Marine biological excursion

University of Innsbruck & Kiel

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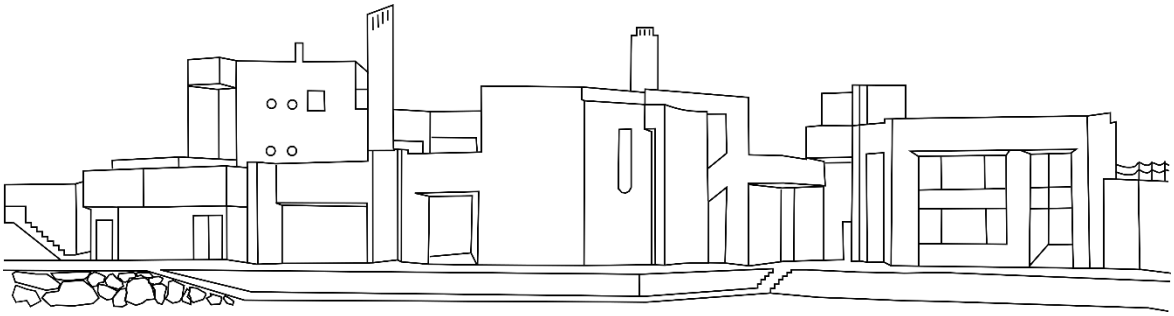

Design: Philip Bertemes

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Introduction

The “station de Recherche Océanographiques et sous Marines” (STARESO) founded by the university of Liege is a marine biological center on the North-Western coast of Corsica that offers direct access to the sea combined with a research facility for scientists and students from all over the world. Here students and scientist are welcome to study the marine environment under various aspects. The station offers two dry and one wet lab as well as sleeping quarters that serve amongst others the University of Innsbruck and Kiel as base for a cooperative marine biological excursion.

The course, that takes place every two years, gives students the possibility to get an insights in the biodiversity of the Mediterranean Sea as well as in the existing habitats and their different environmental conditions. Therefore the participants get background information in daily lectures and learn to determine diverse species during snorkeling sessions in the first week. In the second week the students work on individual projects in groups. In the STARESO lab the students are able to perform their own experiments in the field of developmental biology and fish biology.



Daily protocols



Algea

Serra Örey, Rafael Meichßner

Introduction

Macroalgae are a polyphyletic group of photosynthetic organisms consisting of Phaeophyta (brown algae), Chlorophyta (green algae) and Rhodophyta (red algae). These groups are phylogenetically far apart descending from different unicellular ancestors (Cock *et al.* 2010). As a result they have different accessory photosynthetic pigments which are responsible for their typical color. From polar to subtropical regions macroalgae dominate shallow benthic areas with hard substrate (Lüning 1985). In places like the island of Corsica with a lot of rocky coastline algae represent the main benthic primary producers of the shallow water ecosystem (Ballesteros 1989). On our excursion to the Marine Biological Station STARESO in the northwest of Corsica we spent one day on exploring the local macroalgal flora.

Generally, the ecological niche of an algal species in coastal habitats is defined by its resistance to desiccation on one hand and its light demand on the other hand. Due to these two factors algal communities arrange in girdles of typical, sometimes almost monospecific composition (Lüning 1985). In our study area in Corsica, there were no significant tidal changes to create advantage for desiccation resistant species. Therefore, in Stareso, we expected light to be the main driver and hence macroalgal species to arrange along a light gradient. As light conditions change with depth and also with the morphology of the rocks, we aimed to assess the present algal community with respect to different depth zones and daylight exposure.

Methods

The explored habitat was a boulder field with larger rocks in adjacent areas, also including a harbor wall. Sampling was done by collecting the algae species by snorkeling. Three depth zones were decided as following: 0-1 m, 1-3 m and 3-5 m. Each zone was sampled by two people twice. During sampling, it was noted if the sampled algae were from a shadowed area or an area highly exposed to direct daylight. Specimens were brought to land, stored in buckets filled with seawater and identified with the help of Riedl (1983).

RESULTS

There were in total 19 algae species identified during the study. We found four species in all water depth zones that were: *Dictyota dichotoma, Halopteris scoparia, Padina pavonica, Udotea petiolata*.

More than half of the species were specific to their depth zone. The only species never found in shadowed conditions was *Chaetomorpha sp.*. On the other hand the following species were only found in areas of limited light exposure due to rock structure: *Peyssonnelia squamaria, Lithothamnion sp., Caulerpa racemosa, Codium adherens, Codium bursa, Cryptonemia sp., Halimeda tuna, Pseudolithophyllum expansum.*

Table 1- Total species list with distribution among depths and different light conditions

Phylum	Class	Species	Total distribution	Light Conditions		
				0-1m	1m-3 m	3m-5m
Ochrophyta	Phaeophyceae	*Dictyota dichotoma*	All	Both	Light	Light
Ochrophyta	Phaeophyceae	*Halopteris scoparia*	All	Both	Both	Both
Ochrophyta	Phaeophyceae	*Padina pavonica*	All	Both	Both	Light
Chlorophyta	Ulvophyceae	*Udotea petiolata*	All	Both	Both	Both
Rhodophyta	Florideophyceae	*Corallina mediterranea*	Top	Both	-	-
Rhodophyta	Florideophyceae	*Jania rubens*	Top	Both	-	-
Rhodophyta	Florideophyceae	*Peyssonnelia squamaria*	Top	Dark	-	-
Chlorophyta	Ulvophyceae	*Chaetomorpha sp.*	Top-Mid	Both	Light	-
Chlorophyta	Ulvophyceae	*Cladophora prolifera*	Top-Mid	Dark	Both	-
Ochrophyta	Phaeophyceae	*Cystoseira sp.*	Top-Mid	Light	Light	-
Rhodophyta	Florideophyceae	*Lithothamnion sp.*	Mid	-	Dark	-
Rhodophyta	Florideophyceae	*Amphiroa rigida*	Mid-Deep	-	Dark	Both
Ochrophyta	Phaeophyceae	*Dictyota linearis*	Deep	-	-	Light
Chlorophyta	Ulvophyceae	*Caulerpa racemosa*	Deep	-	-	Dark
Chlorophyta	Ulvophyceae	*Codium adherens*	Deep	-	-	Dark
Chlorophyta	Ulvophyceae	*Codium bursa*	Deep	-	-	Dark
Rhodophyta	Florideophyceae	*Cryptonemia sp.*	Deep	-	-	Dark
Chlorophyta	Ulvophyceae	*Halimeda tuna*	Deep	-	-	Dark
Rhodophyta	Florideophyceae	*Pseudolithophyllum expansum*	Deep	-	-	Dark

DISCUSSION

In general, the results reveal that light is a driving factor for the species distribution on the rocky shore of Corsica, because the species composition differed between sites of different light regime. However, the species number did not change with depth or daylight exposure. There were similar numbers of species (6-9) in all depth zones as well as in shadowed and light exposed sites in each respective depth zone.

The uppermost 30 cm close to the water surface were dominated by *Corallina mediterranea,* especially on wave-exposed sites (Figure 2). More sheltered sites also showed specimens of *Dictyota dichotoma* and *Jania rubens* (Figure 1) Filiform green algae dominate in even more sheltered areas like rock holes (Figure 2). Reasons may be that this species can survive desiccation but doesn´t have a very high light demand and is therefore able to live in holes. On the other hand it may be less resistant to strong water movement and therefore be not able to grow on exposed (and hence sunny) sites. This is a good example for the weakness of the used method, because species composition in areas with different light levels can be explained by other factors (water movement, grazing, substrate type etc.), as well (Ruitton *et al.* 2000). These factors were not recorded and therefore cannot be part of the analysis. Consequently, the results will be interpreted with respect to light. The reader should keep in mind that other factors can be drivers for the distribution of species, too.

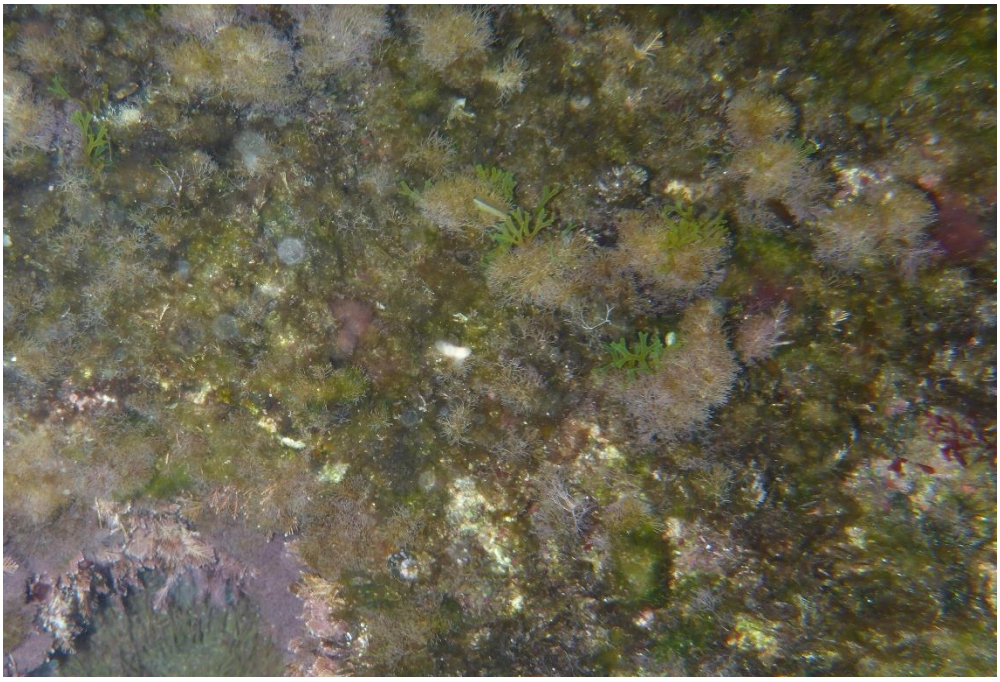

Figure 1-: Typical algae community close to the water surface with J. rubens and D. dichotoma

Figure 2- C. mediterranea, growing in a hole close to the surface

Below 30 cm down to 5 m depth the algal community was mostly dominated by *P. pavonica* and *H. scoparia*. Although single individuals of both species were found at all sites, a clear pattern was observed while snorkeling. *P. pavonica* was dominant at exposed and sunny sites on top of larger rocks while *H. scoparia* rather colonized the shadowed sides of the rocks (Figure 3). Here again light as well as water movement can be the driving factor. However, the fact that *H. scoparia* was very rare in the sun lit upper zone (0-1 m) and *P. pavonica* did not occur in 3-5 m deep shadowed sites, supports the importance of light for this pattern. Other species were not as dominant being found rather as single individuals than as whole stands. Some species can be regarded as generalists with a still high light demand because they were found everywhere except in 3-5m shadowed areas (*P. pavonica, U. petiolata*). Others are specialists with respect to light, being found only at the surface (*J. rubens, C. mediterranea, P. squamaria*) or only at deeper sites (*H. tuna, C. bursa, P. expansum, C. adherens*). Interestingly, some rocks at deeper sites were covered with a yellowish mucus layer at the top that could not be assigned to a certain species (Figure 4). These layers had not been observed in the years before (pers. comm. Prof. Dr. Reinhold Hanel). We assume that it is some kind of benthic marine mucilage, the occurrence of pelagic marine mucilage has increasingly been observed within the last 20 years in the Mediterranean which has been attributed to warming (Danovaro *et al.* 2009).

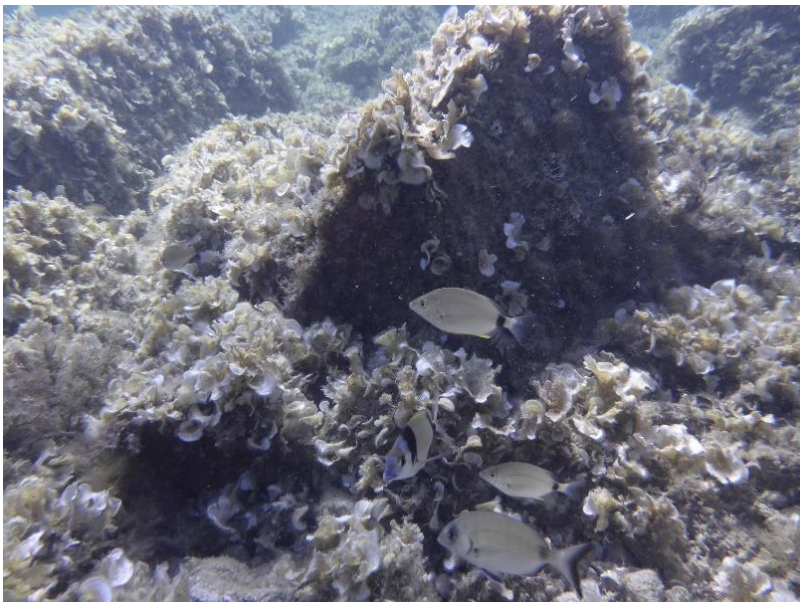

Figure 3-: Larger rocks in 1-3m depth with H. scoparia growing at the side and P. pavonica on top

Figure 4- Rock with P. pavonica and a yellowish mucus layer

The general distribution pattern of the dominant algal species was very obvious and light plays a governing role in their distribution. However, the results still need to be treated with caution, because other species may have been overlooked, especially in the deeper zones where snorkeling was more difficult and the sampling time was restricted due to experience of the free diver.

