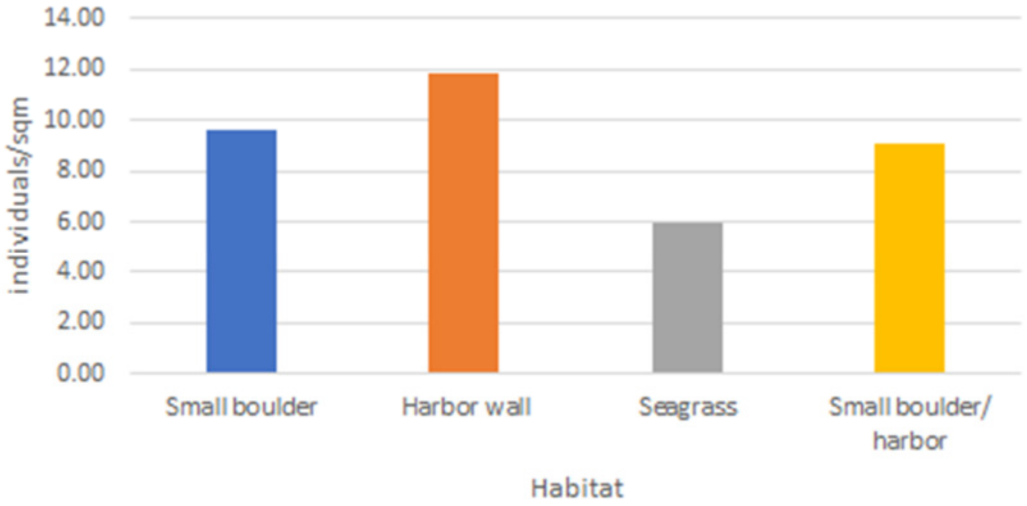

Figure 7: Fish density across all families within the different habitats

Abundance over the Course of the Day

The most abundant fish families occur in highest abundances during daytime (Fig. 8), where Sparidae reach abundances of up to 300 individuals for instance and are less abundant at night between 10 pm and 6 am, where the abundances of Pomacentridae and Labridae even drop to zero. Atherinidae are an exception to this and show highest abundances at nighttime, especially at 10 pm and 4 am, but also at the second observation at 2 pm.

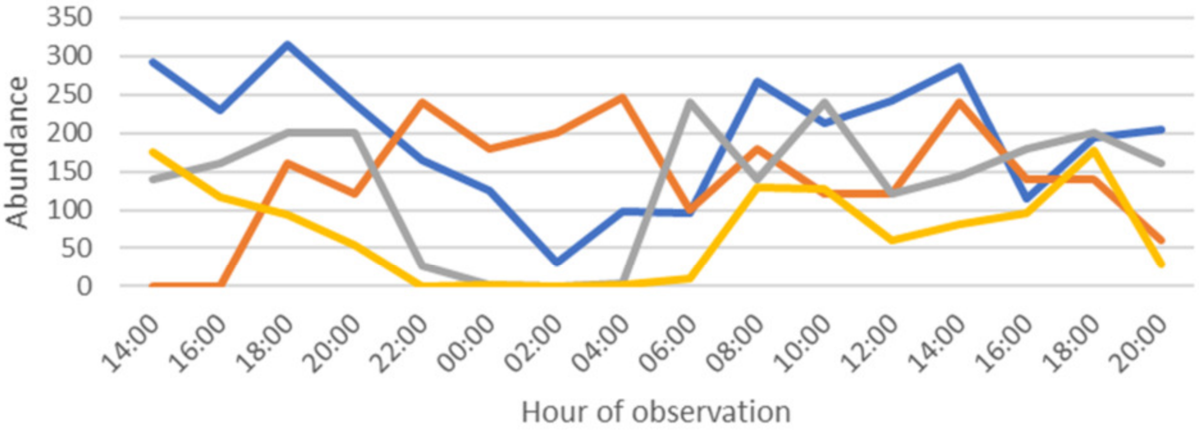

Figure 8: Fish abundance of most abundant families over the course of the day

The abundance of Mugilidae fluctuates strongly over the day with the highest peak at midnight (Fig. 9). Serranidae seem to appear in the late afternoon and early evening and show lowest abundances at nighttime. Most Apogonidae could be observed between 2 and 6 am (Fig. 9).

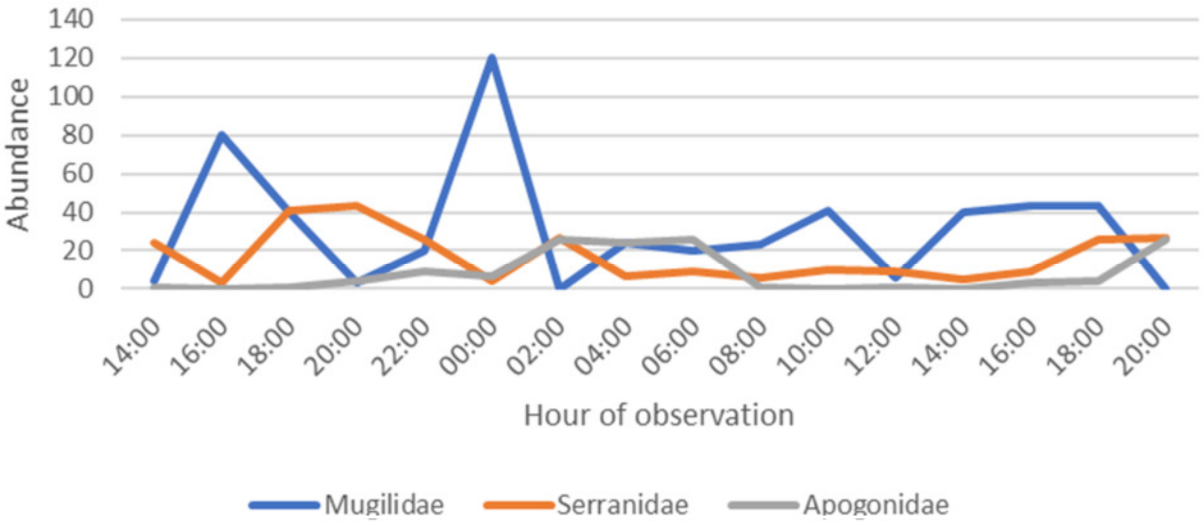

Figure 9: Fish abundance of intermediately abundant families over the course of the day

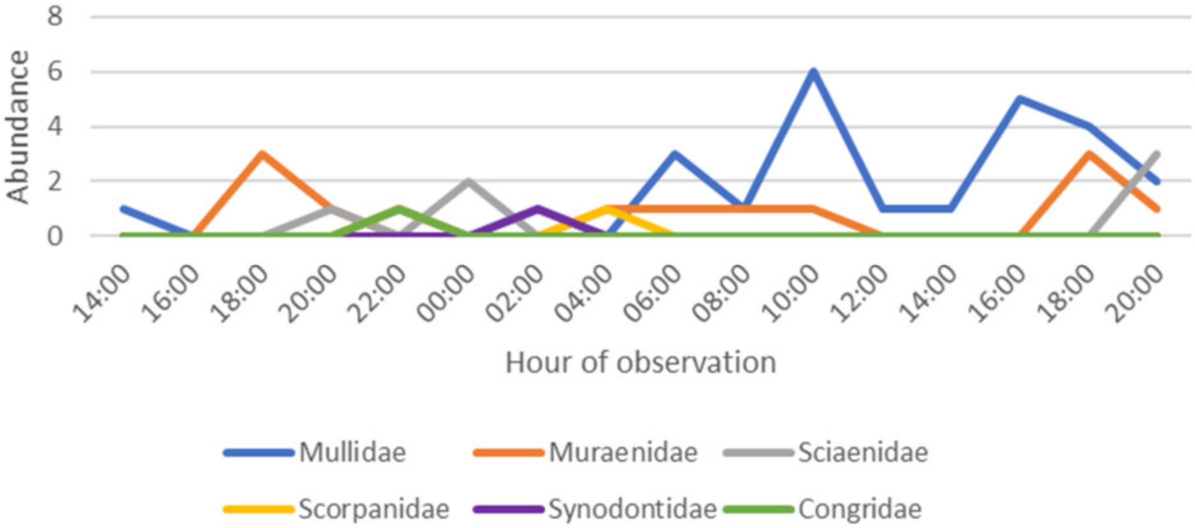

Figure 10: Fish abundance of least abundant families over the course of the day

One can barely make out diurnal patterns for the least abundant fish families (Fig. 10), as their abundances change by very few individuals only. Mullidae, however, seem to appear from the early morning (4 am) to the evening (8 pm), while Muraenidae were mostly observed in the early evening around 6 pm and in the early morning and before noon between 4 and 10 am. Sciaenidae were only observed in the evening and at night, and Synodontidae and

Scorpaenidae with only one observed individual each, only appeared at 2 and 4 am. Congridae also only appeared once during nighttime at 10 pm.

Fish Biomass

The fish biomass per square meter differs significantly between fish families. While abundant families with small individuals like Atherinidae and Pomacentridae contribute little to the total fish biomass in the transect and are hence excluded from Figure 11, the far less abundant Muraenidae with large individuals are among the five fish families with the highest biomass per square meter. Sparidae by far have the highest biomass per square meter with around 180 grams per square meter at the harbor entrance. They are followed by Labridae, Muraenidae, Mugilidae and Serranidae, which all display a comparable average biomass per square meter (10-30 grams per square meter). Each of the five families clearly shows the lowest biomass per square meter in the seagrass, while highest biomass per square meter was determined at the harbor entrance for Sparidae and Muraenidae, at the harbor wall for Mugilidae, and at the small boulder field for Labridae. This observation matches the biomass per square meter of all fish families combined (Fig. 12), which is highest at the harbor entrance (around 300 grams per square meter), followed by the harbor wall (slightly above 200 grams per square meter), the small boulder field (around 180 grams per square meter) and, with a great distance, the seagrass (about 20 grams per square meter).

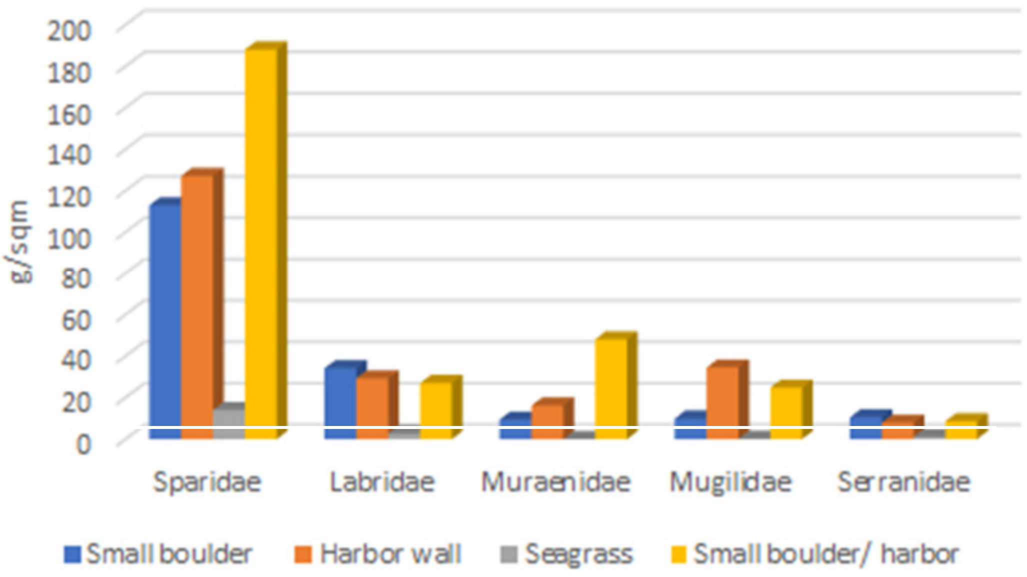

Figure 11: Biomass per area of most abundant fish families within the different habitats

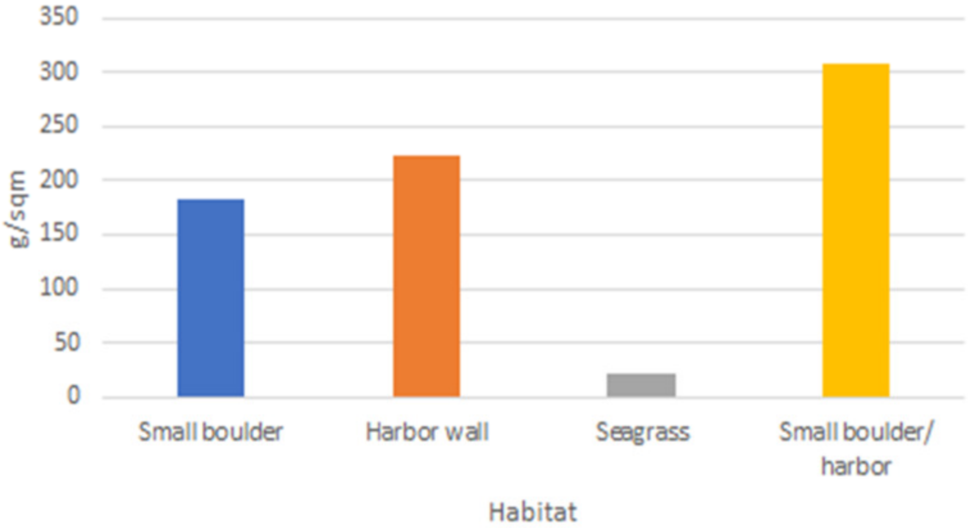

Figure 12: Biomass per area across all fish families within the different habitats

Sparidae contributed 67% to the total fish biomass calculated with around 135 kg (total fish biomass = 200.8 kg), followed by Labridae with 26.4 kg (13%), Mugilidae (21 kg or 11%), and Serranidae (8 kg; 4%). All other fish families only contribute 1% or less (Fig. 13). In the approach with the common length method, Sparidae make up 48% of the total biomass with 514 kg, Labridae have a contribution of 17% with 185 kg, and Muraenidae, Mugilidae, Pomacentridae, Serranidae and Atherinidae each contribute by a few percent (6 to 8%, 6 to 8 kg see Fig. 14).