An additional remark concerns the genus *Cystoseira*. Species of this genus are able to form large stands which provide habitat for a huge variety of benthic organisms (Figure 5). However, these stands have drastically declined in the Mediterranean which is attributed to different human impacts (Thibaut *et al.* 2005). In our study area, which was a human impacted harbor, we could hardly find any *Cystoseira sp*. individuals. In contrast, we found *Cystoseira* -stands in less disturbed areas outside the harbor (Figure 6). This pattern underlines the negative impact of human activity on *Cystoseira sp*. distribution. However, the pattern can also be explained by the absence of large flat shallow water rocks in the harbor, which were the main substrate for *Cystosira sp.* in the areas where it was observed.

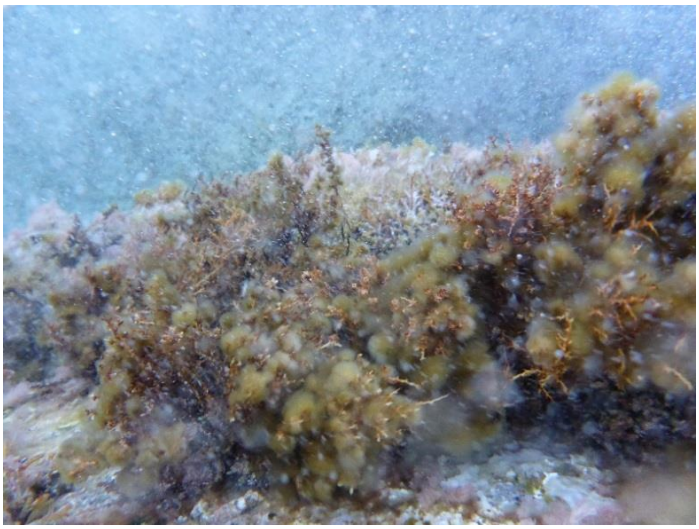

Figure 5- Cystoseira sp., overgrown by J. rubens

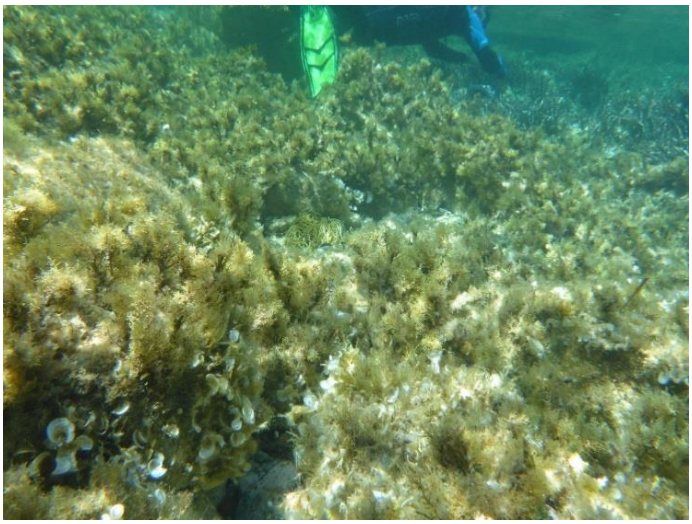

Figure 6- Cystoseira- stand found close to the sandy beach in the Bay of Revelata

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Boulder Field

Daniela Spielmann, Julia Vorhauser

Introduction

During this study trip the boulder field was chosen as one of the areas of investigation. It was selected on the one hand because of its appearance just in front of the STARESO-station and on the other hand because of its dominance around Corsica. Generally, 50-55% of the Mediterranean Sea coast are cliffy; in contrast to that only 15% of the coasts worldwide consist of boulder field (Hofrichter, 2002). Therefore, in other parts of the world, the sediment coasts seem to be dominant. Boulder fields are part of steep coasts and they are defined as coasts with no continuous transition from the continent to the sea.

A very important component of the boulder field formation is the basic material of the coast. The western part of Corsica is mostly composed of granite, which has a hard character and because of that, it degrades slowly. The main force of the boulder field formation is the abrasion, which includes the erosion of ebb and flow, the weather and the wind. Therefore, the boulder field is ground off continually. The part where the abrasion acts is called “cavetto-Hohlkehle” and above this layer the abrasion has less impact on the coast; thus, an overhang starts to form. With increasing time this erosion of the coast leads to the breaking of this overhang due to its own weight. The broken material accumulates along the cliff and gets continuously excavated through the wave activity (Hofrichter, 2002).

With increasing time, the cliffs move back and an abrasion-platform develops. In the case of Corsica this material gets fragmented into boulders because of the hydrodynamic forces. In the boulder field the rocks are sorted by their size more or less. Near the surface the big rocks are located, followed by rubble and finally sand. If the current is strong enough to keep the fields free of sediment underwater landscape with a high diversity develops.

A closer look inside the boulder field shows different levels of diversity, in regard to algae and sessile organism, depending on the size of the rocks. Small and big boulders both show a low level of diversity: while small boulders contain only early successional communities, big boulders are covered only by a few dominant species, which displace other species. The midsize boulders, however, contain the highest level of diversity (Sousa, 1979). This is due to the disturbance level, which is defined by the frequency of their being overturned by the current. The disturbance level it is very high with small boulders and very low with big boulders; midsize boulders underlie intermediate disturbance, which is why more species can become established and which is therefore, why midsize boulders have the highest diversity. This means consequently, that in the boulder field, the theory of intermediate disturbance is confirmed.

Material and Methods

As sampling area the boulder field in the bay in front of the STARESO station was chosen, as well as the adjacent areas. The sampling took place on two half-days via snorkelling. In accordance to the sampling method, the sampling depth was mainly between 0 and 3 m. Species on top of stones as well as underneath the stones in the boulder field were collected by hand and nets, transferred into small plastic bags and afterwards kept in small basins for the following determination. Species identification was conducted by eye, in some cases additionally using stereo microscopes for closer examination. As determination literature mainly “Fauna und Flora des Mittelmeers” by Riedl (1983) was used, only occasionally drawing on specialized literature, such as “Das Mittelmeer” by Hofrichter (2003) or popular identification books like “Was lebt im Mittelmeer” by Bergbauer and Humberg (2009).

In addition to the species found during the two half days reserved for boulder field, species that were found in this habitat during other investigations, such as night-snorkelling or transsect-sampling, were added to the list.

Results

Through the sampling method the main focus of the sampling clearly lies on benthic species, therefore species of certain groups, such as fish or some cnidaria, were not included for this habitat, even when they appeared within the realm of the boulder stones. All in all, 39 species were found (Table 1). In the pie chart in Figure 1 the species-richness of the phyla are compared, however differences in the actual species' frequency were not taken into consideration. This can therefore only be seen as a qualitative investigation, not as a quantitative one.

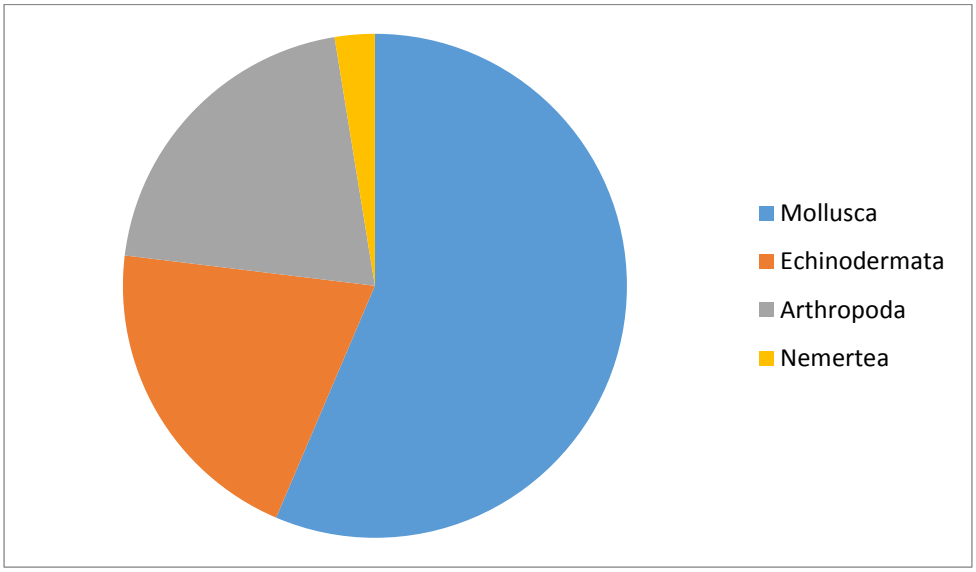

Figure 1- species diversity and distribution in the boulder field of Stareso bay

*Table 1- Species list of the boulder field. The * indicates that the species were determined using literature deviating from Riedl (2012)*

Phylum	Division	Class	Species	German Name
Mollusca	Conchifera	Gastropoda	*Patella coerulea*	Gewöhnliche Napfschnecke
Mollusca	Conchifera	Gastropoda	*Gibbula varia*	
Mollusca	Conchifera	Gastropoda	*Gourmya vulgata*	Gemeine Seenadelschnecke
Mollusca	Conchifera	Gastropoda	*Pisania striata*	
Mollusca	Conchifera	Gastropoda	*Thais haemastorma*	Rotmund-Leistenschnecke
Mollusca	Conchifera	Gastropoda	*Haliotis lamellosa*	Seeohr
Mollusca	Conchifera	Gastropoda	*Bittium reticulatum*	Netzhornschnecke
Mollusca	Conchifera	Gastropoda	*Monodonta turbinata*	Turbanschnecke
Mollusca	Conchifera	Gastropoda	*Conus mediterraneus*	Mittelmeer-Kegelschnecke
Mollusca	Conchifera	Gastropoda	*Thuridilla hopei*	
Mollusca	Conchifera	Gastropoda	*Acanthochitona sp.*	
Mollusca	Conchifera	Gastropoda	*Trunculariopsis trunculus*	Purpurschnecke
Mollusca	Conchifera	Gastropoda	*Collumbella rustica*	
Mollusca	Conchifera	Bivalvia	*Pecten jacobaeus*	Jakobsmuschel
Mollusca	Conchifera	Bivalvia	*Manupecten pesfelis*	
Mollusca	Conchifera	Bivalvia	*Cerastoderma edule*	Gewöhnliche Herzmuschel
Mollusca	Conchifera	Bivalvia	*Ostrea sp.*	Auster
Mollusca	Conchifera	Bivalvia	*Venus verrucosa*	Raue Venusmuschel
Mollusca	Conchifera	Bivalvia	*Loripes lucinalis*	
Mollusca	Aculifera	Placophora	*Chiton olivaceus*	Grüne Käferschnecke
Mollusca	Aculifera	Placophora	*Middendorffia caprearum*	
Echinodermata		Echinoidea	*Paracentrotus lividus*	Steinseeigel
Echinodermata		Echinoidea	*Spaerechinus granularis*	Violetter Seeigel
Echinodermata		Echinoidea	*Arbacia lixula*	Schwarzer Seeigel
Echinodermata		Asteroidea	*Ophiomyxa sp.*	Schlangenstern
Echinodermata		Asteroidea	*Echinaster sepositus*	Purpurstern
Echinodermata		Asteroidea	*Asterina gibbosa*	
Echinodermata		Ophiuroidea	*Ophioderma longicaudum*	
Echinodermata		Holothurioidea	*Holothuria polii*	Weißspitzen-Seegurke
Arthropoda		Crustaceae	*Palaemon serratus*	
Arthropoda		Crustaceae	*Xantho poressa*	
Arthropoda		Crustaceae	*Catapaguroides timidus*	
Arthropoda		Crustaceae	*Pagurus sp.*	Einsiedlerkrebs
Arthropoda		Crustaceae	*Pisidia longicornis*	Schwarzer Porzellankrebs
Arthropoda		Crustacea	*Eriphia verrucosa*	
Arthropoda		Crustacea	*Pachygrapsus marmoratus*	
Arthropoda		Decapoda	*Herbstia condyliata**	
Nemertea		Anopla	*Lineus geniculatus**	

Discussion

When compared to the species lists from the last years a resemblance in the species composition can be found. Also in the last excursions mostly molluscs were sampled, followed by Echinodermata and Arthropoda. Molluscs represent one of the most species-rich phylum inside of the protostomia. This phylum includes the class of the bivalvia, which represents the second most common group that was found during this course. Even though all six bivalvia species as listed above were found on the rocks, only one of them, the species *Ostrea sp*., actually lives on rocks, whereas the others are either free-living (*Pecten sp*. and *Manupecten sp.*) or live in the sand. Hence the shells of all species other than *Ostrea sp.* have most probably been introduced into the boulder field by external forces. Nevertheless it could be observed, that the bivalvia, which naturally live on rocks, were more present on bigger rocks than on the smaller ones. However, there was no obvious difference in their frequency on middle- and big-sized rocks. So based on this investigation the initial theory about the intermediate disturbance could be partly confirmed. Another possible explanation for their staying on the larger rocks could be the proximity to the water surface.