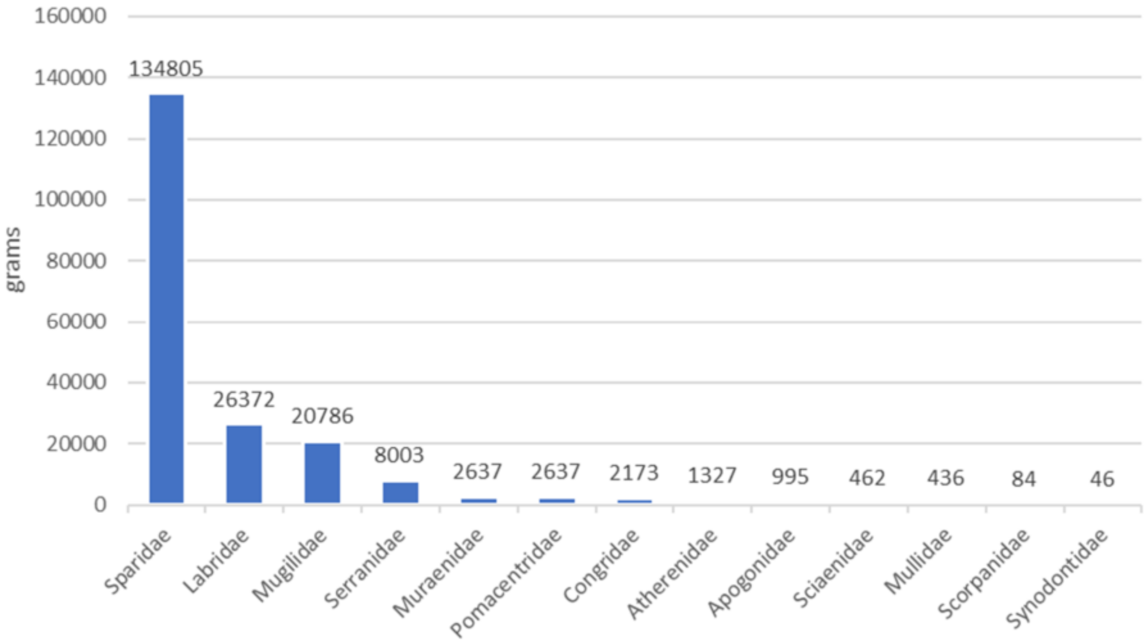

Figure 13: Total fish biomass of the observed fish families (using the observed weight)

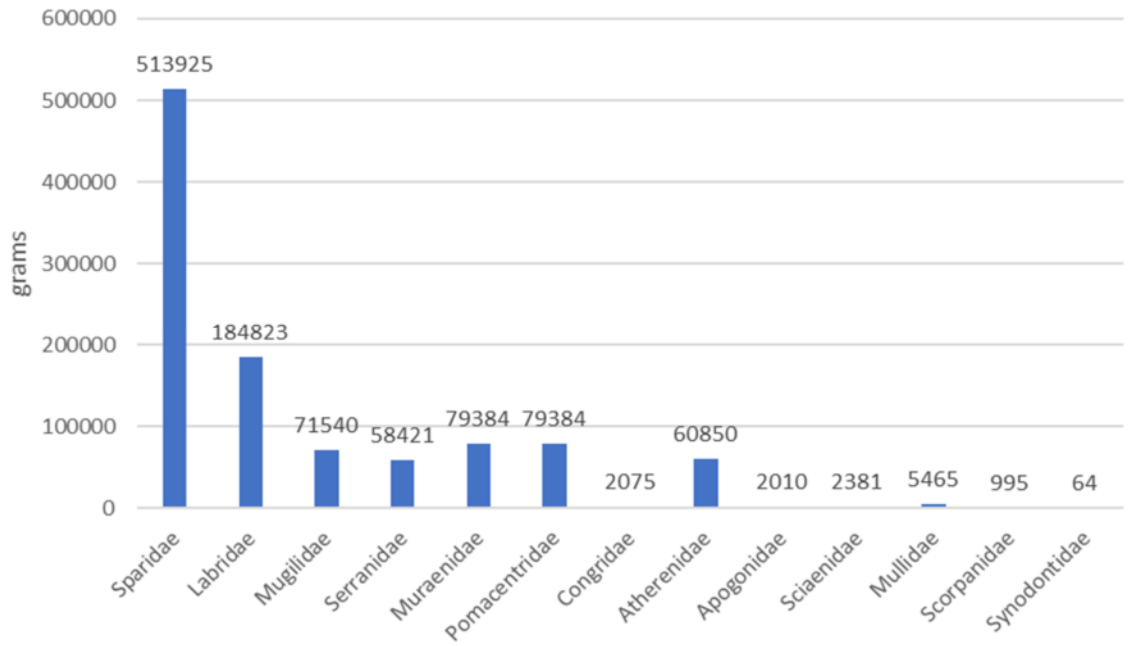

Figure 14: Total fish biomass of the observed fish families (using the common weight)

Discussion

Fish Species List

A decline in abundance of cryptobenthic fish can be seen in our data, for which some publications list reasons. The territorial behavior of blennies could lead to rapid community shifts due to strong spatial competition (Koblmüller et al., 2015; Pastor & Francour, 2010). In addition, stronger heat periods could induce migrations, e.g. to greater depths (Marengo et al., 2021). Nevertheless, the most likely reason for the seeming decline in cryptobenthic fish species we observed is the lack of attention they received in the recent field courses (R. Hanel, personal communication, August 2023). Consequently, the newly recorded black-faced blenny *Tripterygion delaisi* and *Tripterygion melanurus* simply could have been overlooked during the last field courses.

The disappearance of *S. cabrilla* is especially surprising when considering the similar ecology of the sympatric *S. scriba,* which remained common in the waters around STARESO (Vandewalle et al., 1999). One might hypothesize an increased competition, in which *S. scriba* is favored, which might result from declining prey for instance. There might be other factors that specifically impact the fecundity and survival of *S. cabrilla*, like decreased prey availability in general, or changes in physicochemical parameters. Similarly, the disappearance of the small red scorpionfish *S. notata* co-occurs with the first record of the closely related *S. scrofa.* One might also suspect a change in prey availability potentially connected to increased interspecific competition here. Ordines et al. (2017) highlight a correlation of algal biomass with the somatic condition and the abundance of *S. notata* in the Balearic Islands, which, in this study, seems to be explained by increased prey availability in red algae beds. Combined with the observed decline of coralligenous red algae close to STARESO, which comprise red algae beds to a large part, the possible linkage of the disappearance of *S. notata* to decreased prey availability seems more likely. However, the assessment of both the Corraligène and the fish population only appeared at very small spatial scales, and much more detailed studies would be required here.

Some general causes for the decline of the fish abundance that is observed over the years (R. Hanel, personal communication, August 2023) could be increased recreational fishing, pollution, increasing ocean temperature, and (associated) life history changes, with ocean temperature being the abiotic factor with the greatest impact on fish. Increasing temperatures due to global warming lead to more intense thermal stratification near the coast as well as longer and warmer summers in general. Both can lead to lower success in maturation, spawning, and egg survival of fish. As mentioned, temperature changes may also indirectly affect ecosystems by shifting populations' habitats and altering their community structure, as it induces fish migrations to climate refugia at different areas or greater depths (Marengo et al., 2021).

Transect

Abundance and Density

Unsurprisingly, swarm fishes like (juvenile) Sparidae, Atherinidae and Pomacentridae occurred in high abundances in the transect, as they, if present, come in large numbers and are spotted more easily than solitary fishes. That the density of fish was highest at the large boulders of the harbor wall might be accounted for by the fact that this habitat is relatively sheltered, offers many hiding places, and is less affected by fish traffic than the harbor entrance. The low fish abundance and density in the seagrass might be a result of potentially stronger currents in this less sheltered habitat, and fewer hiding places except from in the seagrass itself, where fishes then easily escape the observer's eyes. Fish observation in this habitat is further complicated by its depth and darkness. Furthermore, the feeding preferences of fish might deter them from the seagrass. Many of the observed species, such as many members of the Labridae and Sparidae, feed on epibionts and other organisms that are associated with rocks and macrophytes (Koblmüller et al., 2003; Osman & Mahmoud, 2009; Taieb et al., 2013a; Taieb et al., 2013; Pallaoro et al., 2003; Chaouch et al., 2014). Despite Sarpa salpa, whose herbivory we commonly observed, a significant contribution of seagrass to the diet is only reported for D. puntazzo (Havelange et al. 1997; Chaouch et al., 2013; Jadot et al., 2002). Neither S. salpa nor D. puntazzo did however appear in larger abundances over the seagrass compared to the other habitats. As relatively large and mobile fish they might exclusively go there for feeding. The Pomacentridae, with the damselfish Chromis chromis as sole representative, were the only fish family that displayed the highest density over the seagrass meadow. C. chromis might avoid swarms of larger fishes (Sparidae) that occupy the other habitats. In addition, the dark color of C. chromis might serve as good camouflage from predators from above over the dark seagrass. It might also be the planktivorous diet of C. chromis that attracts them to the seagrass, as they preferentially forage in well-lit open surface waters (Bracciali et al. 2012).

Abundance over the Course of the Day

The fact that some of the most abundant fish families (Sparidae, Pomacentridae and Labridae) clearly decreased in abundance during nighttime hints at diurnal behavior in which they feed during the daytime, in schools in the case of Sparidae and Pomacentridae, while they rest in isolation and hide at the seafloor and between rocks at night (Koblmüller et al., 2003, Bracciali et al., 2012. The disappearance of the Labridae was most prominent, they appear to hide deeper between stones than the other fish families. Some fish might also be deterred by the unexpected light from the diving torch, however, the fishes captured in the light beam were clearly resting and didn’t flee.

The increase of Apogonidae, namely *Apogon imberbis*, presumably results from their nocturnal feeding (Bussotti et al., 2018). The reason for the clear increase of Atherinidae at night remains unclear. They also appeared to be resting individually during the night, while forming large and close schools at daytime. A more even distribution of Atherinidae in the water might increase the chance of observing them in the transect, which might increase their abundance. Alternatively, their abundance might have been overestimated systematically due to their omnipresence. This is rather speculative though.

Especially for the less abundant fish families, it is rather difficult to infer conclusions about their feeding behavior and diurnal activity. This might require closer studies of the individual fish species.

Biomass

Sparidae contribute the most to the total fish biomass, as they did not only occur in high abundances in the transect, but also are of a relatively large body size. Except for Sparidae, only Labridae, Mugilidae, Serranidae and Muraenidae contribute to the biomass by a few percent. Though all except for Labridae occurred in rather little abundances, all four families have a relatively large body size in common. There is a non-linear, exponential relationship of the length and weight in fish, meaning that an increase in length corresponds to a disproportionately large increment of the weight (Bohnsack & Harper, 1988). This explains why the contribution of large fish species to the total biomass is disproportionately large in relation to their abundance. The opposite is true for small, highly abundant fish families like Pomacentridae and Atherinidae, which contribute little to biomass while having high abundances.

As evident in the results, the biomass calculations differ strongly when taking the presence of juvenile/small fish into account. Sparidae contribute significantly less to the total biomass with the common length method (48% vs. 67%), and the rest of the biomass is distributed more evenly among the other families. Also, the total values vary a lot (135 kg vs. 514 kg of Sparidae with the observed length vs. common length method, respectively). The common length method might lead to an overestimation of the biomass of many species, as the common length could be biased by the fact that only fish that is caught is used for deriving the common length. Small fish are however caught less easily, and hence, the common length itself could be overestimated. This might explain the higher contribution of small fish like Atherinidae and Pomacentridae to the total biomass as well as the higher total biomass derived from the common length method. Furthermore, we might have had a disproportionately large fraction of juvenile fish in the transect, as they might seek shelter near the coast. By estimating the length of the fish observed in the transect, we consequently might have achieved more accurate biomass estimations, as the common length method could be particularly inaccurate in this case. However, our method is strongly biased by the observers. Hence, the results obtained by this method applied by different people might be less intercomparable than the ones derived from the common length method. We therefore suggest that both methods will be used in the future, and that the results within each method are compared to see whether the method affects how well the results match.

Our calculated fish biomass values (accounting for juvenile/small individuals) are much higher than values in literature. Sala et al. (2012) found a maximum fish biomass of 118 grams per square meter in marine protected areas (shallow rocky reefs, 8-12 m depth) with the highest degree of protection (well-enforced protection, no-take reserves). McClanahan and Sala (1997) suggest a fish biomass of 41 grams per square meter for herbivorous (*S. salpa, D. puntazzo* and *D. sargus*) and 40 grams per square meter for invertivorous fish species (mostly Serranidae, Sparidae and Labridae also found in our transect). These numbers are derived from field data, the model used by the authors suggests around 50% lower values for both groups. In contrast, we calculated a total fish biomass ranging between 175 and more than 300 grams per square meter in three out of four habitats. The reasons for the low fish abundance and hence biomass in the seagrass habitat were discussed in the *Abundance and Density* section and will therefore be neglected here. With values between 10 and 30 grams per square meter, the biomass calculated for Serranidae and Labridae is comparable to the invertivorous fish biomass by McClanahan and Sala (40 grams per square meter). However, the calculated Sparidae biomass is up to four times higher than the invertivorous fish biomass of Clanahan and Sala (about 100-180 grams per square meter vs. 40 grams per square meter).

Several reasons may explain the much higher fish biomass we calculated. Firstly, the harbor around STARESO may serve as a refuge and attract high quantities of fish. The harbor is sheltered and except for occasional spearfishing, there are no fisheries. This would be in line with Sala et al. (2012), who found a strong correlation of the degree of protection and fish biomass. Secondly, the fish biomass at the harbor of STARESO may be incomparable to the biomass found in the study of Sala et al. (2012). The water depth in our transect barely reached 8 meters. In contrast, Sala et al. (2012) censused at depths between 8 and 12 meters, where less sheltering may explain lower fish densities and hence biomass. Lastly, fish biomass is shown to fluctuate seasonally, for instance due to recruitment of piscivorous fish in late summer (Sala et al., 2012). However, quantifying potential seasonal fluctuations of the fish biomass at STARESO would require further experiments.

We conclude that the deviation of our fish biomass from literature values may be a result of different census depths and may highlight the importance of shallow coastal waters as shelter for fish. However, larger-scale and longer-time experiments are required for solid conclusions.

Comparison with previous years

Note: It is not possible to achieve a complete overlap of the defined transects between the different years due to practical reasons. Therefore, transect length differs (137m in 2018, 155m in 2021, 177m in 2023), and hence also the extent of the four different habitats within the transect. This affects the comparability of the total numbers (biodiversity, abundance and total fish biomass).

When comparing the results of the fish transect experiment with the previous years (Hamer et al., 2018, Bioly & Nenning, 2021), some general patterns of the fish composition and distribution are quite similar. In all three years (2018, 2021, 2023), Sparidae represent the most abundant fish family, and the abundance of many fish families clearly declines during nighttime (most evident for Sparidae, Labridae and Pomacentridae). The abundance of Atherinidae, however, is highest around midnight in all three years. The diversity of fish is comparable with 29 species in 2018, 27 in 2021 and 30 in 2023. There is a high overlap of fish species between 2018 and 2023 (no list of fish species observed in the transect is available for 2021). A total of 35 fish species were observed in both years, with 25 species being common to both.