The most frequently detected class of the molluscs was the gastropoda. The gastropoda represent the biggest group within the molluscs and are characterized by a great variety of shapes (http://www.spektrum.de/lexikon/biologie-kompakt/gastropoda/4578, 19.09.2016).

In contrast to the last years Tunicata, Annelida, Echiurida and Porifera are missing in the list. Different reasons can be the answer for that. First of all, during the sampling for the boulder field we did not pay attention to sponges (Porifera) as our eyes were fairly unskilled at that point of time. Their presence and diversity was determined in other fields, like the “Algae” or the “Coralligene”.

For the other groups like the Tunicata, Echiurida and the Annelida, it must be taken into consideration that the results are highly dependent on the sampling method and therefore on the skills of the students who do the sampling. Most of the students hadn’t snorkelled before the excursion and had to learn it from scratch, which might have led to a species list, which deviates from the species actually present in this habitat. This could be why we found fewer species than for example the students in 2012. But, of course, also in the former excursions amateurs performed the sampling, which could lead to a false confirmation of the species present.

The fact that mostly molluscs were found in the excursion could therefore be led back to the fact that molluscs are animals that move rather slowly and are therefore easy to catch in comparison to rapidly-moving groups, such as crabs. Another explanation for their putative abundance could be their houses often survive much longer than the animal itself in contrast to other species and in the case of snails for example are used by hermit crabs; we still counted the species when the animal itself didn't live anymore.

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Coralligène

Alica Ohnesorge, Shaomin Chen

Introduction

Coralligène is defined as secondary biogenic hard bottom formed mainly by calcifying red algae of the genus *Lithophyllum*. It is usually characterized by a clear water phase and being below the zone of providing sufficient light for macrophytes. Moreover, a permanent pycnocline prevents the water column from mixing although strong currents can occur. Hence, the water temperature remains cold throughout the whole year.

One can distinguish two different types of coralligène: the first growing on rocks whereas the second can be found on coastal detritus termed 'platform coralligène'. The latter is the form which occurs in Calvi bay and is commonly located deeper. Especially the region around Corsica is well known for its particular clear water and coralligène habitats being as deep as 80 m. Depending on their associations, coralligène can also be devided into pré-coralligène, *Cystoseira zosteroides*-associations, *Parazoanthus axinellae*-facies, *Asteroides calycularis*-facies, *Gorgonia*-facies, transitional facies to detritic sedimentary bottoms, and facies of the Platform-coralligène. Many not well fixed fragments are the result of the counter game between bio-erosion and re-cementing or newly formed plaques. Net growing rates are extremely slow with 0.5-0.8 mm/year and almost zero in deeper waters; some coralligène therefore being more than 10,000 years old.

In this type of habitat, light and velocity of water are the most important factors determining the settlement of organisms. The coralligène's biodiversity is considered to be very high and is characterized by mainly sessile organisms. This might be related to the diverse habitats characterized by many fissures and holes of different sizes. Due to the poor light conditions, animals are more important than plants in terms of biomass. Nevertheless, the alga family Corallinaceae comprises the main constructors. While Rodophyta are quite common, only very few Chlorophyta like *Ulva* can be expected. Further Coralligène builders are calcareous Chlorophyta, Serpulidae, Bryozoans as well as some Crustaceans. Several sponge species are able to agglutinate smaller pieces of calcareous substratum. In contrast, common taxa responsible for bioerosion are for instance the Polychaeta *Polydora*, the piddock *Lithophaga lithophaga*, and sponges of the genus *Cliona*.

The diverse biocenosis consists mainly of sessile taxa attached to the substratum or digging within it. Also certain cnidarians (especially hydrozoans) can be found frequently. Many worm-like taxa such as plathelminthes, nemertini, echiurida (e.g. *Bonellia viridis*), polychaeta, and sipunculida take advantage of the jointed habitat. Mollusca are represented by gastropoda (including nudibranchia) and bivalvia. Among the crustaceans, the calcifying genus *Balanus* can be expected, as well as harpacticoida, amphipoda, and decapoda. Also potentially contributing to the calcification process are bryozoans.

Since many organisms are hidden within the coralligène, one has to analyze the substratum in detail to detect them.

Materials & Methods

The sampling date was August 27th, 2016. It was the same dredge that was used in the previous years, having the following dimensions:

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

The location was chosen being about 500 m off the Bay of Revellata. Sampling depth was approximately 40-50 meters. It was dredged twice for 5 minutes with a speed of 2 knots. The dredged material was collected in baskets, transported to Stareso Station, and distributed to smaller dishes for further analysis within the next 24 hours.

Results

In our investigation of the coralligène, 19 species were identified, nearly half of which belonged to the algae group (Table.1). Some of the species are shown below (Fig.1 and 2).

Table 1- Species list of coralligène

Phylum/Division	Class	Species
Rhodophyta	Florideophyceae	*Vidalia volubilis*
		Scinaia furcellata
		Botryocladia botryoides
		Lithophyllum racemus
		Rytiphloea tinctoria
		Gracilaria graciis
		Mesophyllum alternans
Chlorophyta	Florideophyceae	*Caulerpa racemosa*
	Ulvophyceae	*Udotea petiolata*
Phaeophyta	Phaeophyceae	*Stilophora rhizoides*
Annelida	Echiura	*Echiurid worm (undef.)*
Annelida	Sipunculida	*Sipunculid worm (undef.)*
Arthropoda	Malacostraca	*Inachus dorsettensis*
Echinodermata	Holothuroidea	*Holothuria polii*
		Holothuria tubulosa
	Ophiuroidea	*Ophioderma sp.*
		Ophiomyxa sp.
Mollusca	Gastropoda	*Aplysia depilans*
		Aplysia parvula

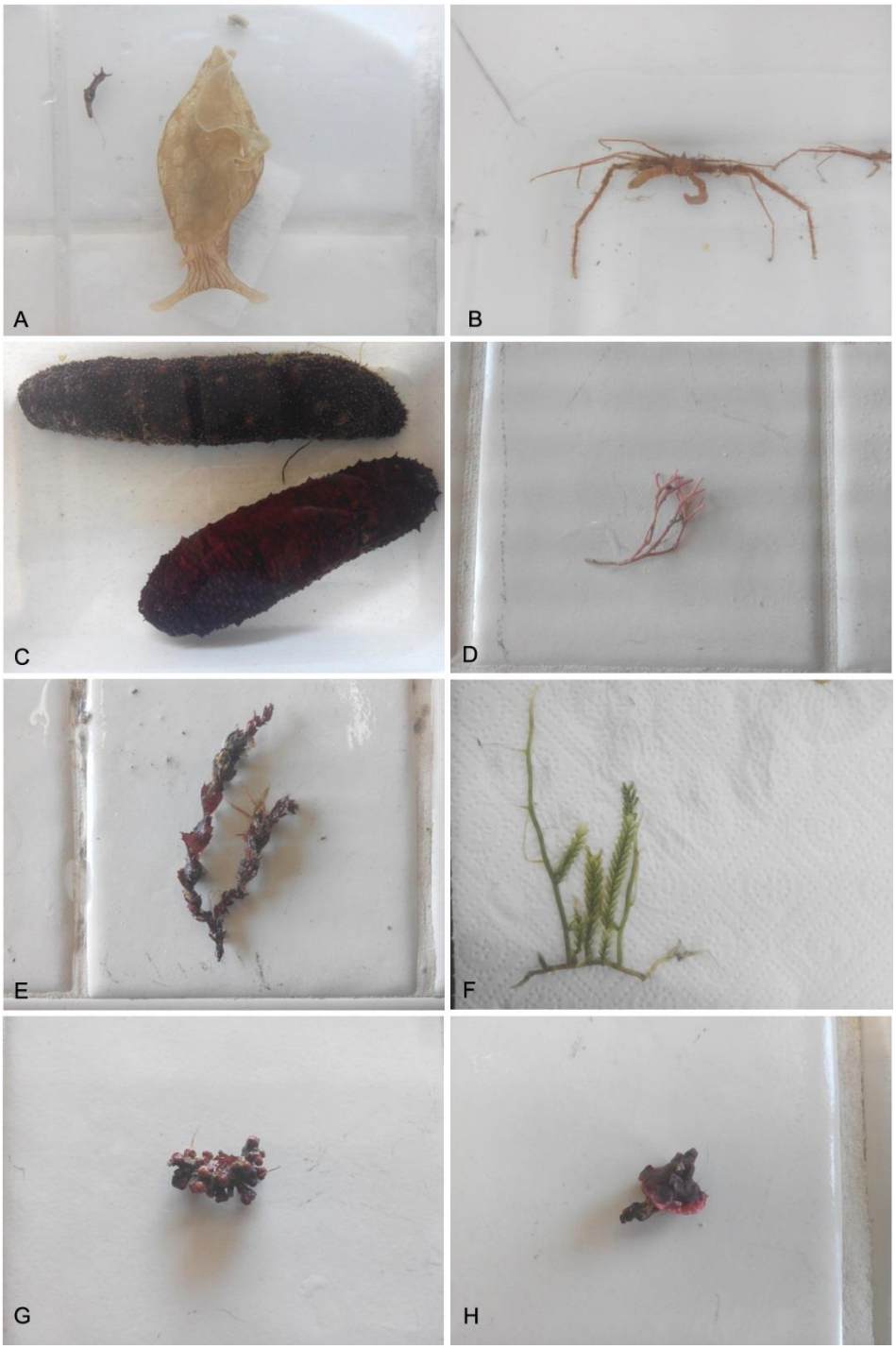

Figure 1- Collected species; A- Sea slugs Aplysia parvula (left) and Aplysia depilans (right); B- scorpion spider crab Inachus dorsettensis; C- sea cucumbers Holothuria polii (top) and Holothuria tubulosa (bottom); D- red algae Scinaia furcellata; E- red alga; F- green algae Caulerpa racemose; G- red algae Lithophyllum racemus; H- red algae Mesophyllum alternans

Discussion

As compared with the results from 2014, representatives of the phyla Chordata, Cnidaria and Nemertea were missing from the list, and in total less species were found this year. This is probably caused by in inefficient dredge sampling, which did not reach the target stone area. Failure in identification could be another reason for the reduction in species number. New species such as *Aplysia depilans* and *Aplysia parvula* (Fig.1a) were also found in our samples. Compared with the species list given by literatures, apparently the dredge sampling was by far not able to collect all the common species in this habitat.

Algae comprised the majority in the species composition of our samples from coralligène which was also found highly diverse in previous studies (SPA, 2003). Importantly, the invasive species *Caulerpa racemosa* (Fig.1f) could be seen in our samples, which was considered as a threat to the biodiversity of coralligène (Verlaque et al, 2003). Unlike in 2014, Molluscs did not dominate the species composition this time, which also could be a result of incomplete species collection by the dredging.

A well planned execution of sampling is the prerequisite for the gain of good and meaningful sampling material which is representative for the target habitat. To consider are the careful preselection of the sampling method as well as the sampling site including depth. If either of them is not appropriate, there might be the risk of low experimental outcome. The efficiency of dredges was already debated in 1958, pointed out by Birkett that dredges collected a maximum of 70 % of the aimed sample volume. Moreover, the sampling depth is very critical since there are big differences of intra- versus inter-specific burrowing activities.

In conclusion, due to the limitation of the sampling methods, our investigation of the coralligène gave us a rough insight into the species composition and did not fully reflect the actual species diversity of the target habitat.

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Figure 2- Undetermined, ca. 40 cm long sipunculid worm

Fish diversity

Nora-Charlotte Pauli, Fabian Wolf

Introduction

The Mediterranean Sea is an almost entirely enclosed basin with a latitudinal range from 45°48'N (Monfalcone, Italy) to 31° 12'N (Sirte, Lybia). Among other factors, it is characterized by its high salinity, which ranges from 35 in the west near the Strait of Gibraltar up to 40 in the most eastern parts of the Levantine basin. Even though the Mediterranean makes up only 1 % of the ocean's surface, it features an astonishing amount of marine diversity. A recent study reported 17,000 species in the Mediterranean Sea including 20 % of endemic species (Coll *et al.*, 2010). According to the online database fishbase (Froese and Pauly, 2016), 750 different fish species occur in the Mediterranean. When addressing biodiversity in the Mediterranean, its history has to be considered. The Mediterranean in its present form goes back to the Tethys Sea, which formed a connection between the Atlantic and the Indian Ocean. After losing its connection to the Indian Ocean some nine million years ago, the Mediterranean basin desiccated several times during the Messinian salinity crisis around six million years ago. Due to this event, most of the species at that time got extinct. About 5 million years ago, the Mediterranean basin was refilled with water from the Atlantic.

Within the scope of our marine biological excursion, we aimed to investigate the fish diversity in the bay of Revellata, Corsica.

Methods

The study site was located on Corsica in the Tyrrhenian Sea off the west coast of Italy. All observations were conducted at STARESO, a marine and oceanographic research station, and in adjacent areas. It is located in the golf de la Revellata opposite to the city of Calvi on the west coast of Corsica. The area surrounding the headland of Revellata is a marine protected area. All fish species that were identified in the entire two weeks were recorded. Furthermore, there was also one day of fish identification training. This single-day identification was conducted between 8 am and 6 pm in the harbor bay of STARESO and adjacent areas by snorkeling.

The identification was based on morphological and behavioural chracaters. In addition, the habitat was of importance, as specific species prefer specific environments. Taxonomic classification was conducted following the World register of Marine Species (WoRMS Editorial Board, 2016) and GBIF (GBIF Directory, 2016).

Results

Overall, 68 fish species (examples in Figures 1 and 2) belonging to 28 families were identified within the period of two weeks. The most diverse families were the Labridae with 12 species and the Sparidae with 13 species. Three species were found for the first time in this area: *Gobius fallax*, *Gymnammodytes* cicerelus and *Pegusa nasuta.*

During the single-day identification, 29 fish species from 12 families were observed. The Labridae with 12 species and Sparidae with seven species were the most diverse families.

Taxonomy of all identified fish species – Species identified during the single-day identification are marked in red.

Kingdom: Animalia

Phylum: Chordata

Class: Elasmobranchii

Order: Myliobatiformis

Family: Dasyatidae

Dasyatis pastinaca (Linnaeus, 1758)

Class: Actinopteri

Order: Anguilliformes

Family: Muraenidae

Muraena helena (Linnaeus, 1758)

Order: Aulopiformes

Family: Synodontidae

Synodus saurus (Linnaeus, 1758)

Order: Gadiformes

Family: Phycidae

Phycis phycis (Linnaeus, 1766)

Order: Atheriniformes

Family: Atherinidae

Atherina boyeri (Rossi, 1810)

Atherina hepsetus (Linnaeus, 1758)

Atherinidae (silversides) are shoal fish feeding mainly on zooplankton (Neumann and Paulus, 2005). *A. hepsetus* is noticeably larger than *A. boyeri* and may be distinguished by a lateral blue stripe.

Order: Beloniformes

Family: Belonidae

Belone belone (Linnaeus, 1761)

Order: Scorpaeniformes

Family: Scorpaenidae

Scorpaena porcus (Linnaeus, 1758)

Scorpaena notate (Rafinesque, 1810)

Scorpaena scrofa (Linnaeus, 1758)

Order: Perciformes

Family: Mugilidae

Oedalechilus labeo (Cuvier, 1829)

Family: Moronidae

Dicentrarchus labrax (Linnaeus, 1758)

Family: Serranidae

Epinephelus marginatus (Lowe, 1834)

Serranus cabrilla (Linnaeus, 1758)

Serranus scriba (Linnaeus, 1758)

In the Mediterranean there are 14 species of the family Serranidae. Most of them are demersal predators, characterized by their mouth, massive habitus and long dorsal fin (Neumann and Paulus, 2005). The most abundant species during our survey was *Serranus scriba* (painted comber).

Family: Apogonidae

Apogon imberbis (Linnaeus, 1758)

Family: Carangidae

Seriola dumerili (Risso, 1810)

Family: Sparidae

Boops boops (Linnaeus, 1758)

Dentex dentex (Linnaeus, 1758)

Diplodus annularis (Linnaeus, 1758)

Diplodus puntazzo (Walbaum, 1792)

Diplodus sargus sargus (Linnaeus, 1758)

Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)

Oblada melanura (Linnaeus, 1758)

Sarpa salpa (Linnaeus, 1758)

Sparus aurata (Linnaeus, 1758)

Lithognathus mormyrus (Linnaeus, 1758)

Pagellus acarne (Risso, 1827)

Pagellus erythrinus (Linnaeus, 1758)

Spondyliosoma cantharus (Linnaeus, 1758)

Sparidae was one of the two most species rich families during the survey. There are 23 species in the Mediterranean, which often make up the majority of coastal fish communities. Many species are shoal fish, e.g. *Diplodus vulgaris* and *Oblada melanura* (Neumann and Paulus, 2005). *Sarpa salpa* is the only species known to feed on the endemic seagrass *Posidonia oceanica* (Jadot *et al.*, 2002).

Family: Centracanthidae

Spicara smaris (Linnaeus, 1758)

Family: Sciaenidae

Sciaena umbra (Linnaeus, 1758)

Family: Mullidae

Mullus surmuletus (Linnaeus, 1758)

Family: Pomacentridae

Chromis chromis (Linnaeus, 1758)

Chromis chromis (damsel fish) is the only species of the family Pomacentridae in the Mediterranean Sea. The damsel fish is the dominant shoal fish in shallow waters. It occurs above hart bottoms and seagrass meadows in 3 to 35 m depth (Neumann and Paulus, 2005).

Family: Labridae

Coris julis (Linnaeus, 1758)

Labrus viridis (Linnaeus, 1758)

Labrus merula (Linnaeus, 1758)

Symphodus cinereus (Bonnaterre, 1788)

Symphodus mediterraneus (Linnaeus, 1758)

Symphodus melanocercus (Risso, 1810)

Symphodus ocellatus (Linnaeus, 1758)

Symphodus roissali (Risso, 1810)

Symphodus rostratus (Bloch, 1791)

Symphodus tinca (Linnaeus, 1758)

Thalassoma pavo (Linnaeus, 1758)

Xyrichtys novacula (Linnaeus, 1758)

Labridae (wrasses) was one of the most species rich families during our survey. There are 21 species of this family in the Mediterranean Sea. Labridae are extremely diverse in morphology, size and colouration. Therefore, identification can be challenging. The most abundant species during this survey was *Symphodus tinca* (peacock wrasse).

Family: Ammodytidae

Gymnammodytes cicerelus (Rafinesque, 1810)

Family: Trachinidae

Trachinus draco (Linnaeus, 1758)

Family: Tripterygiidae

Tripterygion tripteronotum (Risso, 1810)

Family: Blenniidae

Parablennius gattorugine (Linnaeus, 1758)

Parablennius sanguinolentus (Pallas, 1814)

Parablennius rouxi (Cocco, 1833)

Parablennius zvonimiri (Kolombatovic, 1892)

Salaria pavo (Risso, 1810) (Linnaeus, 1758)

Lipophrys trigloides (Valenciennes, 1836)

Lipophrys fluviatilis (Asso, 1801)

Aidablennius sphinx (Valenciennes, 1836)

Coryphoblennius galerita (Linnaeus, 1758)

There are 20 species of Blenniidae in the Mediterranean Sea. The representatives of this family are characterized by a lack of scales, head tentacles and one long undivided dorsal fin (Neumann and Paulus, 2005).

Family: Gobiidae

Gobius bucchichi (Steindachner, 1870)

Gobius fallax (Sarato, 1889)

Gobius paganellus (Linnaeus, 1758)

Gobius geniporus (Valenciennes, 1837)

Family: Gobiescoidae

Gouania willdenowi (Risso, 1810)

Diplecogaster bimaculata (Bonnaterre, 1788)

Lepadogaster lepadogaster (Bonnaterre, 1788)

Family: Callionymidae

Callionymus pusillus

Family: Sphyraenidae

Sphyraena viridensis (Cuvier, 1829)

Order: Pleuronectiformes

Family: Bothidae

Bothus podas (Delaroche, 1809)

Family: Soleidae

Pegusa nasuta (Pallas, 1814)

Discussion

Overall, we were able to identify 69 different fish species within two weeks, which accounts to 9.1 % of the total fish diversity in the Mediterranean Sea. On one day which was especially dedicated to fish identification, 29 fish species were identified. This is the same number as in 2012, which was the last year with comparable data (Table 1). Eight species, which were found this year, were not observed in 2012. This includes the big-scale sand smelt (*Atherina boyeri*), the brown meagre (*Sciaena umbra*), the black scorpionfish (*Scorpaena porcus*) and the dusky grouper (*Epinephelus marginatus*). On the other hand, the common dentex (*Dentex dentex*) and the European seabass (*Dicentrarchus labrax*) were only identified in 2012. However, these differences between the years are mainly caused by a lack of experience of the identifying scientists. Moreover, certain species are difficult to observe and/or restricted to specific habitats. *Scorpaena porcus* for example is a night active predator, which is mainly found in rocky habitats such as large gravel. Adult specimens of *Epinephelus marginatus* are hardly observed while snorkeling since they are found in greater depth on rocky bottoms. Only juveniles occur in shallower waters near coast.
Overall, purely bottom living species, as well as fish species of the open ocean are underrepresented in our results as we were limited to the coastline and observation from the surface.

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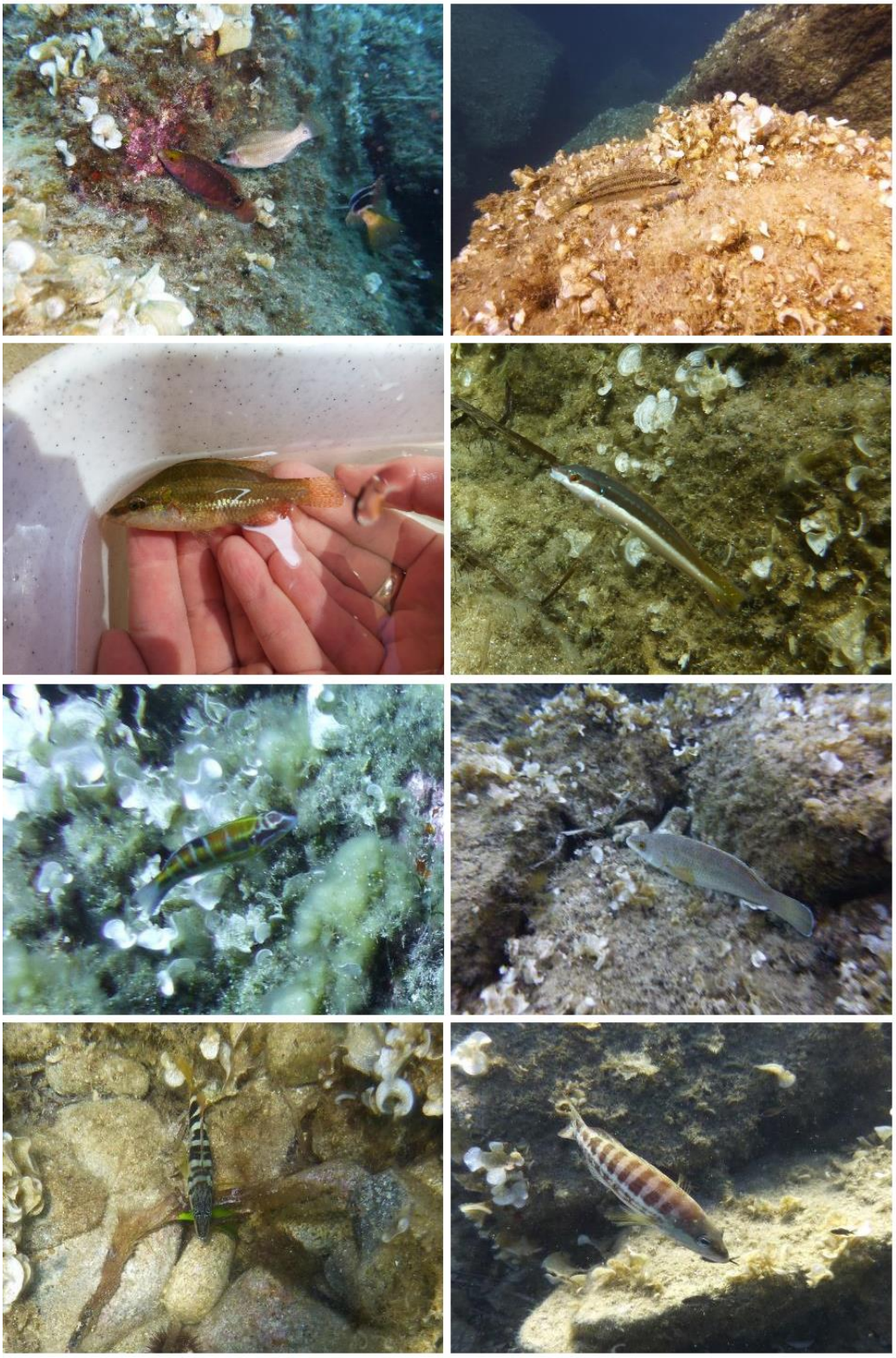

Figure 1- From left to right and top to bottom: Symphodus mediterraneus, Symphodus tinca, Symphodus ocellatus, Coris julis, Thalassoma pavo, Labrus merula, Serranus scriba, Serranus cabrilla (Pictures © Fabian Wolf)