The contribution of the different fish families to the total fish abundance is also very similar between 2021 and 2023 with Sparidae making up around a third of the total abundance, followed by Atherinidae contributing about a quarter, Pomacentridae a fifth, and Labridae around 12 % to the fish abundance. However, while fish abundance was highest in the harbor followed by the harbor wall in 2023 (around 3500 vs. 3000 individuals), fish abundances were highest at the large boulders of the harbor wall in 2018 and 2021, followed by the harbor ("Harbor/Small boulder"). In 2018 and 2021, the abundances in both habitats barely differed though (around 2500 vs. 2000 individuals). It remains unclear why the total abundances increased and why the number of individuals was that much higher at the harbor compared to the harbor wall in 2023.

While the fish density was highest at the large boulders, followed by small boulders, the harbor and the seagrass in 2023, the small boulders were the most densely populated habitat in 2021, followed by the harbor, the large boulders and the seagrass eventually (fish density in 2023 (2021): large boulders: 12 (6) individuals per square meter; small boulders: 10 (8.5) individuals per square meter; harbor entrance 9 (8) individuals per square meter; seagrass: 6 (4.5) individuals per square meter). The fish density apparently increased in every habitat in 2023, most prominently at the large boulder field. An explanation of the shift of the densely populated habitats (from small boulders and harbor in 2021 to large and small boulders in 2023) could partially be explained by the termination of food scrap tossing by the station (personal communication with employees of STARESO, August 2023) However, the most prominent increase of fish density in all habitats lacks a comprehensible explanation.

A direct comparison of fish biomass between 2021 and 2023 would not be meaningful as the total numbers from 2021 are lacking and as different methods were used for calculation of the biomass per area. However, in both years, Sparidae clearly represent the fish family with the highest total biomass, followed by Labridae with some distance, matching their high abundances and relatively large body sizes. Both years also have in common that all other fish families contribute very little to the biomass.

Methodological Issues

There are some biases that unfortunately cannot be avoided during the assessment of a transect, for instance the individual swimming speed. Observers who swim more slowly might spot more fish. We therefore measured the swimming times through the transect to ensure that it took approximately the same amount of time for the different swimmers. Our average swimming time was 26 minutes with the maximum time of 34 minutes and a minimum time of 19 minutes. The variance of swimming times by more than 50% may have affected both, the abundance and number of fish species observed by different swimmers and therefore must be considered when interpreting the results. In addition, different people may identify the fish differently or incorrectly. Unfortunately, it is not possible to correct misidentifications that result from inexperience (Prato et al., 2017). As mentioned before, cryptobenthic fish are often underestimated in a transect, which is why we did not include them. In addition, individual fish behavior can lead to a bias in fish abundance, as shy fish are easily overlooked and fish with a low density may not be spotted at all in the transect and are therefore missing in the results (Caldwell et al., 2016; Marengo et al., 2021; Prato et al., 2017). To rule out this bias to a certain degree, the transect could be enlarged or the observation frequency and/or duration could be increased. Additionally, the transect method could be combined with other UVC methods that use cameras for instance (Prato et al., 2017).

Conclusion

Like in previous years, the most prominent change in fish diversity is the ongoing disappearance of crypto-benthic fish species like gobies and blennies. This is most likely a result of little attention that has been paid to these species in the last years.

In the fish transect, some minor changes in the fish distribution to different habitats occurred. However, the community structure does not appear to have undergone major changes, as the same fish families dominate the abundance (Sparidae, Atherinidae and Pomacentridae) and biomass (Sparidae, Labridae) as in previous years.

Though both projects do not represent a detailed stock assessment of the fish species found around STARESO, we did provide valuable observations for monitoring, as larger and potentially alarming changes will not go unnoticed as long as the field courses resume. Eventually, our projects foster countless interesting research questions about the observed fish species. It would be interesting to assess how they are affected by possible physico-chemical or ecological changes in the area. Furthermore, for many fish species, little is known about their feeding ecology and behavior. Given their importance in Mediterranean food webs and the strong impact climate change has on them, further research should aspire to close those gaps.

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Behavioural response of a Mediterranean fish community to different bait, changing light regime, and predator presence in a baited underwater video (BUV) study

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Abstract

This study investigates coastal fish biodiversity and behaviour in the Northwest Mediterranean Sea near Calvi, Corsica, using baited underwater videos (BUVs). We address questions regarding species' attraction to various bait and bait exposure, the impact of changing light regimes, and the presence of predators on feeding behaviour. Our findings revealed that BUVs attracted a diverse range of species, with sea urchin bait preferred during the day and fish bait at night. The species' occurrence and activity patterns varied at different times of day and the presence of a predator influences fish behaviour, although recognition remains uncertain. Despite some challenges, our research highlights the effectiveness of BUVs for studying coastal fish communities and provides valuable insights into their dynamics and interactions.

Keywords: fish behaviour, shallow rocky ecosystem, underwater videos, bait, Mediterranean Sea, feeding interaction

Introduction

Local knowledge about coastal fish biodiversity, interactions within the community and species-specific behavioural patterns is very important for conservation and management related issues (Cappo et al. 2003, Mittelbach et al. 2014). Besides the more commonly used methods, such as diver-based surveys, fishing and acoustic monitoring, underwater videos, with improving techniques, have been used increasingly for more than sixty decades to gain this knowledge (Mallet & Pelletier 2014). Nevertheless, Mallet & Pelletier (2014) reviewed underwater video techniques for the first time. In the Mediterranean Sea, the first observations via video were made in the 1970s (Fedra & Machan 1979). Whereas also amateur made underwater videos help to understand the appearance and distribution of fish in the Mediterranean Sea, e.g., non-indigenous species (Stern et al. 2019, Deidun et al. 2011), also baited underwater videos (BUV) contribute to this research (Mallet & Pelletier 2014). Baited underwater videos are also criticized for some disadvantages, but they are a good choice for studying spatial and temporal changes in fish assemblages and relative abundances of individual species within the community (Harvey et al. 2007). Being able to assess the health of a shallow rocky ecosystem by understanding the structure of the community and the interactions between species at diverse levels, helps to implement meaningful conservation measures to improve conditions for the whole community (Sala et al. 2012). Since species within a community might have different feeding preferences, BUVs could be useful to investigate individual behavioural responses to changes in the experimental set-up and bait type. The freshness of the bait and the odour plume, which determines the attraction radius, might change the results of the study.

While it was thought that anti-predator behaviour is mostly inherited, because fishes need to be able to recognize and escape a predator from the moment they hatch, predator-prey interactions are more complex than that and it is assumed that prey can learn about predators and accustom their behaviour accordingly (Kelley and Magurran, 2006). This allows prey to react to highly variable predation risk (Helfman, 1989) and to balance the risk against other activities that influence fitness, for example feeding (Sih, 1980, 1988). Most examples of associative learning combine alarm cues with predator odour, but prey can be conditioned to respond to visual cues from predators, too (Chivers & Smith, 1994). Previous studies showed that physical predator characteristics presented to prey species can induce anti-predator behaviour (Karplus et al. 1982).

Bosch et al. (2017) showed that the methodology influences the ability to detect biodiversity patterns. For local studies, underwater visual census is recommended due to the cost-efficiency, while BUVs are time-efficient and provide more detailed biodiversity information.

By using baited underwater videos, our study investigates the behavioural response of fish species in the local coastal community of the bay of the STARESO research station near Calvi, Corsica. With our study approach, we are addressing the following research questions: Which species can we attract with bait? How does bait type and bait exposure affect fish feeding behaviour? How does a changing light regime under water at different times of day affect the occurrence of species, their abundance and their behavioural response? How does the presence of a predator (predator dummy) affect the occurrence of the species, its abundance and its behavioural response?

Material and Methods

To observe the occurring fish species, their abundances & their feeding behaviour in the Mediterranean Sea, baited underwater videos (~30 minutes) were recorded using GoPro cameras. The study area was the harbor of the STARESO Institute near Calvi, Corsica. The video recordings took place between the 8th and 11th of August 2023, at different times of the day in order to attract different fish species. Table 1 lists the videos made and their conditions.

Over the four days of experimentation, the experimental approach changed in order to improve our experimental set-up, make more successful recordings and test different influencing factors. The first video experiments were made with eel traps (Figure 1a), which were mounted on a rack together with the GoPro cameras. To attract the fish, bait, fish scraps and crushed sea urchin, was placed in the traps. After four experimental runs, we improved our experimental set-up by building smaller racks without the traps and only the GoPro cameras and bait, on which the baits were placed loosely, or with the help of ropes (Figure 1b). Our racks were attached to buoys with lines.

With this experimental set-up, we carried out BUV recordings in a time series and investigated the influence of different light regimes at different times of day. To differentiate between the light regimes, we decided to label the videos after lunchtime, the “midday” videos with strong sunlight, the “afternoon” videos with already less strong sunlight, the “evening” videos where our study side was exposed in the shade, and the “night” videos in the dark.

On the last experiment day, we wanted to observe the influence of a present predator and attached on our racks a fish dummy of the predatory *Dentex dentex* and *Epinephelus marginatus* (Figure 1c). The GoPro racks were deployed by snorkeling. The snorkelers left the camera site and returned after more than 30 minutes to collect the racks, secure the videos and prepare the set-up for the next run. In each experiment there were two replicates (fish bait vs. sea urchin, present predator vs. absent predator). Thus, always two GoPro racks were deployed at an appropriate non-impacting distance from each other, in the same water depth. For our analysis, we identified the species appearing in the videos and the type of interaction they showed ("no" interaction, "interest" interaction or "feeding" interaction). We recorded the number of individual fish appearing per species per video and additionally categorised the numbers into four abundance classes (A= 1, B= 3-5, C= 6-30, D= >30 individuals).

Table 5: Analysed bait underwater videos. Our three main studies were categorised into primary, secondary and tertiary experiments and the experiment names indicate which videos were included for which experiments. Information on the day, time, depth, experimental set-up, bait type and predator are also listed.

Primary experiment	Secondary experiment	Tertiary experiment	Date	Time	Depth	Setup	Bait type	Predator
Bait type/exposure	-	-	09.08.2023	15:50	5,5	Trap	Fish	-
Bait type/exposure	-	-	09.08.2023	15:50	5,5	Trap	Seaurchin	-
Bait type/exposure	-	-	09.08.2023	18:30	7	Trap	Fish	-
Bait type/exposure	-	-	09.08.2023	18:30	7	Trap	Seaurchin	-
Bait type/exposure	-	-	10.08.2023	11:11	7	No trap	Fish	-
Bait type/exposure	-	-	10.08.2023	11:11	7	No trap	Seaurchin	-
Predator presence/absence	-	-	10.08.2023	13:45	7	No trap	Seaurchin	Epinephelus marginatus
Predator presence/absence	Time series	-	10.08.2023	13:45	7	No trap	Seaurchin	-
Predator presence/absence	-	-	10.08.2023	16:45	4,5	No trap	Seaurchin	Epinephelus marginatus
Predator presence/absence	Time series	-	10.08.2023	16:45	4,5	No trap	Seaurchin	-
Predator presence/absence	-	-	10.08.2023	19:04	4,5	No trap	Seaurchin	Epinephelus marginatus
Predator presence/absence	Time series	-	10.08.2023	19:04	4,5	No trap	Seaurchin	-
Predator presence/absence	-	Bait type/exposure	10.08.2023	21:05	4,5	No trap	Fish	Epinephelus marginatus
Predator presence/absence	Time series	Bait type/exposure	10.08.2023	21:05	4,5	No trap	Fish	-
Predator presence/absence	Time series	Bait type/exposure	10.08.2023	21:45	4,5	No trap	Fish	-
Predator presence/absence	-	-	11.08.2023	14:35	4,5	No trap	Seaurchin	Dentex dentex
Predator presence/absence	Time series	-	11.08.2023	14:35	4,5	No trap	Seaurchin	-
Predator presence/absence	-	-	11.08.2023	18:30	4,5	No trap	Seaurchin	Dentex dentex
Predator presence/absence	Time series	-	11.08.2023	18:30	4,5	No trap	Seaurchin	-

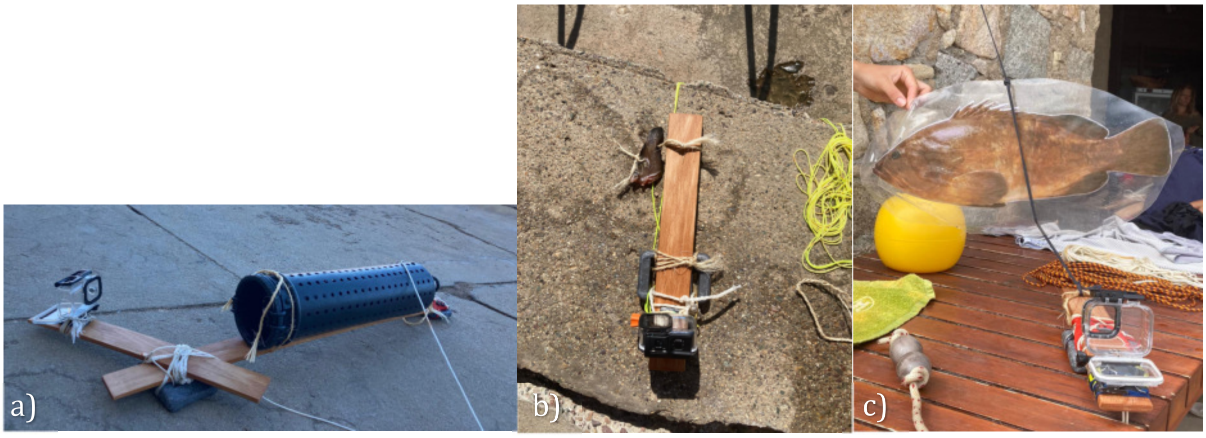
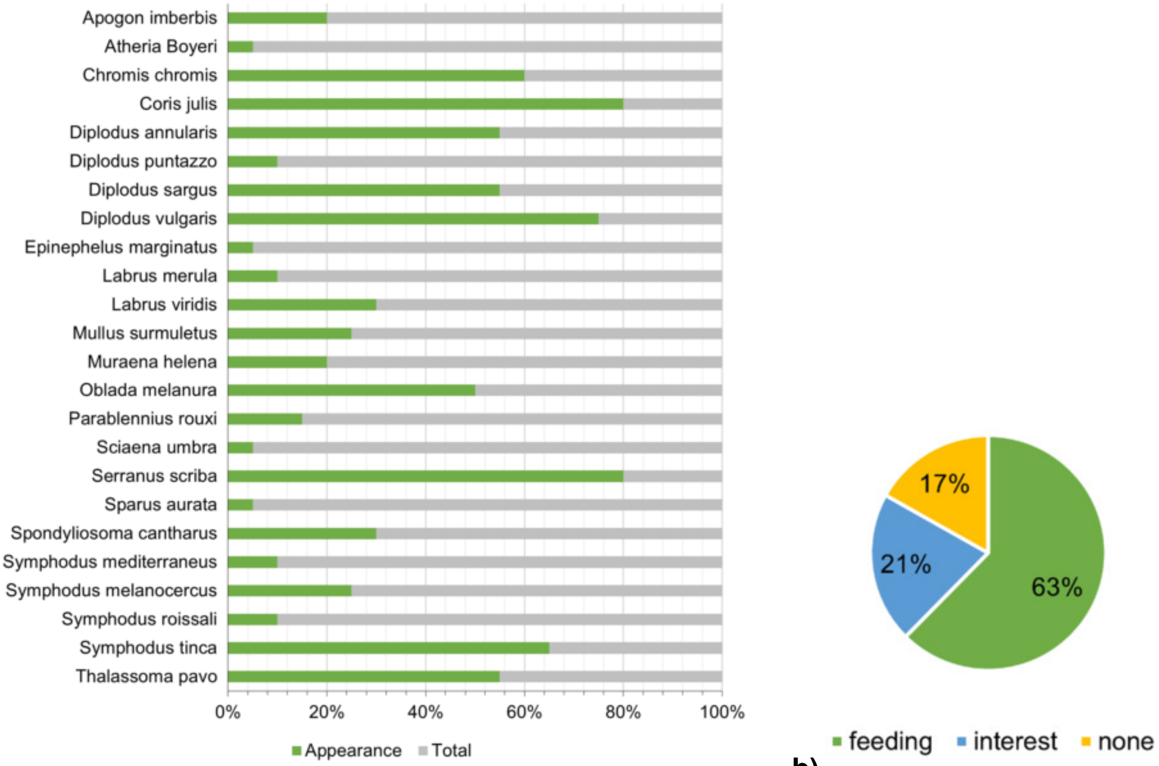


Figure 28: a) First experimental set-up, with eel trap, GoPro camera protection cover, weights and ropes with buoys. **b)** Improved experimental set-up including camera case, weight, bait, ropes and buoy. **c)** Experimental set-up with predator dummy.

Results

Species composition

In total, 24 species appeared in front of the cameras (Figure 2a). The most abundant five species appeared in 60% to 80% of the videos (*Chromis chromis*, *Coris julis*, *Diplodus vulgaris*, *Serranus scriba*, *Symphodus tinca*). Four species appeared in 40% to 60% of the videos (*Diplodus annularis, Diplodus sargus, Oblada melanura, Thalassoma pavo*), six species occurred in 20% to 40% of the videos (*Apogon imberbis, Labrus viridis, Mullus surmuletus, Muraena helena, Spondyliosoma cantharus, Symphodus melanocercus*), and nine species were seen in less than 20% of the videos (*Atherina boyeri, Diplodus puntazzo, Epinephelus marginatus, Labrus merula, Parablennius rouxi, Sciaena umbra, Sparus aurata,* *Symphodus mediterraneus, Symphodus roissali*). It differed among species, how many individuals were seen and which interaction type was shown. The majority of species that were detected in front of the cameras (63%) were feeding (figure 2b). 21% showed interest in the set-up, and only 17% of the fish that appeared were neither feeding nor showed interest.



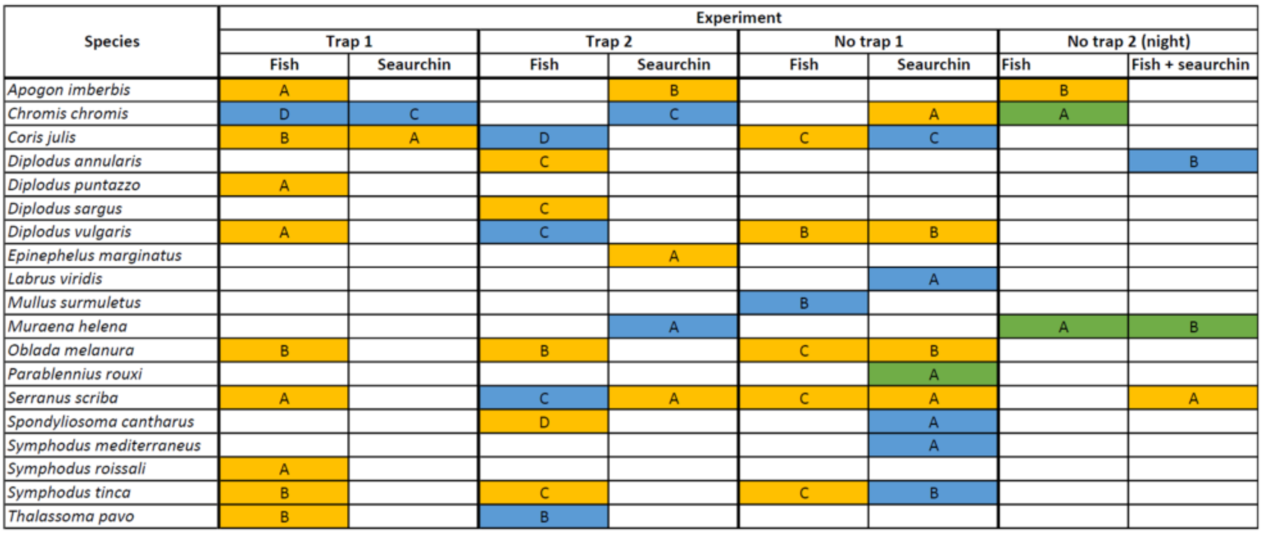
a) **b)**

Figure 29: a) Relative appearance of fish species in the videos. 1,0 if the species was seen in all videos (20 out of 20 videos). **b)** Relative number of species showing the corresponding interaction types. Averaged over all videos.

Bait type and bait exposure

Four experiments were carried out to test which bait type (fish or sea urchin) and which bait exposure (eel trap or no trap) are preferred by the fish community. In two experiments, eel traps were used, while in the others, the bait was presented openly. Per experiment, two camera set-ups with either fish or sea urchin (or a combination of both at night) were deployed. The abundance classes and interaction types of fish species in the different experiments are displayed in table 2. From the outcome of the set-ups with the eel trap it can be seen that a higher number of species appeared when fish was used as bait. However, most of the species were not interested. Without the eel trap, the picture changes. Many more species showed interest in the sea urchin-baited set-up than in the fish-baited one, and one species (*Parablennius rouxi*) was even feeding on the sea urchin. Due to previous observations regarding *Muraena helena* presence at night, we chose to take a combination of both bait types in one of the nightly videos ("Sea urchin (+fish)"). While *Muraena helena* and *Chromis chromis* fed on the fish at night, *Diplodus annularis* only arrived after sea urchin was added to the set-up.

Table 6: Abundance classes and interaction types of fish species in the bait type and exposure experiments. Abundance classes: A = 1, B = 2-5, C = 6-30, D = >30 individuals. Interaction types: green = feeding, blue = interest, yellow = none.



Species	Experiment							
	Trap 1		Trap 2		No trap 1		No trap 2 (night)	
	Fish	Seaurchin	Fish	Seaurchin	Fish	Seaurchin	Fish	Fish + seaurchin
Apogon imberbis	🟨 A			🟨 B			🟨 B	
Chromis chromis	🟦 D	🟦 C		🟦 C		🟨 A	🟩 A	
Coris julis	🟨 B	🟨 A	🟦 D		🟨 C	🟦 C		
Diplodus annularis			🟨 C					🟦 B
Diplodus puntazzo	🟨 A							
Diplodus sargus			🟨 C					
Diplodus vulgaris	🟨 A		🟦 C		🟨 B	🟨 B		
Epinephelus marginatus				🟨 A				
Labrus viridis						🟦 A		
Mullus surmuletus					🟦 B			
Muraena helena				🟦 A			🟩 A	🟩 B
Oblada melanura	🟨 B		🟨 B		🟨 C	🟨 B		
Parablennius rouxi						🟩 A		
Serranus scriba	🟨 A		🟦 C	🟨 A	🟨 C	🟨 A		🟨 A
Spondyliosoma cantharus			🟨 D			🟦 A		
Symphodus mediterraneus						🟦 A		
Symphodus roissali	🟨 A							
Symphodus tinca	🟨 B		🟨 C		🟨 C	🟦 B		
Thalassoma pavo	🟨 B		🟦 B					

Figure 3 shows the relative relationship of interaction types in the different bait type and exposure videos using percentages of species numbers. Seen in this context, sea urchin was the most preferred bait in almost all of the videos, because most of the species in the fish-baited set-up were not interested. The nightly fish-baited set-up is the only exception.

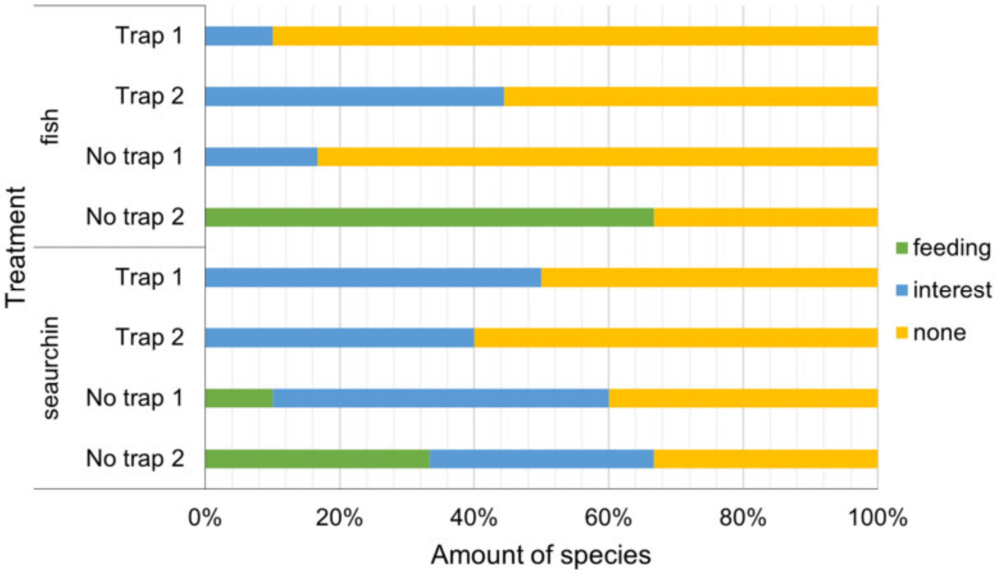
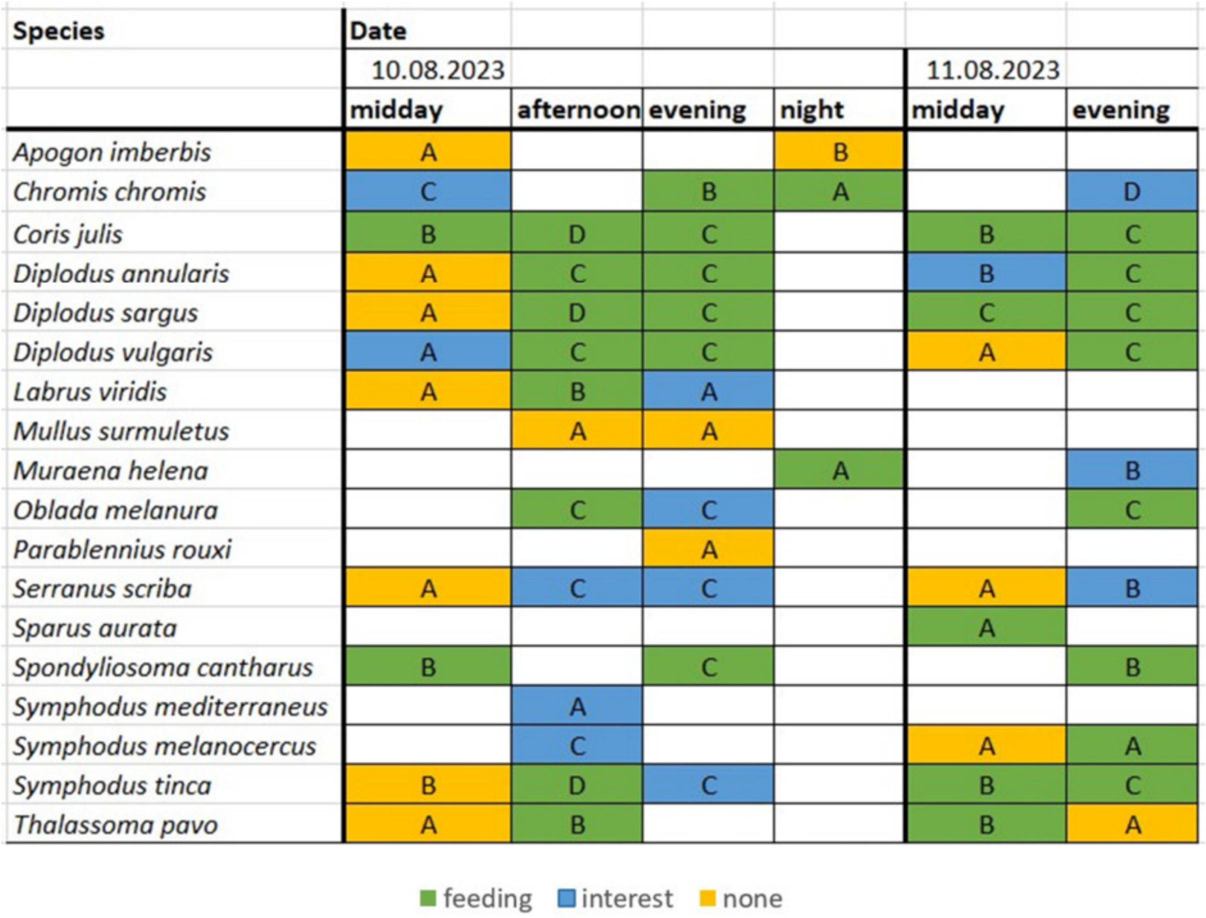


Figure 30: Relative relationship of interaction types in the different bait type and exposure videos using percentages of species numbers.

Light regime (time series)

A total of 18 species were attracted in our time series experiments (Table 3). Some of them, *Coris julis, Diplodus annularis, Diplodus sargus, Diplodus vulgaris, Serranus scriba, Symphodus tinca*, appeared always when light was present regardless of whether there was midday, afternoon, or evening light on both days. *Mullus surmuletus, Muraena helena, Oblada melanura, Parablennius rouxi* and *Symphodus mediterraneus* were never seen during midday. Although *P. rouxi* and *S. mediterraneus* were only seen once in all the videos. *M. helena* was only seen in the evening or at night, when the species also showed a very aggressive predator/scavenger behaviour. The night was different from the other times of the day, as only three species (*M. helena, Apogon imberbis, Chromis chromis*) were sighted here and also only a few individuals in the abundance classes A and B. All other species exhibited no particular light regime pattern.