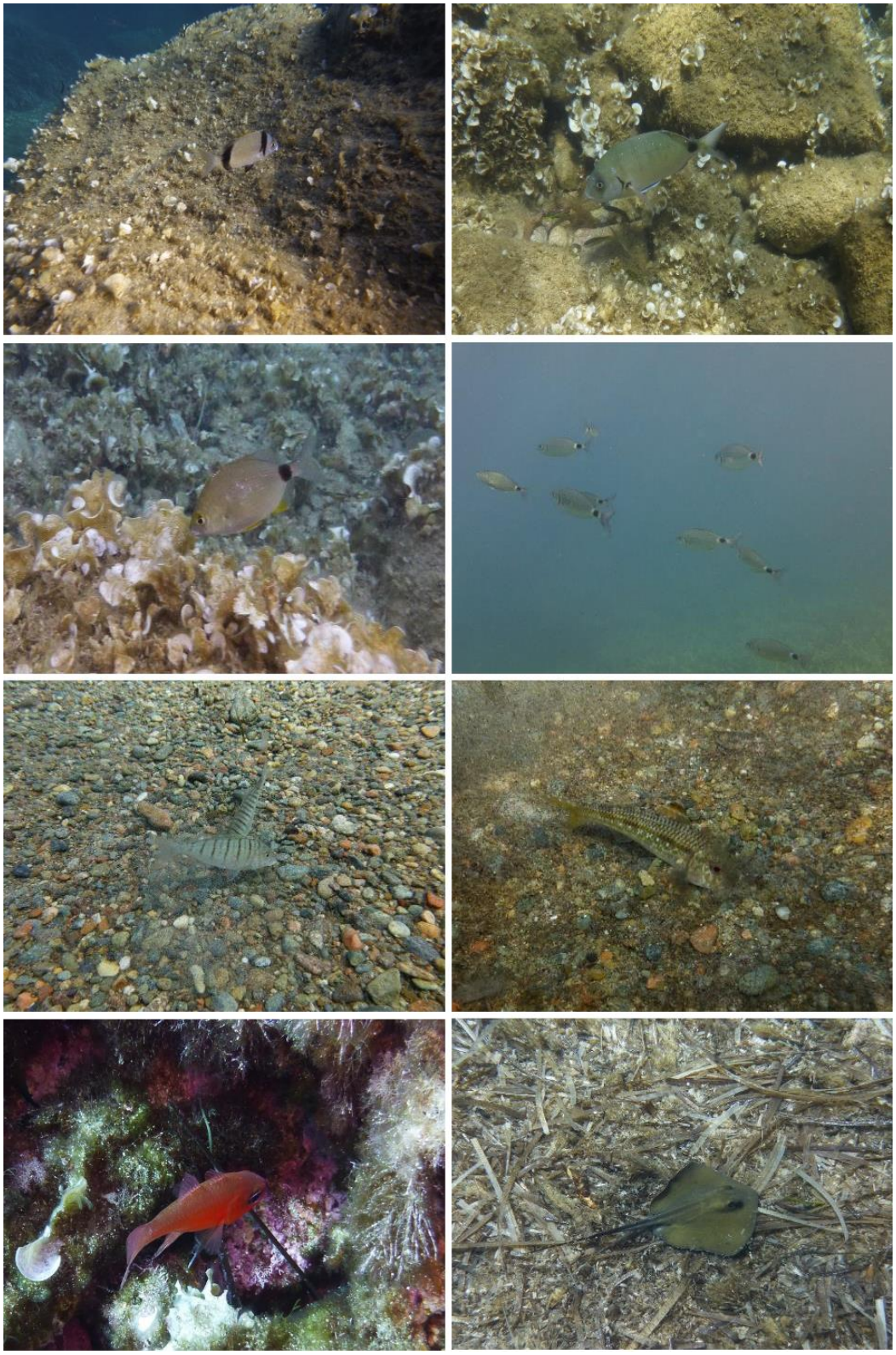

Figure 2- From left to right and top to bottom: Diplodus vulgaris, Diplodus sargus sargus, Diplodus annularis, Oblada melanura, Lithognathus mormyrus, Mullus surmuletus, Apogon imberbis, Dasyatis pastinaca (Pictures © Fabian Wolf)

Table 1- Appendix- Species List

Art	2016	2014	2012	2010	2008	2006	2004	2002	2000	1998	1996
Torpedo marmorata				x							
Dasyatis pastinaca	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	
Conger conger		x			x		x		x	x	
Anguilla anguilla						x	x		x	x	
Engraulis encrasicolus		x				x					
Synodon saurus	x	x	x	x							
Phycis phycis	x	x	x			x		x			
Oedalechios labeo	x	x	x	x	x	x	x	x			
Liza aurata			x	x	x	x	x	x	x	x	
Atherina boyeri	x	x	x	x	x	x	x	x	x	x	
Atherina hepsetus	x	x	x	x	x	x	x		x		
Belone belone	x	x	x	x	x	x		x			
Hippocampus guttulatus										x	
Syngnathus typhle								x			
Dactylopterus volitans				x							
Scorpaena porcus	x	x	x	x	x	x	x	x	x		
Scorpaena notata	x	x		x	x	x	x	x	x	x	
Scorpaena scrofa	x	x		x	x	x		x		x	
Chelidonichthys sp.							x				
Dicentrarchus labrax	x		x	x			x				
Epinephelus marginatus	x	x	x	x	x	x	x	x	x	x	
Serranus cabrilla	x	x	x	x	x	x	x	x	x	x	
Serranus scriba	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	
Seriola carpenteri			x	x	x	x					
Seriola dumerili	x	x				x	x				
Trachinotus ovatus						x					
Trachurus mediterraneus		x					x				
Pomadasys incisus				x							
Boops boops	x	x	x	x	x	x	x	x			
Dentex dentex	x	x	x	x	x	x	x	x		x	
Diplodus annularis	x	x	x	x	x	x	x	x	x	x	x
Diplodus puntazzo	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
Diplodus vulgaris	x	x	x	x	x	x	x	x	x	x	x
Oblada melanura	x	x	x	x	x	x	x	x	x	x	x
Sarpa salpa	x	x	x	x	x	x	x	x	x	x	x
Sparus aurata	x	x	x	x	x	x	x	x	x	x	x
Lithognathus mormyrus	x	x	x	x	x	x	x	x		x	
Pagellus acarne	x	x	x		x		x	x			
Pagellus erythrinus	x	x			x				x	x	
Pagrus pagrus								x			
Spondyliosoma cantharus	x	x	x	x	x	x	x	x	x		x
Spicara maena			x		x	x		x	x		
Spicara smaris	x	x		x							
Sciaena umbra	x	x	x	x		x	x	x			
Mullus barbatus						x	x				
Mullus surmuletus	x	x	x	x	x	x	x	x	x	x	
Chromis chromis	x	x	x	x	x	x	x	x	x	x	
Coris julis	x	x	x	x	x	x	x	x	x	x	x
Labrus viridis	x	x	x	x	x	x	x	x		x	x
Labrus merula	x	x	x	x	x	x	x	x	x	x	x
Symphodus cinereus	x	x	x	x	x	x	x	x		x	x
Symphodus mediterraneus	x	x	x	x	x	x	x	x	x		

Symphodus melanocercus	x	x	x	x	x	x	x	x	x	x	x
Symphodus ocellatus	x	x	x	x	x	x	x	x	x	x	x
Symphodus roissali	x	x	x	x	x	x	x	x	x	x	x
Symphodus rostratus	x	x	x	x	x	x	x	x	x	x	x
Symphodus tinca	x	x	x	x	x	x	x	x	x	x	x
Symphodus doderleini							x				
Thalassoma pavo	x	x	x	x	x	x	x	x	x	x	
Xyrichthys novacula	x	x									
Gymnammodytes cicerelus	x										
Trachinus draco	x	x	x	x	x	x					
Trachinus araneus		x								x	
Trachinus radiatus		x									
Uranoscopus scaber					x	x					
Tripterygion tripteronotus	x	x	x	x	x	x	x	x		x	
Parablennius gattorugine	x	x	x	x	x	x	x	x		x	
Parablennius pilicornis				x							
Parablennius sanguinolentus	x	x	x	x	x	x	x	x		x	
Parablennius rouxi	x		x	x	x		x	x		x	
Parablennius zvonimiri	x	x	x	x	x		x	x		x	
Salaria pavo	x						x	x		x	
Lipophrys nigriceps		x		x	x	x	x				
Lipophrys trigloides	x					x	x				
Lipophrys fluviatilis	x	x	x	x	x	x	x				
Adiablennius sphynx	x	x	x	x	x	x		x		x	

Coryphoblennius galerita	x	x		x	x						
Clinitrachus argentatus							x				
Gobius bucchichi	x					x	x	x		x	
Gobius cobitis		x			x	x	x	x			
Gobius fallax	x										
Gobius paganellus	x					x					
Gobius geniporus	x						x				
Pomatoschistus minutus		x		x	x	x					
Lepadogaster candollei					x	x	x	x		x	
Diplecogaster bimaculata	x	x		x							
Lepadogaster lepadogaster	x			x							
Opeatogenys gracilis			x			x	x				
Gouania wildenowi	x			x							
Callionymus pusillus	x		x	x	x						
Sphyraena viridensis	x	x	x	x	x	x	x				
Bothus podas	x	x	x		x	x					
Phrynorhombus regius				x	x		x	x			
Arnoglossus kessleri										x	
Solea lascaris				(x) *	x	x					
Pegusa nasuta	x										
Ballistes capriscus					x					x	

* identified as *Solea sp.*

Plankton

Laura-Sophie Frommelt, Stephanie Waich

Introduction

In 1887, Viktor Hensen introduced the term “plankton” for describing organisms that float or drift in the open water column. The entire community of marine plankton is characterized by its high diversity. To get an overview of this community of various unicellular and multicellular organisms, there are different categories including phytoplankton and zooplankton as well as bacterioplankton and virioplankton. As the size spectrum of planktic organisms comprises six orders of magnitude (µm to m), their size range is another possibility to distinguish between them (Table 1.).

Table 1- Classification of plankton by size slightly modified form Larink, Westheide (2011)

Term	Size range	Representatives
Mega- or Megaloplankton	0.2 – 2 m	jellyfish and tunicates
Makroplankton	2 – 20 cm	krill, arrow-worms
Mesoplankton	0.2 – 20 mm	copepods, larvae, hydromedusae
Mikroplankton	20 – 200 µm	tintinnids, ciliates, diatoms
Nanoplankton	2 – 20 µm	flagellates
Ultraplankton	< 2 µm	viruses, bacteria

The terms holoplankton and meroplankton allow another differentiation, as holoplankton describes organisms that spend their entire life in the pelagial and meroplankton comprises all animal species that spend only parts of their life cycles (e.g. developing stages) in the open water column and then adapt to an endobenthic or epibenthic life.

The natural turbidity of the water and its viscosity help small planktic organisms to stay in the water column to some extent, but considering the inability of planktic organisms to execute long-distance directed movements, they had to acquire strategies to avoid sinking into deeper water layers. Therefore, some forms store lighter substances (fats, oil, and gas) in their body in order to reduce their density.

Also the relative surface of the organisms plays an important role, as does the formation of body appendices like bristles, fine hair or spines. Other strategies to avoid sinking would be flattening, joining together, building chains, and the reduction of calcium carbonate in the skeleton or the shell.Using these strategies, planktic organisms are able to perform vertical migration depending on seasons and daytime. The main factors for this migration are temperature and light intensity. There is also a difference between migration depending on daytime and migration depending on ontogenesis. Most of the zooplanktic forms have constant migration-patterns.

Plankton is dependent on water-temperature, salinity, light-intensity, water chemistry and other factors that influence its abundance and composition. Significant changes in plankton abundance and composition have massive impact on the food chain since plankton represents the basis of this highly fragile network. The photoautotrophic phytoplankton with its huge abundance represents the basis of the marine food chain and is the main nutrient supply for the heterotrophic zooplankton which in turn is the main food resource for bigger zooplankton and other organisms like fish, whales or sponges. Phytoplankton in its capacity as a primary producer is an important component in the global production of oxygen.

Material and Methods

The most common method catching plankton is a net made of silk, nylon or polyester with a mesh size between 0.05 mm and 0.35 mm. There are even finer nets for catching smaller organisms and also bigger vessels for the Mega- or Makroplankton.

Since most planktic organisms are very fragile, the sampling had to be conducted carefully. A small area in the open water, about 1 km off the coast, was sampled by boat at low speed. The sample was taken with a fine net in a depth of approximately six meters. On the following day another sample was taken from the inner harbor basin in a depth of approximately one meter with the same net but this time pulled by a kayak at very low speed. The samplings took place around 09:30 a.m. and in both cases the net was pulled horizontally through the water for about ten minutes. In the laboratory the samples were transferred into smaller dishes for observation and determination. Therefore regular binoculars were used as well as the microscope for taking pictures of selected organisms. The determination was conducted with the help of appropriate literature (see below).

Results

Table 2 shows the collected systematic data of all students from the open water column sampling by boat. Organisms contained therein were classified to the species level, if possible, using the two marine classification books "Costal Plankton" from Larink, Westheide (2011) and "Fauna and Flora of the Mediterranean Sea" from Abel et al. (1983).