Table 7: Occurring fish species by day and daytime (midday, afternoon, evening and night) in the seven videos of the time series. Letters indicate the abundance classes (A= 1, B= 3-5, C= 6-30, D= >30 individuals) and colors the interaction types (yellow = "none", blue = "interest", green = "feeding") of the fish species that appeared.



Species	**Date**					
	10.08.2023				11.08.2023	
	midday	**afternoon**	**evening**	**night**	**midday**	**evening**
Apogon imberbis	🟨 A			🟨 B		
Chromis chromis	🟦 C		🟩 B	🟩 A		🟦 D
Coris julis	🟩 B	🟩 D	🟩 C		🟩 B	🟩 C
Diplodus annularis	🟨 A	🟩 C	🟩 C		🟦 B	🟩 C
Diplodus sargus	🟨 A	🟩 D	🟩 C		🟩 C	🟩 C
Diplodus vulgaris	🟦 A	🟩 C	🟩 C		🟨 A	🟩 C
Labrus viridis	🟨 A	🟩 B	🟦 A			
Mullus surmuletus		🟨 A	🟨 A			
Muraena helena				🟩 A		🟦 B
Oblada melanura		🟩 C	🟦 C			🟩 C
Parablennius rouxi			🟨 A			
Serranus scriba	🟨 A	🟦 C	🟦 C		🟨 A	🟦 B
Sparus aurata					🟩 A	
Spondyliosoma cantharus	🟩 B		🟩 C			🟩 B
Symphodus mediterraneus		🟦 A				
Symphodus melanocercus		🟦 C			🟨 A	🟩 A
Symphodus tinca	🟨 B	🟩 D	🟦 C		🟩 B	🟩 C
Thalassoma pavo	🟨 A	🟩 B			🟩 B	🟨 A

🟩 feeding 🟦 interest 🟨 none

Comparing the interaction types between the different light regimes, feeding was the main interaction in the afternoon with around 90%, and during evening light with around 80% (Figure

4). If no feeding were exhibited, at least the species were mostly interested. Responsible for the high feeding activity were the three *Diplodus* species, *C. julis*, *S. tinca*, which were feeding on the bait in the afternoon and on both days during the evening. During midday, the activity of these species varied between the three interaction types and at night they were not present at all. Whenever the species, *C. julis* and *Spondyliosoma cantharus*, were present, they always fed on the bait. *O. melanura* seems to be a bream that only reacted to the bait and appeared in the afternoon and evening. At night *M. helena* (one individual) and *C. chromis* (one individual) were feeding on the fish bait, whereas several *A. imberbis* were appearing but without interest for the bait. At midday the majority (around 40%) of the fish seen expressed an interest in the bait, a slightly smaller proportion fed on the bait, while around 20% were also present but displayed no interest. The species *A. imberbis* (only present at midday and night), *M. surmuletus* and *P. rouxi* (only present at afternoon and evening) only appeared in the surrounding area of the bait, without any interaction, whereas *S. scriba* and *S. melanocerus* displayed at least some interest.

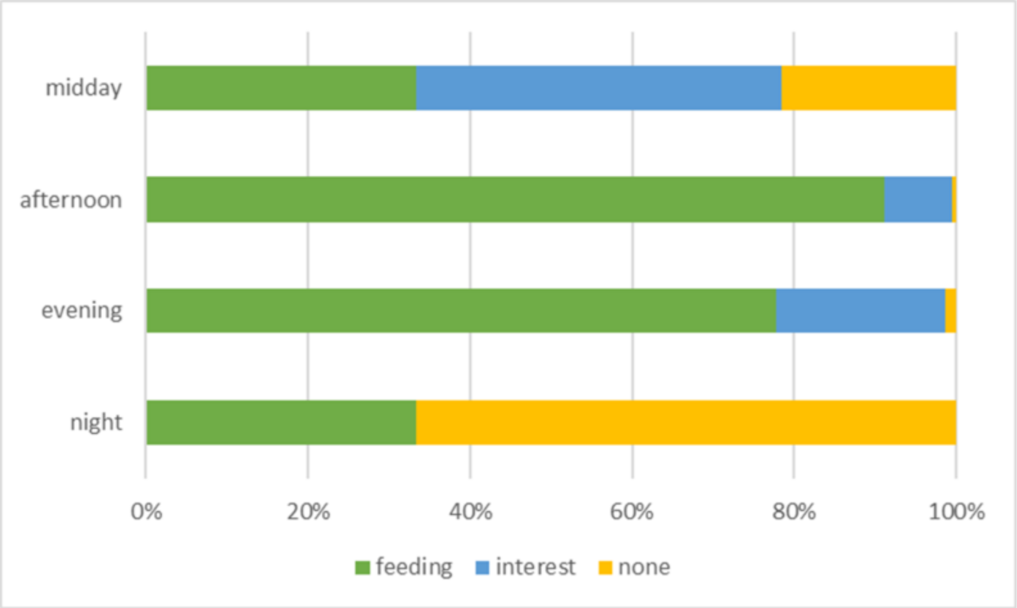


Figure 31: Percentage of interaction types +(yellow = "none", blue = "interest", green = "feeding") performed across all species that appeared in the seven time series videos separated by daytime (noon, afternoon, evening, and night).

Predator presence/absence

A total of 20 species appeared at the set-up with the predator (Figure 5), while 19 species were recorded at the site without a predator (Figure 6). Apart from a few differences, the species composition is very similar. The species *Atheria boyeri*, *Sciaena umbra*, *Diplodus puntazzo*, *Symphodus roissali* were recorded only at the site with predator, while the species *Apogon imberbis*, *Symphodus mediterraneus* and *Sparus aurata* were exclusively seen at the site without a predator.

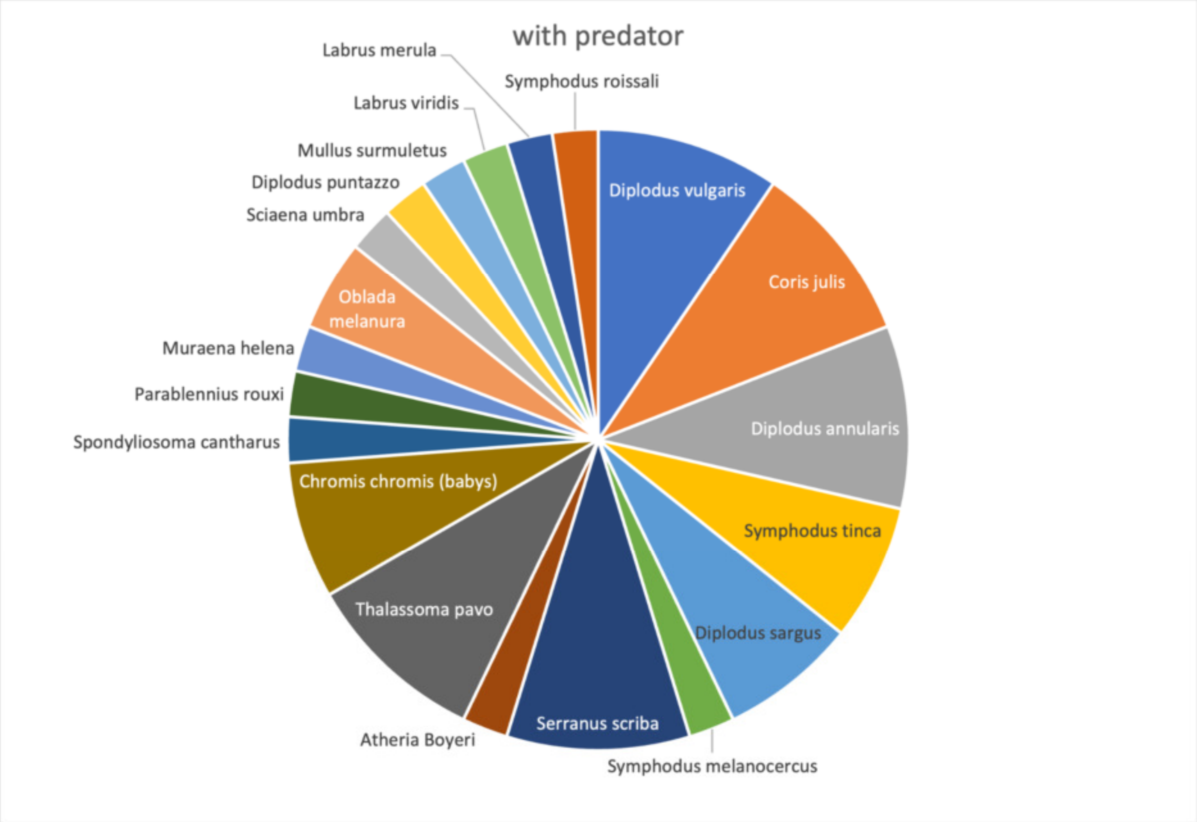


Figure 32: Appearing fish species in the experiment set-up with the predator.

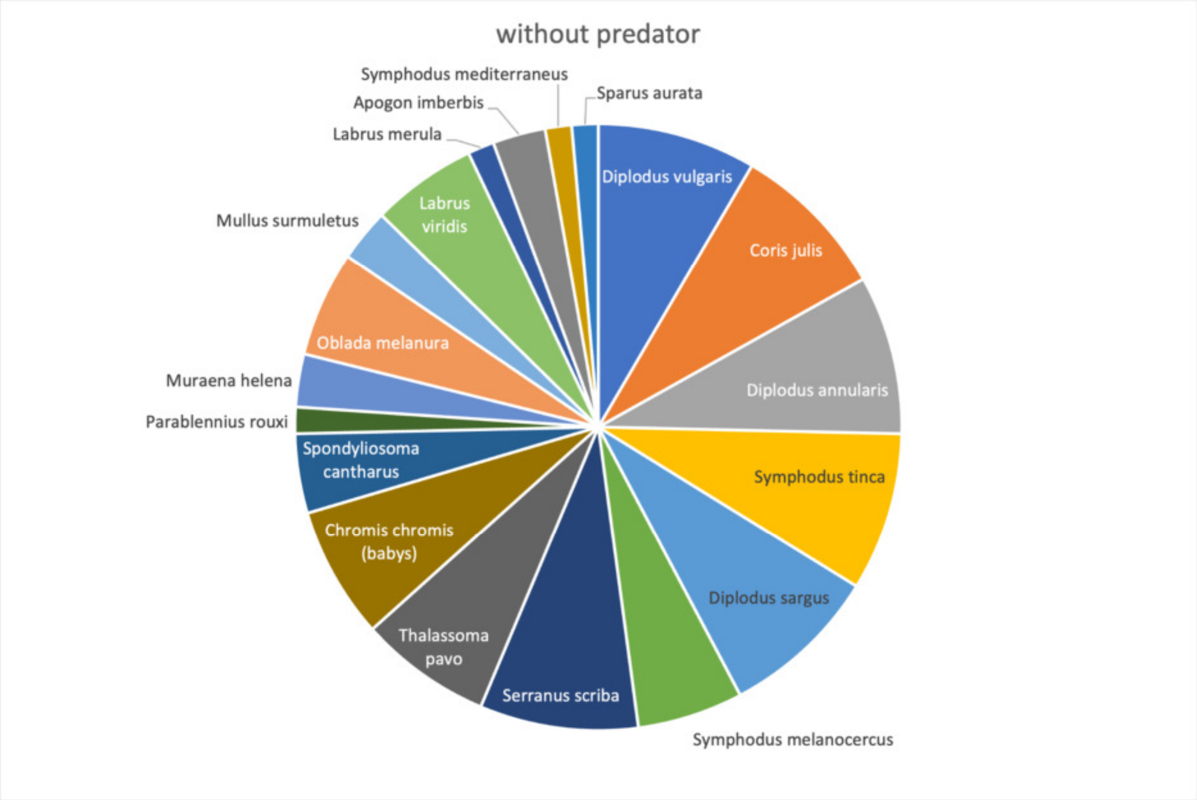


Figure 33: Appearing fish species in the experiment set-up without the predator.

While the species composition was fairly similar, a big difference can be seen in the number of total individuals (Figure 7). Counted over all experiments with and without a predator a total of 236 individuals were counted at the site with a predator, while a total of 532 individuals were counted at the site without a predator.

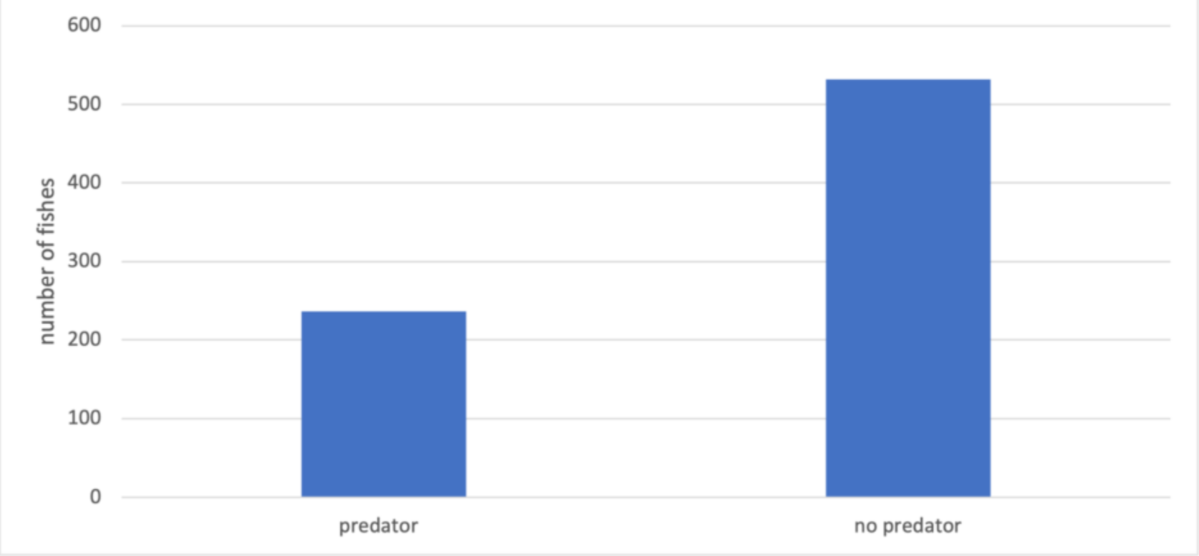


Figure 34: Total number of counted fish individuals across the predator presence and the predator absence set-ups.

All of the three interaction types could be seen at both sites (Figure 8). However, feeding was the interaction type that was observed the most. While a lot of species seemed shy in the recordings and did not feed at the site with the predator, most species fed at the site without a predator.

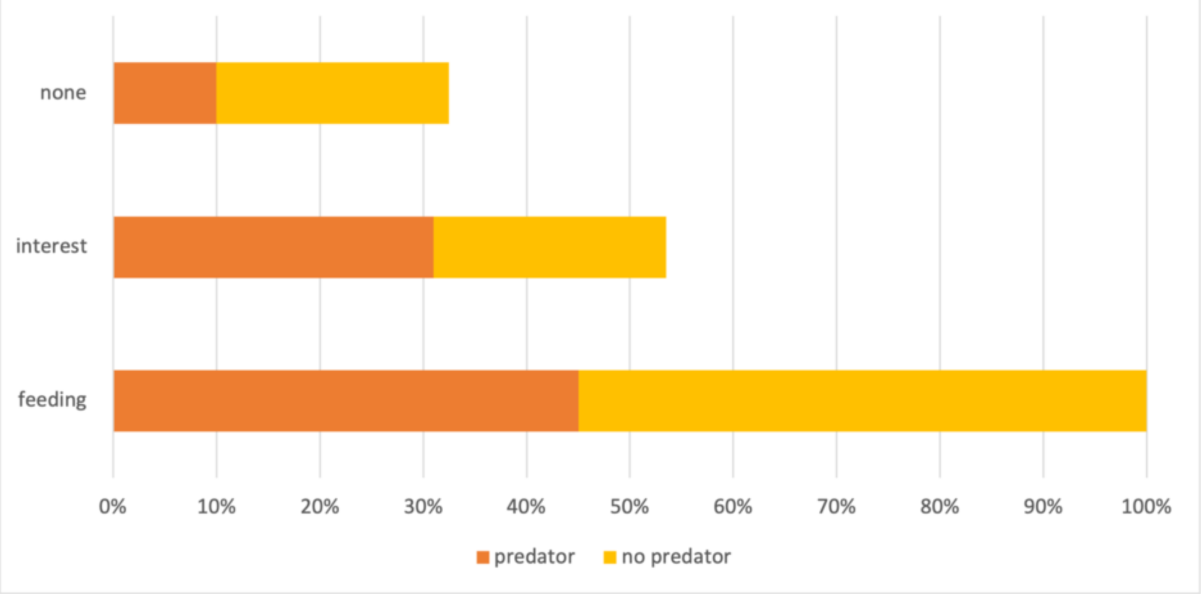


Figure 35: Interaction types performed across all species that appeared at set-up with predator presence and predator absence.

Discussion

Species composition

Many of the species that appeared often in the videos (40% - 80%), are carnivores like *Coris julis, Diplodus annularis, Diplodus sargus, Diplodus vulgaris,* and *Symphodus tinca.* La Manna et al. (2017) found a similar pattern and stated that some of the planktivores and herbivores (*Chromis chromis* and *Oblada melanura*) might have gotten attracted by the movement of many fishes around the camera unit. This might also apply, in our case, to *Serranus scriba*, *Thalassoma pavo,* and *Symphodus* species.

Due to the limited comparability of the environmental conditions during sampling times, we have refrained from doing a statistical analysis. With higher amounts of data, a non-metric multidimensional ordination (NMDS) combined with Bray-curtis similarity or Shannon-Wiener index as measurements for diversity and community composition would have been the most informative approach. La Manna et al. (2021) conducted a large study to test the efficiency of BUVs, underwater visual census (UVC) and fish traps, but they sampled at multiple, geographically distant sites. As our study site, methodology and diving experience defined that the camera units had to be relatively close to each other, statistical analyses would not have shown large dissimilarities in community composition. It seemed therefore more useful to us to compare absolute numbers of fish sightings and abundances.

Due to the relatively small video frame of a single camera, it was sometimes difficult to assess whether many individuals of the same species entered the frame one time or whether it was the same individual that entered the frame multiple times. This insufficient information is a common problem for baited underwater video (BUV) abundance assessments (Stobart et al. 2015, La Manna et al. 2021). Still, our method to count individuals not more than once to obtain the minimum number of individuals known to occur within one video, is widely used and conservative (Stobart et al. 2015).

Bait type and exposure

Our results show that different bait exposure influences how interested fish are in the set-up. When openly displaying the bait during the day, sea urchin is the preferred bait. At night, when *Muraena helena* is more active, it is reasonable to use fish, or a combination of both bait types, as the fish is clearly preferred by *M. helena*. The relative relationship of the interaction types using percentages of species numbers was displayed to get a clearer idea of which bait is actually preferred by the species that appeared in the respective videos. The diagram has to be interpreted with caution but shows that a high number of disinterested species, as in experiment 1 (“Trap 1”) can skew the outcome.

Another factor that might explain that sea urchin was preferred over fish is the consistency of the bait. Other BUV studies used crushed, oily fish, crustaceans, or bivalves as bait for different target species (Stobart et al. 2015, La Manna et al. 2021, Bosch et al. 2017). The fish we used as bait was dissected before and stored in the refrigerator until shortly before the camera deployment. The sea urchin on the other hand was freshly crushed and immediately used as bait. The smell might have spread further than thawing or thawed fish and therefore attract more species. In this context, it is necessary to place adjacent cameras apart from each other to separate the attraction radiuses and avoid pseudo-replication of fish counts (Bosch et al. 2017).

As the experiments considering the bait type and exposure have been conducted as one of the first, our set-up was not yet fully developed. The trap we used was an eel trap and therefore not adapted to the local fish community at STARESO. The efficiency of various methods to measure abundance and community composition has been tested before (Bosch et al. 2017, La Manna et al. 2021, Nalmpanti et al. 2021, Stobart et al. 2015). Bosch et al. 2017 found that video recordings (BUVs) are indeed much more efficient than fish traps and also than UVC, because several camera units can be deployed simultaneously, as has been done in our study.

In our study, more videos with openly presented fish as bait could have been taken during the day to investigate the preferred bait type more thoroughly. However, videos taken for the other experiments (light regime and predator disturbance) showed that sea urchin attracts many different fish species under varying conditions and thus support our results that sea urchin is a well-suited bait for underwater video analysis. The feeding behaviour of *Muraena helena* implies that the light regime, which is discussed in the next paragraph, has an additional impact on the best bait type to attract fish. Other abiotic parameters, such as depth or habitat characteristics, might influence the feeding behaviour and thus the preferred bait, as well. It would be informative to test these assumptions in future studies.

Light regime (time series)

Our observations revealed differences in the species occurrence, abundance, and activity pattern at different times of day and under different light regimes. It is known that fish from tropical and temperate regions generally feed in two ways in terms of time, during the day or at night, or they are feeding during twilight (Emery 1978). According to Arndt and Evans (2022) almost 40% of native Mediterranean display fishing activity during the day, whereas around 20% are active at night and only a small proportion of fish species forage at crepuscular time. Since such feeding patterns are also linked to prey activity patterns, the presence of predators and/or food optimization (Daan 1981), using underwater videos we were able to investigate changes in the interaction types over the day in the natural environment and the local fish community. In the fish community of the STARESO bay, we found the highest feeding activity in less intense sunlight in the afternoon and in the evening, when our study site was already in the shade. At that time, it was interesting to observe that all species of one genus (*Diplodus* sp.) showed the same feeding behaviour at the same time. Furthermore, as we observed while snorkeling that during night the fish are less active, our night experiments aimed only to attract the predators/scavengers with bloody fish bait. This worked perfectly, as we attracted *M. helena* within seconds. The moray showed aggressive feeding behaviour and attacked the fish bait until it was finally able to detach the bait and feed on it. As a result, we lost our bait in the night experiments within minutes. The differences in daylight at different times and at different light intensities suggest that future studies that measure precise light intensities and investigate fish activity could provide interesting insights into the biological behavioural cycles of Mediterranean fish species.

Predator presence/absence

While the species composition was fairly similar, there were clearly less fish at the site with a predator. However, it cannot be assumed with certainty that the fish recognized the predator as predator. Most examples of associative learning combine alarm cues with predator odour, but prey can be conditioned to respond to visual cues from predators, too (Chivers & Smith, 1994). In a detailed analysis of facial features, fishes with a large mouth and widely spaced eyes tended to be predatory (Karplus & Algom, 1981). Models with these facial features presented to fish (*Chromis caeruleus*) got a stronger anti-predator response, than models without these features (Karplus et al., 1982). Since it was a two-dimensional picture without any chemical alarm cues in this experiment, it could also be neophobia, a phenomenon where prey exhibits an avoidance response to any novel cue (Sneddon et al., 2003). To test if the prey species recognize the predator as predator, one could perform more experiments where instead of a photograph of the predator there are different shapes and colors, like a brown circle or a red triangle. If the response of the fish differed significantly our predator was recognized as a predator.

On the recordings, one can see big differences in feeding behaviour with predator presence or absence. While the fish start to feed right from the start at the site without a predator, when a predator was present, the fish seemed more shy and cautious at first. Thus, both inter- and intraspecific differences in behaviour have been found.

The behaviour of each individual depends on two things: its motivational state and the immediate environmental incitement (Budaev and Brown, 2006). Due to different experiences, the fish often react differently to identical stimuli (Brown & Warburton 1999). Individual fish can even differ considerably within a school (Pitcher & Parrish 1993; Ward et al. 2004; Leblond & Reebs 2006).

Some individuals are therefore more willing to take risks than others. As soon as they have dared to interact with the bait, other fish have also dared to do so. It would be very interesting to conduct further experiments on the different character traits of different species and individuals. However, even under controlled experimental conditions, it is almost impossible to create the same environment for all individuals in order to record the differences in character.

Obstacles

During the sampling process and video analysis, we faced several obstacles. Most importantly to note is that it took some trials and experiments for building and perfecting our set-up. This concerned both the deployment of the cameras and the addition of bait. Timing of camera deployment was a crucial factor due to the risk of battery constraint. A further obstacle with underwater video recording was the extraordinarily high amount of data that had to be stored on various devices, making it difficult to keep an overview.

Some of the videos taken during the development time of the set-up were still used for later analysis, e.g. for bait type and exposure. As the conditions during sampling time might have differed, not all of the videos are easily comparable. However, it was our goal to find a method that can be used in future studies, therefore we had to accept the trade-off between method development and comparability. Since the abundances and interaction types in the videos have been analysed by several people, they could not be interpreted in a standardized way. In addition, the level of experience in determining the species varied and could have led to slightly distorted data. To make the analysis as standardized as possible, we have agreed on using the same abundance classes as have been used for the fish transect, a technique that has been effectively used before (Bosch et al. 2017).

Conclusion

In our study, we showed that species abundance, composition, and behavioural analyses can be conducted in an applicable way when using snorkeling and simultaneously deployed baited underwater videos. Many species of the local community in the Northwest of Corsica have been attracted by our baited underwater experiment, when openly deploying a bait. Sea urchin was the preferred bait during daytime, while at night, fish was more efficient to attract carnivorous species. This was also reflected in the shift between daily and nightly communities, detected when comparing light regimes. The presence of a predator exhibited an influence on the species abundance and individual behaviour, while no effect on species composition was seen. Using underwater videos reveals interesting insights in direct visual fish observations and offer great potential in future studies to investigate interactions between species during bait-influenced feeding, as well as the behaviour of individual characters of fish.

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Diet composition of the Pointed-snout wrasse (*Symphodus rostratus*) and the Green wrasse (*Labrus viridis*)

Georgia Avgerinou, Hanna Rudnick, Smilla Todt

Introduction

Being the world's largest and deepest enclosed sea, the Mediterranean covers 0.82 % of the world's ocean surface (Psomadakis et al., 2012). Despite its small size it shows a rich fish diversity with between 4-18 % of the world's marine species found in Mediterranean waters (Psomadakis et al., 2012). The family of the wrasses (Labridae) represents one of the most species rich fish families in the Mediterranean (next to Gobiidae, Sparidae and Blenniidae) (Psomadakis et al., 2012) with over 20 species (Koblmüller et al., 2003).

Wrasses are commonly diurnal substrate-oriented fish of smaller size, usually found in the coastal zones of the upper littoral, inhabiting seagrass beds, shallow rocky areas and algae meadows (Arigoni et al., 2002,Bell & Harmelin-Vivien, 1983, Hanel et al., 2002, Koblmüller et al. 2003, Rodrigues et al., 2015). This heterogeneous habitat leads to very broad diets (Bell & Harmelin-Vivien, 1983), with wrasses showing species specific use of food resources and specialization for microhabitats (Koblmüller et al., 2003). Even though research on Labridae diet is often contradictory, wrasses are in general found to be carnivorous (Bell & Harmelin-Vivien, 1983, Koblmüller et al., 2003). Their anatomy shows pharyngeal jaws with partly carnivorous dentition, short intestines and the lack of a true stomach (Koblmüller et al., 2023, Quignard, 1966). Past gut content analysis of Labridae identified benthic invertebrates (mainly Crustacea and Gastropoda) as the dominant food source of Mediterranean wrasses (Bell & Harmelin-Vivien, 1983, Koblmüller et al., 2003). Only the bigger wrasse species *Labrus merula* and *Labrus viridis* show a slightly different diet. Gut content analyses of *Labrus viridis* revealed a bigger amount of fish remains, whereas *Labrus merula* feeds primarily on decapods (Bell & Harmelin-Vivien, 1983, Ben Slama et al., 2007). Additional plant material can often be found in the gut contents of most Labridae species (Koblmüller et al., 2003, Kabasakal, 2001). This should however not be interpreted as an omnivorous diet but is most likely due to most wrasses' specialization on algae infauna and epiphytic animals resulting in accidental take up of algae material during foraging (Koblmüller et al., 2003, Ben Slama et al., 2007).

Wrasses belonging to the genera Labrus and Symphodus in the Mediterranean Sea exhibit two distinct morphs, characterized by either brown or green coloration. It has been hypothesized that the observed color morphs in these wrasses may have evolved as an adaptation to the unique habitat conditions specific to the Mediterranean Sea that include a combination of vegetation and substrate types. The green morph is associated with seagrass meadows and the brown one with rocks covered in brown algae (Fark et al., 2022).

During the 2023 course in the bay of Calvi we could regularly observe medium sized individuals of the wrasse species *Symphodus rostratus* swimming with groups of medium sized *Symphodus tinca*. *Symphodus rostratus* is reported to feed primarily on amphipods, carids, copepods and mysids (Bell & Harmelin-Vivien, 1983). This suggests that *S. rostratus* benefits from following schools of *S. tinca* by feeding on evasive prey that *S. tinca* chases away during foraging or ejects after filtering (Calvi report 2012, Zupo & Stübing, 2010, Zander & Sötje, 2001).

The observed feeding aggregation between *S. tinca* and *S. rostratus* could however only be detected in medium sized *S. tinca* groups. In groups of small sized *S. tinca* there could never be seen any accompanying *S. rostratus*. These behavioral observations suggest that *S. rostratus* might not only be specialized in, as so far believed, crustaceans (Bell & Harmelin-Vivien, 1983), but might also show a piscivorous diet and act as a predator of small sized fish. Therefore, medium sized *S. rostratus* might not be allowed in groups of young *S. tinca* because it is seen as a predator. However, in medium sized *S. tinca* groups *S. rostratus* is tolerated for not presenting any harm to bigger individuals.