Table 2- Species list of all planktonic organisms found in the open water column sample

Phylum	Division	Class	Species
Acrania		Leptocardia	*Branchiostoma lanceolatum*
Annelida		Polychaeta	Trochophore larvae
Arthropoda	Crustacea	Malacostrac	*Doliolum sp.*
		Malacostrac	*Scyllarus sp.* (larvae)
		Malacostrac	*Pagurus bernhardus* (larvae)
		Malacostrac	*Mysidacea sp.*(larvae)
		Malacostrac	*Porcellana sp.* (larvae)
		Malacostrac	*Crangon crangon* (larvae)
		Malacostrac	*Carcinus sp.* (larvae)
		Malacostrac	*Processa sp.* (larvae)
		Malacostrac	*Callianassa sp.* (larvae)
		Malacostrac	*Upogebia sp.* (larvae)
		Malacostrac	*Liocarcinus holsatus* (larvae)
		Copepoda	*Oncaea sp.*
		Copepoda	*Labidocera sp.*(larvae)
		Copepoda	Cyclopoida species
		Copepoda	*Euterpina acutifrons*
		Copepoda	*Oithona sp.*
		Branchiopod	*Evadne sp.*
		Branchiopod	Cladocera species
		Ostracoda	*Archiconchoecia striata*
Chaetognat		Sagittoidea	*Sagitta sp.*
Cnidaria		Hydrozoa	*Muggiaea sp.* (juvenile)
		Hydrozoa	*Obelia sp.*
Dinozoa		Dinophycea	*Ceratium sp.*
Echinoderm	Eleutheroz	Ophiuroidea	*Ophiothrix fragilis* (larvae)
		Asteroidea	*Asterias rubens* (larvae)
Mollusca	Conchifera	Gastropoda	*Creseis sp.*
		Gastropoda	*Pneumodermopsis sp.*
		Gastropoda	*Clione sp.* (larvae)
Phycophyta		Diatomeae	*Chaetoceros densus*
		Diatomeae	*Rhizosolenia setigera*
Radiolaria		Acantharia	*Phyllostaurus siculus*
		Acantharia	*Amphilitium sp.*
		Rhizosphaer	*Rhizosphaera sp.*
Rhizopoda		Foraminifer	*Globigerinella sp.*
		Foraminifer	*Orbulina universa*
Rotatoria		Monogonon	*Synchaeta sp.*
Sipunculida		Sipunculidea	*Sipunculus nudus* (larvae)
Tentaculata		Bryozoa	Cyphonautes larvae
Tunicata		Thaliacea	*Doliolum sp.*
		Thaliacea	*Thalia sp.*
		Appendicula	*Oikopleura dioica*

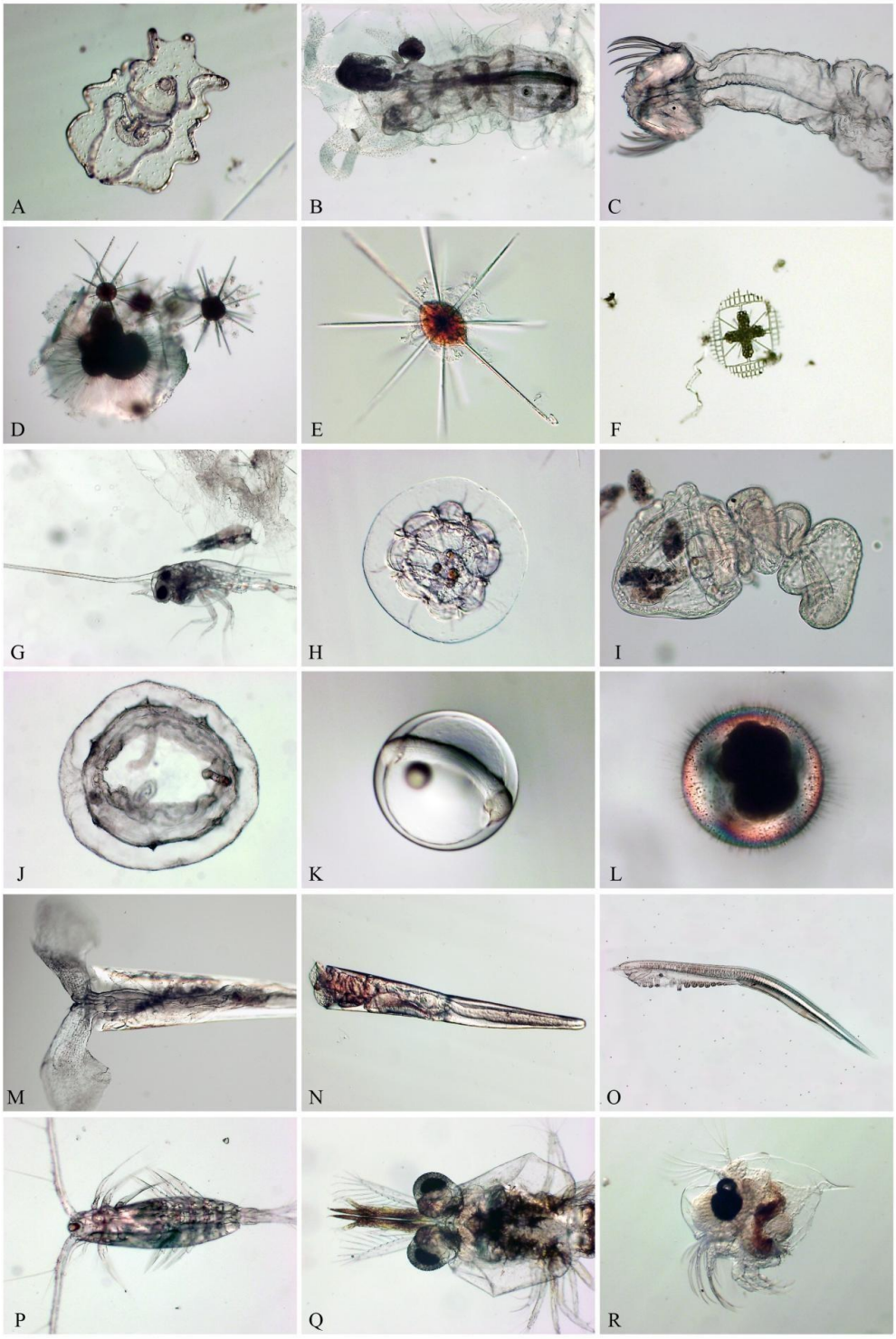

Figure 1- Microscopic images of planktonic organisms found in the open water column sample. A, Bipinnaria larvae;B, Thalia sp.; C, Sagitta sp.; D, Orbulina universa; E, Phyllostaurus siculus; F,L, undetermined Radiolaria species; G, Pagurus bernhardus larvae; H,J, Hydromedusae; I, Sipunculus nudus larvae; K, fish embryo; M, Creseis sp.; N, Creseis sp. larvae; O, Branchiostoma lanceolatum larvae; P, Oithona sp.; Q, Crangon crangon larvae; R, Cladocera species

Figure 1 shows microscopic images of several planktonic organisms which were found in the open water column sample.

Table 3 displays the planktonic species list of the harbor basin sample including the data of all students. The sampling was performed with a fine planktonic net attached to a kayak. Organisms found in this sample were analyzed and, if possible, classified to the species level, again using the two above-mentioned marine classification books.

Figure 2 represents a collection of microscopic images of planktonic organisms found in the harbor basin sample

Table 3- Species list of all planktonic organisms found in the harbor basin sample

Phylum	Division	Class	Species
Annelida		Polychaeta	Hesionidae species
Arthropoda	Cheliceriata	Arachnida	*Copidognathus magnipalpus*
		Arachnida	*Litarachna communis*
	Crustacea	Malacostraca	*Idotea sp.*
		Malacostraca	*Gnathia sp.*
		Copepoda	*Oithona sp.*(larvae)
		Copepoda	*Microsetella norvegica*
		Copepoda	*Euterpina acutifrons*
		Copepoda	*Centropages sp.*
		Copepoda	*Labidocera sp.(*larvae)
		Copepoda	*Longipedia sp.* (larvae)
		Facetotecta	Nauplius larvae
		Ostracoda	*Cypridina mediterranea*
Aschelminthes		Nematoda	undetermined species
Ciliophora		Oligotrichea	*Rhabdonella sp.*
Cnidaria		Hydrozoa	*Muggiaea sp.*(juvenile)
		Hydrozoa	*Obelia sp.*
		Hydrozoa	Hydroid colony
Echinodermata	Eleutherozoa	Ophiuroidea	*Ophiothrix fragilis* (larvae)
Mollusca	Conchifera	Gastropoda	*Clio sp.* (larvae)
		Gastropoda	*Creseis sp.*
		Bivalvia	*Cerastoderma sp.*(juvenile)
Platyhelminthes	Turbellaria	Rhabditophora	Prolecithophora species
	Neodermata	Trematoda	Lepocreadiidae species
Actinopoda		Acantharia	*Acanthostaurus sp.*
Rhizopoda		Foraminifera	*Globigerinella sp.*
Rotatoria		Monogononta	*Synchaeta sp.*
Tentaculata		Bryozoa	Cyphonautes larvae
Tunicata		Appendicularia	*Oikopleura sp.*
Xenacoelomorpha		Acoelomorpha	*Convoluta convoluta*
		Acoelomorpha	*Paraproporus rubescens*

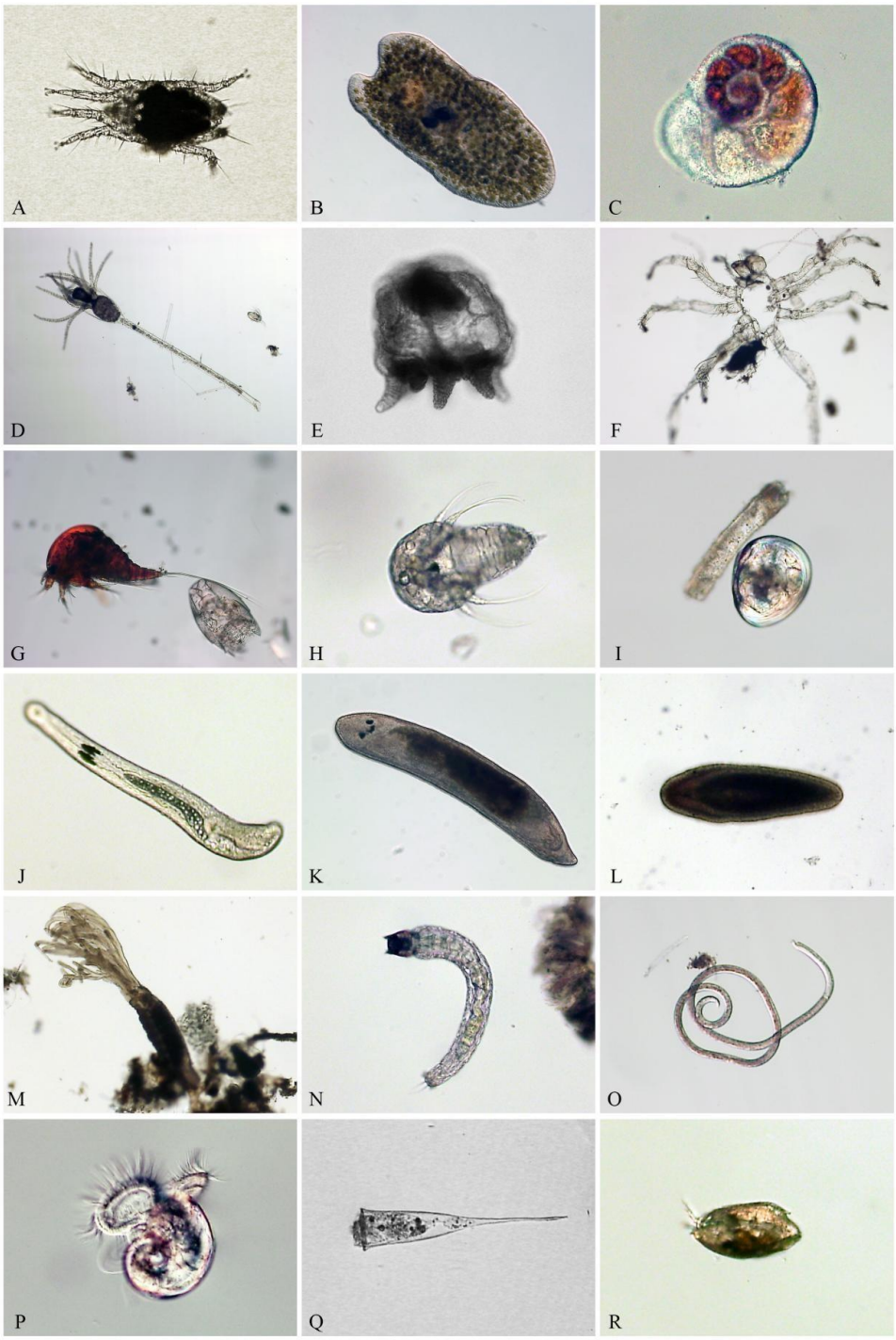

Figure 2- Microscopic images of planktonic organisms found in the harbor basin sample. A, Copidognathus magnipalpus; B, Convoluta convoluta; C, undetermined Pteropoda species; D, part of a hydroid colony – Gastrozoid; E, Hydromedusa; F, Crustacea – larval exuvia; G,H, late and early nauplius larvae; G, undetermined species; H, Facetotecta species; I, Cerastoderma sp (juvenile); J,K, Platyhelminthes species; J, Lepocreadiidae species; K,L Macrostomorpha species; L, Paraproporus rubescens; M,N, Polychaeta species; M, adult individual; N, juvenile individual; O, Nematoda – undetermined speices; P, veliger larvae; Q, Rhabdonella sp.; R, Cypridina mediterranea

Discussion

The different locations and depths, our samples were taken from, and the underlying difference in temperature, chemistry and nutrient content of the water may have led to the dramatically different composition of the two plankton samples (open water column and harbor basin). Also the speed difference between the sample collection by boat or kayak could have influenced the compositions.