As foraging behavior and intra- and interspecific interactions are closely linked to diet composition (Stergiou & Karpouzi, 2002, Mahesh et al., 2018) a widely used tool to evaluate trophic interactions, food and feeding habits and prey selection in fish is the analysis of their gut content (Mahesh et al., 2018). Up to date several methods are available for gut content analysis (Mahesh et al., 2018). Despite the recently introduced more accurate and detailed approaches such as radioisotopes, stable isotope analysis or fatty acid analysis, the direct gut content analysis carried out through dissection and examination via the microscope is still the most commonly used practice (Mahesh et al., 2018). This procedure is easier and less expensive whilst still producing results that are good enough for most biological or ecological studies (Mahesh et al., 2018).

To investigate the feeding behavior of *S. rostratus* and test the above-mentioned hypotheses we conducted a gut content analysis of several *S. rostratus* specimens caught in the area of Calvi Bay. As foraging behavior is also closely connected to habitat (Stergiou et al., 2002, Mahesh et al., 2018), fish were sampled from seagrass/ algae regions as well as from rocky shore regions to compare different habitats. In addition, *Labrus viridis*, another wrasse species, were caught in both habitats to compare its diet with that of *S. rostratus* as *L. viridis* is mentioned in the literature as a partly piscivorous fish (Bell & Harmelin-Vivien, 1983).

Our experiment aims were to: i) find out about the diet composition of *S. rostratus*, ii) compare the diet of *S. rostratus* to the diet of *L. viridis* iii) investigate whether *S. rostratus* can be seen as a further piscivorous wrasse, iv) investigate potential differences in the diet of *S. rostratus* within different habitats (seagrass meadow and rocky shore).

Material and Methods

For gut content analysis fish were caught while snorkeling with hand nets in two habitats. First in the north-east of the peninsula La Revellata in rocky shore areas close to the Station de Recherches Sous-marines et Océanographiques de Calvi (STARESO). Second in the south-east of La Revellata in the Plage de l'Alga in nearshore *Posidonia oceanica* meadows and algae covered rocky substrate. Caught fish were frozen as soon as possible. The dissection of the fish was conducted at STARESO. For the dissection all fish were thawed. Each fish was sized (total length, TL) to the nearest 0.1 mm and weighed (total weight) to the nearest 0.01 g, as well as photographically documented. The gut contents of a total of 19 Pointed-snout wrasses (*Symphodus rostratus*) (Fig. 1) and four Green wrasses (*Labrus viridis*) (Fig. 1) were analysed. An individual ID was assigned to each fish. All fish were opened with scissors on the ventral side. A cut was made from the anus to the throat. The gut was removed with tweezers and cut off at the most anterior end. The gut itself was cut open and all contents were removed in a petri dish. Bile, fat and surrounding tissue were removed. Using a stereomicroscope, the gut contents were identified and categorised. Four types of gut contents were identified. These were 'algae', 'crustacea', 'fish', and 'other'. The category 'other' consisted of e.g. stones and foraminifera. The degree of contribution of each type of content to total gut content in percent was assessed. The length-weight-relationship of *S. rostratus* was calculated. Length weight relationships (LWRs) in fish are defined as $W = a \cdot L^b$. The parameter 'a' is a scaling coefficient for the weight at length of fish, while 'b' is a shape parameter for the body form of fish. Through logarithm this model becomes linear: $\log(W) = \log a + b \log(L)$ (Le Cren, 1951). Thus, 'log (a)' is the intercept and 'b' the slope of the equation. The software R-studio ®, version 4.3.0, was used for the graphical presentation of the results (R Core Team, 2023). The work was completed with version 2.0.0 of the 'tidyverse' package (Wickham et al., 2019). One *S. rostratus* (Fish ID 5) had an empty gut and was removed from the gut content analysis.

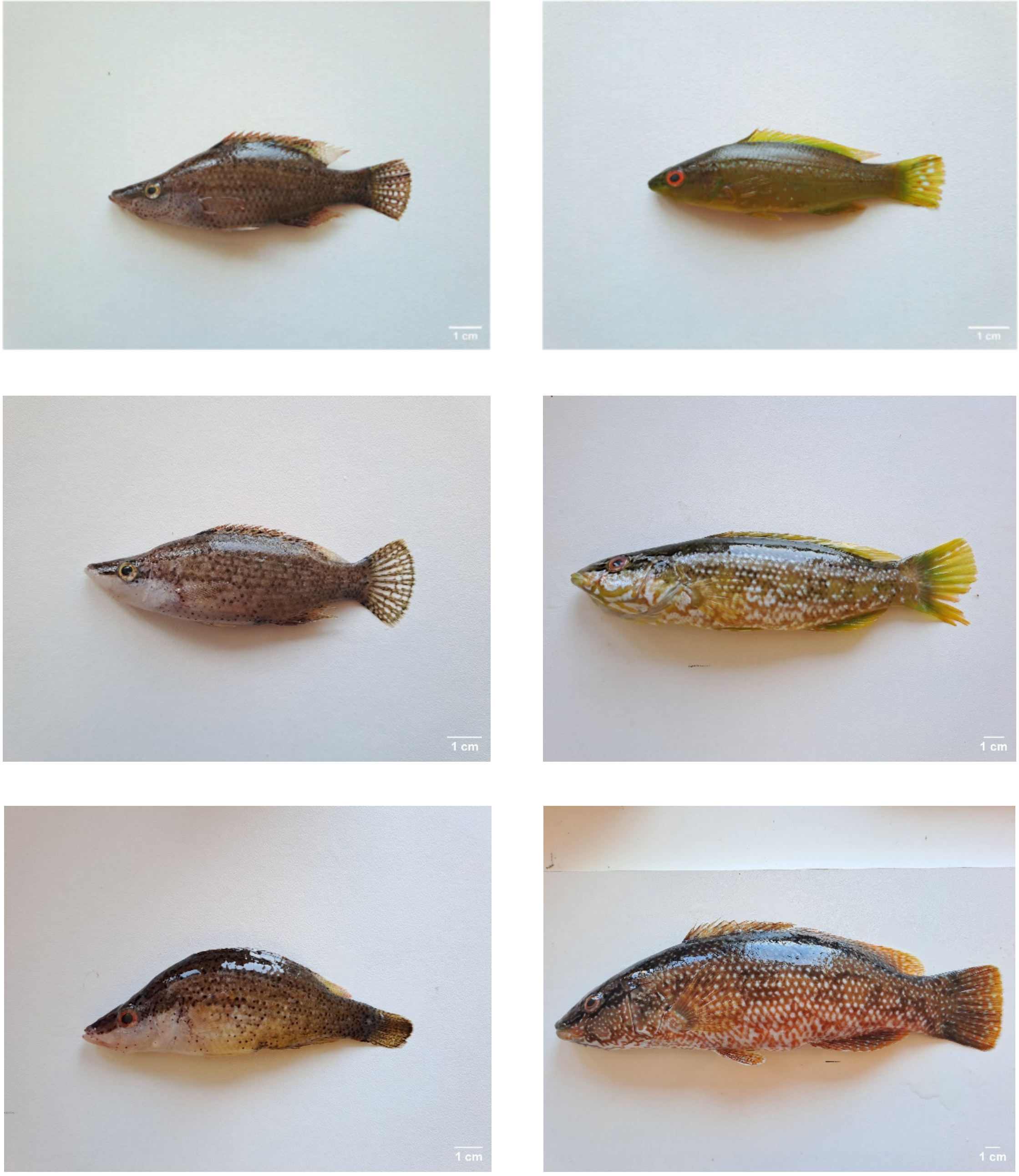

Figure 1: Photos of Pointed-snout wrasses (*Symphodus rostratus*) (left column) and Green wrasses (*Labrus viridis*) (right column) caught north-east of the peninsula La Revellata.

Results

The gut contents of 19 *S. rostratus* were analyzed. The fish used in this study had a length range of 8.2 to 11.5 cm and a weight range of 7.5 to 19.5 g (Fig. 2). The LWR resulted in the following formula: W = 0,0139 * L ^ 2,9750 (Tab. 2).

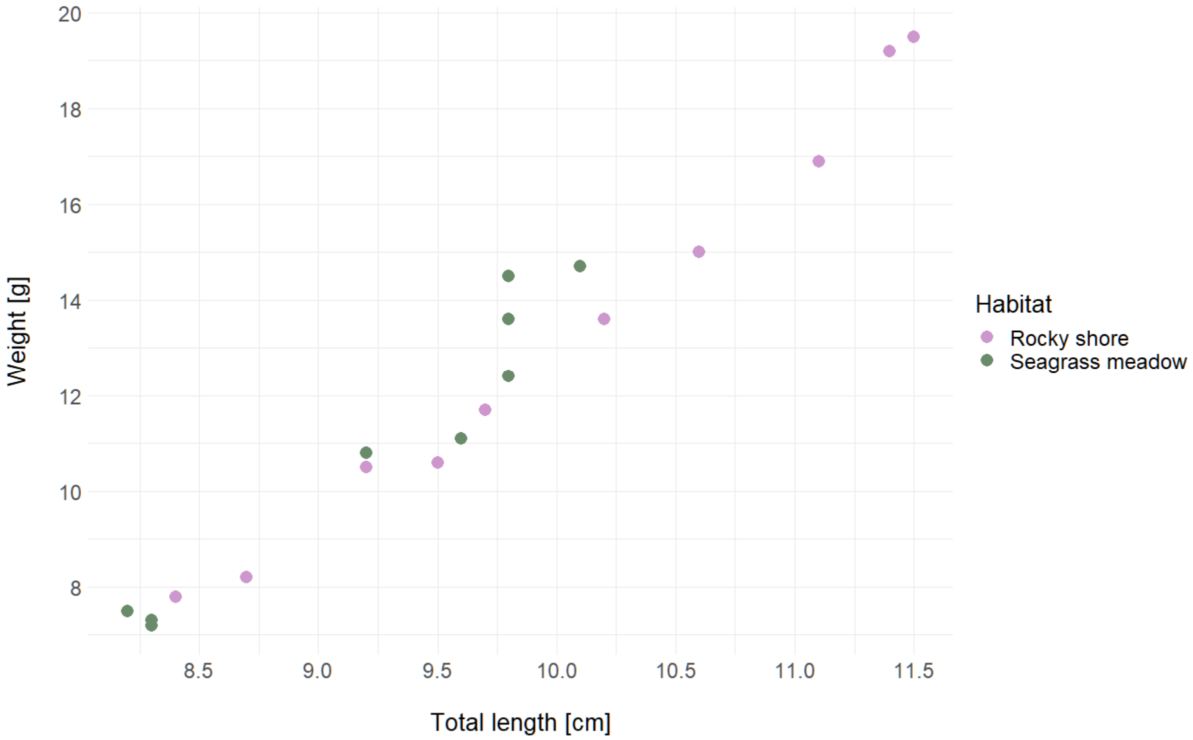

Figure 2: Total length in cm and weight in g of *Symphodus rostratus* caught in *Posidonia oceanica* meadows and algae covered rocky substrate (seagrass meadow) and rocky shore.

Symphodus rostratus was found to predominantly consume crustaceans (Fig. 3). However, there were a few exceptions noted: the gut content of two individuals (Fish ID 9 & 12) consisted exclusively of fish remains and the gut content of another individual (Fish ID 13) consisted of crustaceans and fish in nearly equal proportions. Algae and other organisms, such as foraminifera, were observed in just four cases each, and their quantity was negligible (≤1% of total gut content). An individual (Fish ID 5) contained totally digested material/ material of unidentified origin and will not be taken into consideration in further analyses. A noticeable pattern between *S. rostratus* size and prey preference could not be observed (Fig. 3).

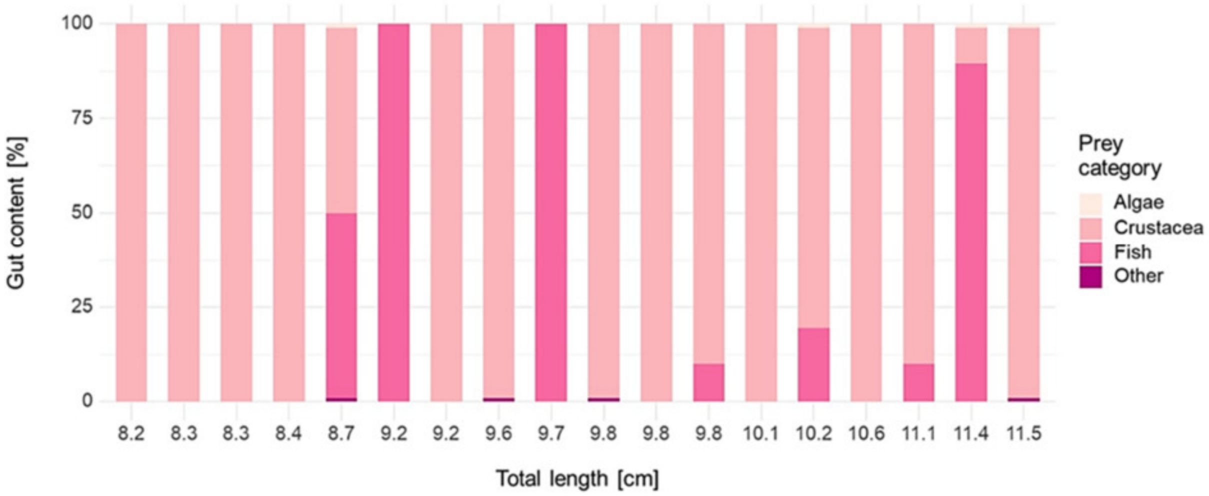

Figure 3: Gut contents of *Symphodus rostratus* in percent of 18 individual fish. Fish were divided according to individual total length in cm. Contents were classified into four prey categories. These categories are 'algae', 'crustacea', 'fish' and 'other'. Proportion of categories in the total content given in percent. Individual '5' had an empty gut and was removed from the analysis.

Fish collected near seagrass meadows fed almost entirely on crustaceans, accounting for 98.6% of their gut content, whereas fish and other organisms constituted only a negligible portion of their gut content (1.1% and 0.2% of the total gut content, respectively). Fish collected near the rocky shore exhibited a higher proportion of fish consumption, accounting for 36.8% of the total content. However, crustaceans were again the dominant component of the gut content (52.6%). The residual content consisted of 0.4% algae and 0.2% other components (Fig. 4).