There were generally less organisms in the harbor basin sample than in the open sea column sample. Furthermore, Crustaceae were dominant in both samples, although overwhelmingly dominant in the open sea column sample. Molluscs and Tunicata, and here in particular Thaliacea, were also strongly represented in both samples featuring a co- dominance.

In the harbor basin sample also non-planktonic organisms were found, because sessile organisms were detached from the ground and drifted into the plankton net due to rough sea and weather conditions. For example, adult stages of substrate organisms like Acari (Arachnida) or Isopods (Crustacea) together with substrate-clusters were detected in the sample. Also single zooids from Bryozoan colonies were observed, which might derive from colonies located in the sea grass population of the harbour basin ground. Furthermore, Platyhelminthes, which are rather benthic, were exclusively found in the kayak sample. Further explanations for the differences in both sample compositions, besides the rough weather, might be the fact that the harbour basin is more influenced by the surrounding area than the open sea, as well as the limited depth of the harbour basin for vertical migration. Thus, the harbour basin features different living conditions for plankton compared to the open sea. For the first time in this course, Cyphonautes larvae of Bryozoa were found in the planktonic sample.

The sample taken by boat was characterized by a high diversity and included huge quantities of zooplankton with many larvae. A possible explanation for this could be that unusual weather conditions resulted in the movement of nutrient-rich water to this region of the Mediterranean Sea.

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Macrofauna of the sandy beach

Baraldo Mattia; Bertemes Philip

Introduction

The costal zones are highly dynamic marine environments where strong physical forces influence the life of fauna and flora. This interface is composed of specific gradients whose dimensions range from a few nanometers to a kilometer or more. Two-thirds of the world's ice-free coastal zones are composed of sandy beaches. According to Alexander Claude Brown and Anton McLachlan, the sandy beach is a marine sandy littoral area open to the sea including the zone of wave shoaling across the near-shore zone, the surf zone with breaking waves, and the swash zone where wave dissipation takes place (Brown, 2006). Due to the constant hydraulic forces given by the waves the sandy beaches are in constant change. This makes it difficult for the fauna and flora to resist the wave's power. Furthermore, the sand plain does not provide many hiding places. Therefore, all organisms populat this dynamic environment have to adapt. Nevertheless, it is possible to find a high biodiversity at the sandy beach including Crustacea, Bivialvia, Cnidaria, different species of fish, algae and seagrass (e.g. *Posidonia oceanica)*.

The geology of a sandy beach is an aggregation of grains of different size, which are mainly composed of quartz. The factors which influence the porosity and conformation of the sandy beach are complex and depend upon grain shapes, grain size, method of deposition and the subsequent processes of compaction solidification (Perkins, 1974). Sand is able to keep the temperature stable and prevent strong temperature oscillations and avoids salt storage. However the selective pressure in the Mediterranean Sea dependent on the concentration of the salinity is higher compared to the Atlantic Ocean. Figure 1 shows a heat map of different salt concentrations for the Mediterranean Sea. Furthermore, the big granularity of the sand and a constant intake of air by the waves allow abundance of oxygen (Nybakken, 2005; Levinton, 2001).

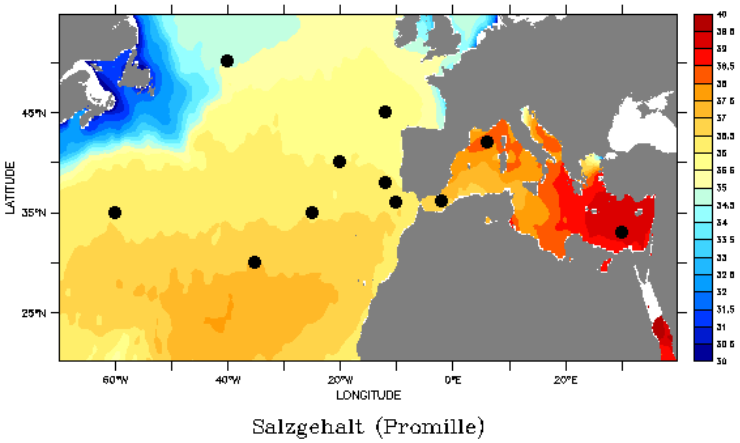

Figure 1- Salinity in Mediterranean Sea and Atlantic Ocean; The salt content (denoted in per mill) is measured in different spots of the Mediterranean Sea and Atlantic Ocean. The results let suggest that the concentration is higher in the Mediterranean Sea (approx. 35per mill in the west to 38.5‰ in the east) compared to the Atlantic Ocean (approx. 37‰ in the south to 31.5‰ in the north)

Fauna

Macrofauna, also referred to as macrobenthos, are animals that live on or in sediments of marine habitats, or attached to hard substrates which are bigger than 0.5 mm (Nybakken, 2005). A correlation between the diversity and abundance of macrofaunal organisms and the particle size and slope of the beach was observed. The mechanical forces originating from the waves have however lower importance (Rodrıguez, 2001). 18 different animal phyla have some representatives in this marine environment, for example annelid worms, bivalves, gastropods, crustaceans, tunicates, and insect larvae (Bell, 1980). Animals living within the substrate need specific forms of adaptation to sandy beach ecosystems. Those animals are also called infauna. The infauna is composed of annelids and bivalves, larval insects, phoronids, amphipod crustaceans, anthozoans, brittle stars and more. The life in the sediment offers considerable protection from predators. While many infauna species are relatively sedentary, others burrow freely through soft sediment (Eleftheriou, 2005). However, even deep burrowers must maintain a connection to water interface in order to obtain oxygen. In opposite to the infauna the epifauna lives on or just above the substrate. They are sessile, relatively sedentary, or highly motile (Gallagher, 1983). Barnacles, tunicates, anthozoans, sponges, oysters, entoprocts, gastropods, crabs, and certain species of amphipods are common to find as epibenthos. Some macrobenthos living organisms are termed “deposit feeders” which means that these animals ingest sediment (sand) and digest associated organic matter (bacteria and microalgae). Marine filter feeding taxa are annelids, tunicates, and hydrozoans, which are highly abundant in coastal marine habitats (Bell, 1980). Another way to hide in places where there are no hiding places is adaptation in form of mimicry. That’s why some organisms in this environment have acquired sand like colors like the European plaice *Pleuronectes platessa*.

Revellata

Southeast of STARESO (station de recherches sous marines et océanographiques) N 42°33'40.8" E 8°43'39.0", the Revellata sandy beach is located. This beach is a special habitat, because it is surrounded by sea grass meadows and boulder fields. These areas provide habitats for other species that only come to the sandy beach to hunt and feed. The species diversity observed in the sandy beach habitat therefore is also influenced by its surroundings, since it is not a closed system. In the north of this ecosystem an access to the open sea is situated where currents provide for sufficient oxygen exchange. The Revellata sandy beach belongs to the category of the so called dissipative beaches, as it has a high wave energy (2-3 m high), the wide surf zone (up to 300-500 m) and the waves dissipate their energy as they break passing over bars

in the surf zone. Furthermore, the wide, low gradient intertidal beach is composed of firm fine sand. However, the beach is prone to extremely high erosion (Nukurangi, 2016).

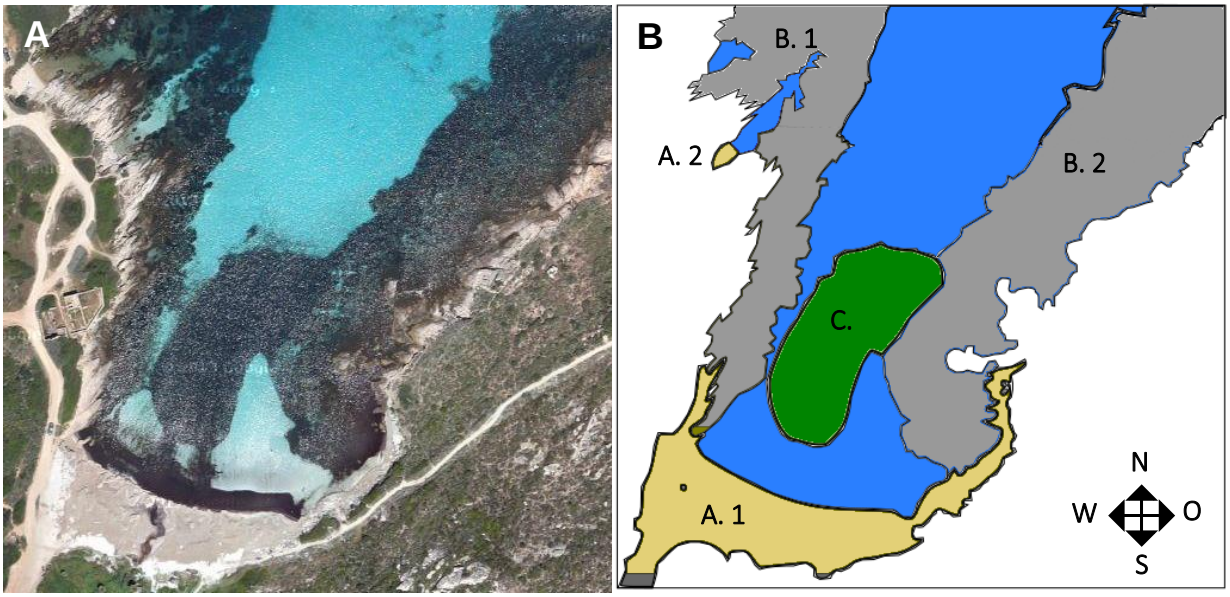

Figure 2- Beach of Revellata from the bird's eye view: the sandy beach is located on the most southern part of the bay. A- Satellite photograph provided by Google Maps; B- The sandy beach is confined by boulder field on the right and left side. Furthermore, a second smaller sandy beach is located at the northwest of the bay. In the middle of the bay a small spot of sea grass meadows determine the local habitat. A.1 & A.2 Sandy Beach; B Boulder Field; C Seagrass Meadow

Materials and Methods

The fauna of the Revellata sandy beach was reported, analyzed and systematically determined on two separate days with different weather conditions. On the first day the sampling was performed on the sandy beach A.2 in the northern part of the bay (see figure 2). The weather conditions were bad the wind coming from the north caused a high wave swell. The waves washed lot of the jellyfish *Pelagia noctiluca* inside the bay and therefore the sampling was interrupted after approximately one hour. The second time the sampling was performed at the more southern situated sandy beach A.1. This time the weather conditions were sunnier and it was less windy.

The main focus was on the determination of the meso- and makrofauna, especially on fishes and mollusks. The meso- and makrofauna was collected in three different manners:

1) Collection by hand: mussel shells were collected and identified at the beach. On the beach and in shallow water, collecting by hands was the main sampling method. The organisms were then placed into plastic zip-lock bags or small plastic containers with screw on lids. This method was mostly applied to larger mollusks and dead shells.

2) Nets and dip nets: This method was primary used to catch fishes. The fishes were then put into some plastic baskets filled with seawater for a better determination. After the systematic identification the animals were released back into the sea. During the whole time the animals were treated in the gentlest possible way and excessive stress was avoided. The animals were only held in the container for a short period of time to avoid stress.

3) Observation: Some animals could be only observed because of different reasons (live in to deep water, protected species, toxic or too fast to catch)

The snorkeling equipment (wet suit, mask, fins and snorkel) was an essential part of the equipment because of a better mobility under water. Furthermore, the systematic book by Riedl was used for the systematic determination (Riedl, 1983).

Results

During the sampling on the Revellata sandy beach, 33 different species were found. There was described one algae species and 32 animal species from sponges till chordates (Table 1).