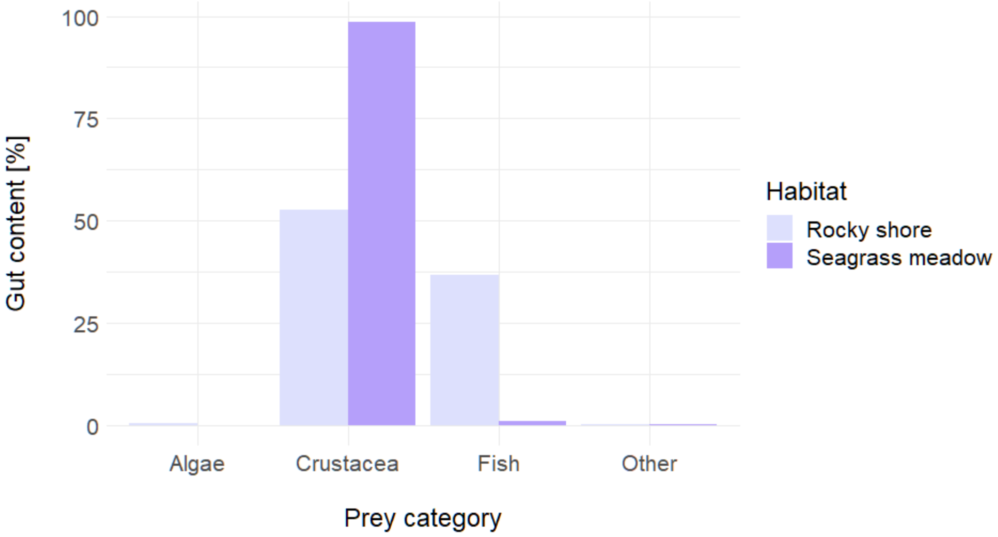

Figure 4: Gut contents of *Symphodus rostratus* in percent in two different habitats. The habitats are *Posidonia oceanica* meadows and algae covered rocky substrate (seagrass meadow) and rocky shore. Contents were classified into four prey categories. These categories are 'algae', 'crustacea', 'fish' and 'other'.

Labrus viridis was found to predominantly consume fish. Only one individual (Fish ID 10) had 90% Crustacea and only 10% fish in its gut (Fig. 5). Fish collected near seagrass meadows fed almost entirely on fish, accounting for 98% of their gut content. Fish dominated the gut content of green wrasses collected near the rocky shore (69.3 %), followed by Crustacea (30%) (Fig. 6).

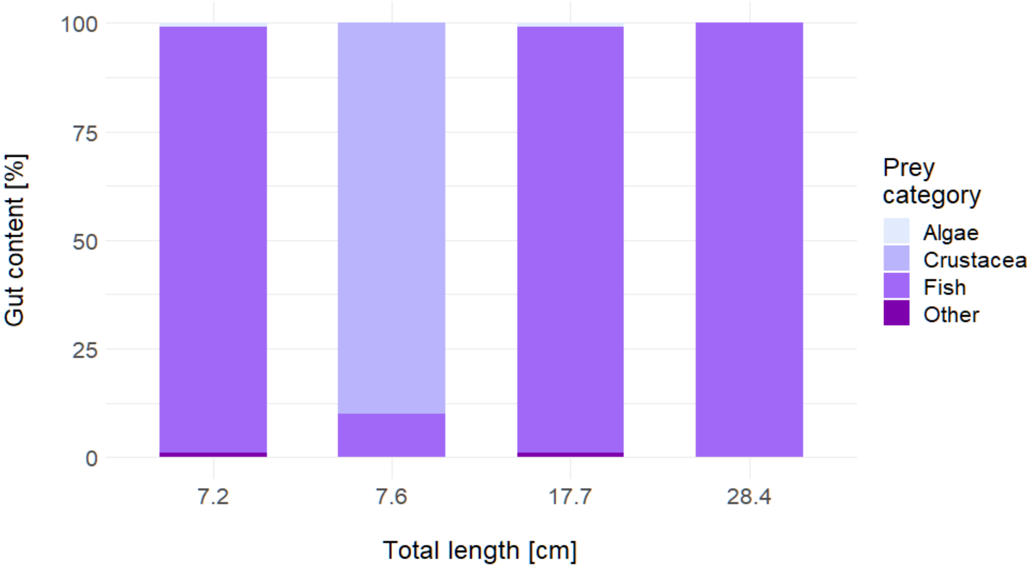

Figure 5: Gut contents of *Labrus viridis* in percent of 4 individual fish. Contents were classified into four prey categories. These categories are 'algae', 'crustacea', 'fish' and 'other'.

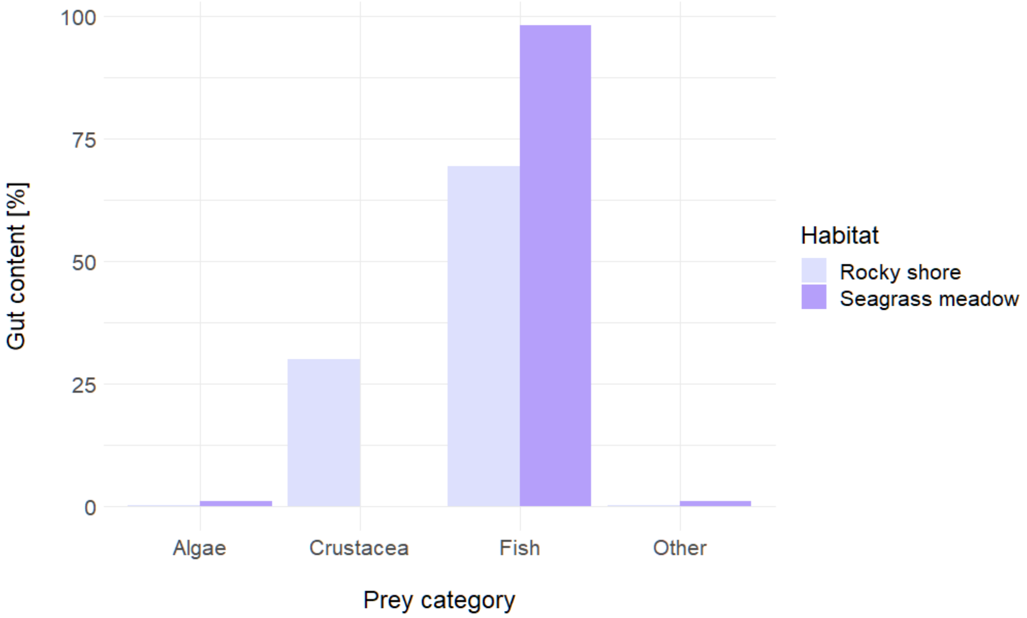

Figure 6: Gut contents of *Labrus viridis* in percent in two different habitats. The habitats are *Posidonia oceanica* meadows and algae covered rocky substrate (seagrass meadow) and rocky shore. Contents were classified into four prey categories. These categories are 'algae', 'crustacea', 'fish' and 'other'.

Discussion

The LWR of *S. rostratus* shows a ‘b’ value of 2,9750, which is close to an ideal value of three, representing isometric growth (Ricker & Carter, 1958). ‘b’ might deviate due to seasonal variations and different environmental conditions affecting growth conditions (Jsir et al., 2018). The ‘b’ value less than three indicates negatively allometric growth, meaning that *S. rostratus* becomes lighter with increasing length. Due to the small deviation of b from the ideal value of three, the growth conditions of *S. rostratus* cannot be assessed negatively. Furthermore, a ‘b’ value smaller than three might indicate that smaller individuals are in better nutritional condition (Kuriakose, 2014). Since the obtained gut content compositions do not show significant differences between different sized *S. rostratus*, one cannot conclude that size-related differences in nutritional condition exist between the sampled fish.

Wrasses are considered mesophagic carnivores and exhibit a broad dietary spectrum including many types of crustaceans, molluscs and fish. Additionally, they demonstrate remarkable flexibility and can adapt their diet spatially and according to the availability of prey (Bell & Harmelin-Vivien, 1983). The two studied wrasse species in the bay of Calvi showed different feeding habits. According to our results, crustaceans dominate *S. rostratus* gut content. Other types of prey were also observed but in smaller proportions. This is an expected result as crustaceans are considered to dominate the diet of wrasses (Bell & Harmelin-Vivien, 1983) and it is in line with previous excursions findings (2010, 2012, 2018 Excursion Reports). Moreover, a study on *S. rostratus* from *Posidonia oceanica* meadows Gulf of Naples which demonstrated a diet consisting mainly of crustacean but no fish (Zupo & Stübing, 2010), also supports our findings. This pattern aligns with the fact that crustaceans are among the most abundant phylum found in seagrass leaves (Bell & Harmelin-Vivien, 1983, Quignard 1966). However, even though *S. rostratus* show comparable densities over *P. oceanica* meadows and algae covered rocky habitats (Guidetti, 2000), information about the feeding habits of this species in algae covered rocky habitats is not available. Furthermore, we reported that fish is dominating *L. viridis* gut content, which is in line with a previous excursion finding (2014 Excursion Report). While research in the bay of Calvi indicates a piscivorous diet in *L. viridis*, studies have shown that this species shows a wide trophic range and can adapt its diet according to prey availability (Zupo & Stübing, 2010). Also, in the Northern Aegean Sea *L. viridis* was found to feed exclusively on molluscs (Karachle, 2017). This information collectively suggests that *L. viridis* diet varies in different locations.

Within the seagrass meadow, *S. rostratus* exclusively consumed crustaceans, while *L. viridis* exclusively consumed fish, according to their gut content. This observation could be an indication for trophic niche partitioning between the two species. Trophic niche partitioning in fish is a major strategy for minimizing competition. Fish can coexist effectively by diversifying their diets and therefore occupying different ecological niches (Macpherson, 1981, Ross, 1986, Abrams, 1987). This has been observed not only within different wrasse species (Davis & Wing, 2012; Sinopoli et al., 2017), but also among wrasses and other fish species (Hielscher et al., 2015).

In an earlier report (2012 Excursion Report), it was observed, that smaller individuals of *S. rostratus*, predominantly feed on crustaceans while individuals exceeding 10 cm exhibit a significant proportion of fish remains were found in their gut, suggesting that *S. rostratus* changes its diet as it is growing. In our case, we did not see a similar pattern. One potential explanation for this could be that specimens were collected from two different habitats, and in the seagrass habitat, very few fish remains were observed in the gut content. Yet, this provides an interesting area for investigation because a shift in diet with increasing length has been noted in other wrasse species as well i.e in *Symphodus (Crenilabrus) melops* (Deady & Fives, 1995), in *Symphodus (Crenilabrus) tinca* (Ouannes-Ghorbel & Bouain, 2006), in *Xyrichtys novacula* (Castriota et al., 2005), in *Thalassoma pavo* and *Coris julis* (Sinopoli et al., 2017).

While the dietary habits of other wrasses have been extensively examined, *S. rostratus* has not received similar attention. In this study, we observed differences in the gut content of *S. rostratus* and *L. viridis*, as well as variations in gut content in fish from rocky shore and seagrass meadow habitats. The hypothesis that *S. rostratus* is a piscivore fish cannot be confirmed. Additionally, understanding whether the absence of larger *S. rostratus* from *S. tinca* groups is connected to their dietary preferences (potential piscivory), further research is essential including complementary methods such as in situ observations and DNA barcoding of the gut content. It is also important to note that this analysis relied on a relatively small sample size and covered a short period, so in order to gain a deeper insight on the dietary habits of these fishes, additional research is needed, involving a longer investigation period, a larger sample size, and taking into consideration various factors that influence the trophic preferences of fish (e.g. sex, maturity status, inter- and intraspecific competition).

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Appendix

Table 1: Gut content in percent, total length in cm and total weight in g of different fish species caught in in the Plage de l'Alga and rocky shore between the 7th and the 10th of August 2023. Each fish was assigned an individual fish ID. Species are sorted alphabetically and according to catch date.

Fish ID	Species	Catch date	Habitat	Total length [cm]	Total weight [g]	Gut content [%]
4	*Labrus viridis*	07.08.2023	Rocky shore	28,4	306,9	Fish (100%)
10	*Labrus viridis*	09.08.2023	Rocky shore	7,6	5,2	Crustacea (90%) Fish (10%)
16	*Labrus viridis*	09.08.2023	Rocky shore	7,2	4,4	Algae (1%) Fish (98%) Other (1%)
29	*Labrus viridis*	10.08.2023	Plage de l'Alga	17,7	63,9	Algae (1%) Fish (98%) Other (1%)
5	*Symphodus rostratus*	07.08.2023	Rocky shore	9,5	10,6	Empty
6	*Symphodus rostratus*	07.08.2023	Rocky shore	11,1	16,9	Crustacea (90%) Fish (10%)
7	*Symphodus rostratus*	07.08.2023	Rocky shore	10,6	15	Crustacea (100%)
8	*Symphodus rostratus*	09.08.2023	Rocky shore	10,2	13,6	Algae (1%) Crustacea (79,5%) Fish (19,5%)
9	*Symphodus rostratus*	09.08.2023	Rocky shore	9,7	11,7	Fish (100%)
11	*Symphodus rostratus*	09.08.2023	Rocky shore	11,5	19,5	Algae (1%) Crustacea (98%) Other (1%)
12	*Symphodus rostratus*	09.08.2023	Rocky shore	9,2	10,5	Fish (100%)
13	*Symphodus rostratus*	09.08.2023	Rocky shore	8,7	8,2	Algae (1%) Crustacea (49%) Fish (49%) Other (1%)
14	*Symphodus rostratus*	09.08.2023	Rocky shore	8,4	7,8	Crustacea (100%)

15	*Symphodus rostratus*	09.08.2023	Rocky shore	11,4	19,2	Algae (1%) Crustacea (9,5%) Fish (89,5%)
18	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	9,8	13,6	Crustacea (99%) Other (1%)
19	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	9,8	14,5	Crustacea (100%)
20	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	8,2	7,5	Crustacea (100%)
21	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	9,8	12,4	Crustacea (90%) Fish (10%)
22	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	9,6	11,1	Crustacea (99%) Other (1%)
23	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	8,3	7,2	Crustacea (100%)
24	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	10,1	14,7	Crustacea (100%)
25	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	8,3	7,3	Crustacea (100%)
26	*Symphodus rostratus*	10.08.2023	Plage de l'Alga	9,2	10,8	Crustacea (100%)

Table 2: Length-weight-relationship for 19 *Symphodus rostratus* caught in the east coast of La Revellata, based on the equation log (W) = log a + b log (L). N (sample size), L min – max [cm] (minimum to maximum total length in cm), W min – max [g] (minimum to maximum total weight in g), a (intercept), b (slope of equation).

Species	*Symphodus rostratus*
N	19
L min – max [cm]	8,2 – 11,5
W min – max [g]	7,5 – 19,5
a	0,0139
b	2,9750
W = a * L ^ b	W = 0,0139 * L ^ 2,9750