Table 1- List of described animals of the sandy beach. 33 animals were described as well as one plant species

Phylum	***Division***	***Class***	***Genus***	***Species***
Heterokontophyta		*Phaeophyta*	*Cystoseira*	*mediterraneus*
Porifera		*Demospongiae*	*Ircinia*	*Ircinia sp.*
Cnidaria		*Anthozoa*	*Balanophyllia*	*europaea*
Arthropoda	*Crustacea*	*malacostraca*	*Clibanarius*	*erythropus*
Mollusca		*Bivalvia*	*Barbatia*	*barbata*
Mollusca		*Bivalvia*	*Dosinia*	*lupinus*
Mollusca		*Bivalvia*	*Arca*	*noae*
Mollusca		*Bivalvia*	*Venerupis*	*aurea*
Mollusca		*Bivalvia*	*Musculus*	*costulatus*
Mollusca		*Bivalvia*	*Pseudochama*	*gryphina*
Mollusca		*Bivalvia*	*Venus*	*verrucosa*
Mollusca		*Bivalvia*	*Lucinella*	*davaricata*
Mollusca		*Bivalvia*	*Loripes*	*lucinalis*
Mollusca		*Gastropoda*	*Cripidula*	*moulinsi*
Mollusca		*Gastropoda*	*Monodonta*	*turbinata*
Mollusca		*Gastropoda*	*Hexaplex*	*trunculus*
Mollusca		*Gastropoda*	*Calliostoma*	*Calliostoma sp.*
Mollusca		*Gastropoda*	*Patellacoerulea*	*cerulea*
Mollusca		*Gastropoda*	*Patella*	*caerulea*
Mollusca		*Gastropoda*	*Stramonita*	*Thais Haemastoma*
Echinodermata		*Asteroidea*	*Asterina*	*gibbosa*
Chordata		*Actinopterygii*	*Atherinidae*	*Atherina hepsetus*
Chordata		*Bothidae*	*Bothus*	*podas*
Chordata		*Trachinidae*	*Trachinus*	*draco*
Chordata		*Sparidae*	*Sarpa*	*salpa*
Chordata		*Sparidae*	*Diplodus*	*annularis*
Chordata		*Sparidae*	*Diplodus*	*sargus*
Chordata		*Sparidae*	*Diplodus*	*puntazzo*
Chordata		*Sparidae*	*Diplodus*	*vulgaris*
Chordata		*Sparidae*	*Lithognathus*	*mormyrus*
Chordata		*Sparidae*	*Oblada*	*melanura*
Chordata		*Labridae*	*Symphodus*	*tinca*

Table 2- List of all collected and classified animals form excursion 2002 until 2014

Phylum	***Class***	***Family***	***Species***
Ciliophora	*Ciliata*		
Plathelminthes	*Aceolomorpha*		*Mecynostomidae*
Plathelminthes	*Aceolomorpha*		*Isodiametridae*
Plathelminthes	*Aceolomorpha*		*Symsagittifera corsicae*
Plathelminthes	*Rhabditophora*		
Plathelminthes	*Macrostomorpha*		*Microstomum sp.*
Plathelminthes	*Macrostomorpha*		*Macrostomum sp.*
Plathelminthes	*Polycladida*		*Prosthiostomum siphunculus*
Plathelminthes	*Polycladida*		*Theama mediterranea*
Plathelminthes	*Proseriata*		*Proseriata sp.*
Plathelminthes	*Proseriata*		*Monocelis lineata*
Plathelminthes	*Rhabdocoela*		*Rhabdocoela sp.*
Plathelminthes	*Rhabdocoela*		*Gyratricinae sp.*
Plathelminthes	*Rhabdocoela*		*Rhabdocoela sp.*
Annelida	*Polychaeta*		*Protodrilus*
Annelida	*Polychaeta*		*Polygordius*
Annelida	*Polychaeta*		*Hesionida*
Annelida	*Polychaeta*		*Dorvilleidae*
Cheathognatha	*Cheathognatha*		*Spadella sp.*
Mollusca	*Gastropoda*	*Buccinidae*	*Mitrella scripta*
Mollusca	*Gastropoda*	*Columbellidae*	*Amohissa scripta*
Mollusca	*Gastropoda*	*Turritellidae*	*Turritella communis*
Mollusca	*Gastropoda*	*Phasianellidae*	*Tricolia speciosa*
Mollusca	*Gastropoda*	*Phasianellidae*	*Tricolia pullus*
Mollusca	*Gastropoda*	*Cerithiidae*	*Bittium reticulatum*
Mollusca	*Gastropoda*	*Nassariidae*	*Cyclope neritea*
Mollusca	*Gastropoda*	*Thaididae*	*Thais haemastoma*
Mollusca	*Gastropoda*	*Patellidae*	*Patella aspera*
Mollusca	*Gastropoda*	*Patellidae*	*Patella caerula*
Mollusca	*Gastropoda*	*Haliotidae*	*Hexaplex trunculus*
Mollusca	*Gastropoda*	*Haliotidae*	*Ocinebrina edwardsi*
Mollusca	*Gastropoda*	*Haliotidae*	*Mitrella sp.*
Mollusca	*Gastropoda*	*Haliotidae*	*Calyptraea chinesis*
Mollusca	*Gastropoda*	*Haliotidae*	*Columbella rustica*
Mollusca	*Bivialvia*	*Veneridae*	*Chamelea gallina*
Mollusca	*Bivialvia*	*Veneridae*	*Venus verrucosa*
Mollusca	*Bivialvia*	*Veneridae*	*Dosinia lupinus*
Mollusca	*Bivialvia*	*Veneridae*	*Paphia aurea*
Mollusca	*Bivialvia*	*Limidae*	*Limaria inflata*
Mollusca	*Bivialvia*	*Glycimeridae*	*Glycimeris glycimeris*
Mollusca	*Bivialvia*	*Glycimeridae*	*Glycimeris pilosa*
Mollusca	*Bivialvia*	*Spondylidae*	*Spondylus gaedeopus*

Mollusca	*Bivialvia*	*Limidae*	*Lima lima*
Mollusca	*Bivialvia*	*Cardiidae*	*Acathocardia tuberculatum*
Phylum	***Class***	***Family***	***Species***
Mollusca	*Bivialvia*	*Chamidae*	*Chama gryphoides*
Mollusca	*Bivialvia*	*Tellinidae*	*Acropagia balaustina*
Mollusca	*Bivialvia*	*Lucinidae*	*Loripes lacteus*
Mollusca	*Bivialvia*	*Lucinidae*	*Neverta josephina*
Mollusca	*Bivalvia*	*Lucinidae*	*Ctena decussata*
Mollusca	*Bivalvia*	*Chamoidea*	*Chama sp.*
Mollusca	*Bivalvia*	*Arcidae*	*Barbatia barbata*
Mollusca	*Bilvalvia*	*Pinnidae*	*Pinna nobilis*
Mollusca	*Bivalvia*	*Arcoidea*	*Arca noae*
Mollusca	*Bivalvia*	*Chamidae*	*Pseudochama gryphina*
Mollusca	*Placophora*	*Chitonidea*	*Chiton olivaceus*
Mollusca	*Cephalopoda*	*Sepiidae*	*Sepia officinalis*
Mollusca	*Cephalopoda*	*Octopodidae*	*Octopus vulgaris*
Arthropoda	*Malacostraca*	*Maiidae*	*Macropodia rostrata*
Arthropoda	*Malacostraca*	*Paguridae*	*Diogenes pugilator*
Arthropoda	*Malacostraca*	*Paguridae*	*Clibanarius erythropus*
Arthropoda	*Malacostraca*	*Paguroidea*	*Pagurus sp.*
Echinodermata	*Echinoidea*	*Parechinidae*	*Paracentrotus lividus*
Echinodermata	*Asteroidea*	*Ecinasteridae*	*Echinaster sepositus*
Chordata	*Chondrichtyes*	*Dasyatidae*	*Dasyatis pastinaca*
Chordata	*Actinopterygii*	*Serranidae*	*Epinephelus marginatus*
Chordata	*Actinopterygii*	*Serranidae*	*Serranus cabrilla*
Chordata	*Actinopterygii*	*Sparidae*	*Sarpa salpa*
Chordata	*Actinopterygii*	*Mullidae*	*Mullus barbatus*
Chordata	*Actinopterygii*	*Mullidae*	*Mullus surmuletus*
Chordata	*Actinopterygii*	*Bothidae*	*Arnoglossus laterna*
Chordata	*Actinopterygii*	*Callionymidae*	*Callionymus pusillus*
Chordata	*Actinopterygii*	*Uranoscopidae*	*Uranoscopus scaber*
Chordata	*Actinopterygii*	*Trachinidae*	*Trachinus draco*
Chordata	*Actinopterygii*	*Labridae*	*Symphodus cinereus*
Chordata	*Actinopterygii*	*Labridae*	*Symphodus rostratus*
Chordata	*Actinapterygii*	*Bothidae*	*Bothus podas podas*
Chordata	*Actinopterygii*	*Sparidae*	*Lithognathus mormyrus*
Chordata	*Actinopterygii*	*Atherinidae*	*Atherina boyeri*
Chordata	*Actinopterygii*	*Sphyraenidae*	*Sphyraena sphyraena*
Chordata	*Actinopterygii*	*Labridae*	*Symphodus roissali*
Chordata	*Actinopterygii*	*Sparidae*	*Diplodus vulgaris*
Chordata	*Actinopterygii*	*Serranidae*	*Serranus scriba*
Chordata	*Actinopterygii*	*Atherinidae*	*Atherina hepsetus*

Discussion

During the fieldwork a high number of different species was found at the Revellata sandy beach. In total 33 different species were observed and described. The most common species were part of the phyla Chordata and Mollusca. Furthermore, Porifera, Cnidaria, Arthropoda, and Echinodermata could be found at the sandy beach.

During the previous seven excursions (from 2002 until 2014) 84 different species were found on the sandy beach of Revellata. The list in Figure 3 shows that a higher amount of different species of the phyla Chordata (fishes), Platyhelminthes and Mollusks were observed. Furthermore some representatives of Annelida, Arthropoda and Echinodermata were described.

A reason why more fish species were found could be that in open water these high motile organisms are better detectible. It is easier to approach the animals in shallow water without allowing them to flee in deeper water. Also mollusks were found in high numbers. The majority of them were bivalves, which were easy to collect in water and along the beach.

The difference in the number of species between years can be explained by the lower number of participants in 2016 compared to the past years.

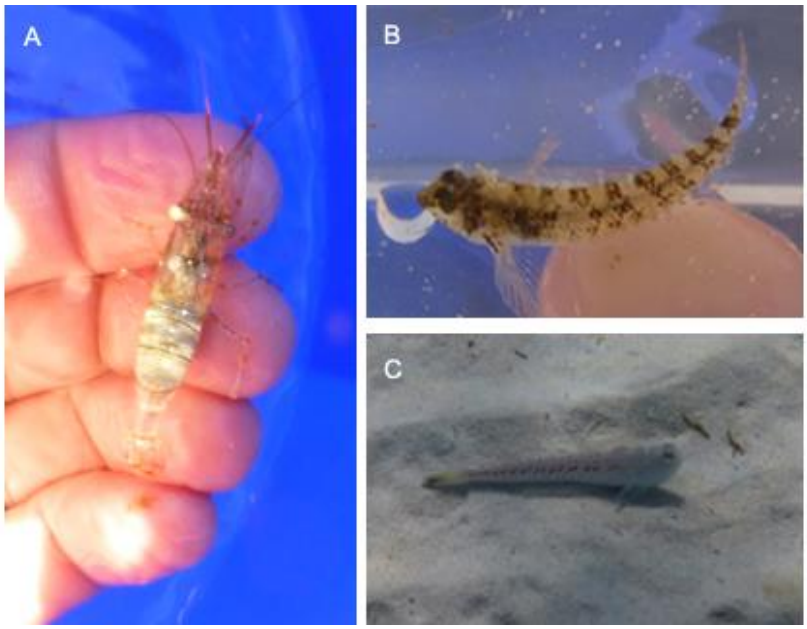

Figure 3- ***[A]*** *Decapoda,* ***[B]*** *Tripterygion tripteronotus* ***[C]*** *Trachinus draco*

Figure 4- Botus podas podas

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Seagrass

Stefanie Pfeifenberger, Jana Ribitsch

Introduction

Seagrass is the only flowering plant in the sea. In the Mediterranean Sea the most important species is *Posidonia oceanica*, where it occurs monotypical, which means it is formed by only one plant species. Seagrass belongs to the Angiosperms and further to the Monocotyledoneae. It has fruits, blossoms and roots (in contrast to algae). The roots fix the plant in the mobile sediment grounds. They live on hard substrate but also on sediment grounds. From the rhizome layer long and thin leaves are growing, which achieve a length from 60 to 80 cm. A basal meristem produces new photosynthetic active leaves all the time. Epibionts colonize the leaves. The structure and diversity of these are dependent on the morphology, life span and the rate of growth of the leaves, but also from the characteristics of the position like light and nutrient availability. The heavily vegetated leaf apex will die and break off. Seagrass grows very slowly with 1 cm per year. The pollination is under water, where the pollen will be drifted away by the sea current.

Worldwide there are 42 Seagrass species known, with only 6 of those occurring in the Mediterranean Sea:

Table 1- Seagrass Species

Species	Characteristics
Posidonia oceanica	endemic in the Mediterranean Sea does not tolerate fresh water
Zostera marina	little tolerance compared to the salinity occurs up to 10 m
Zostera noltii	short roots occurs up to 1 m
Cymodocea nodosa	little tolerance compared to the salinity occurs up to 10 m
Ruppia maritima	occurs in brackish water
Halophila stipulacea	introduced or immigrated does not tolerate fresh water

Posidonia oceanica

It is common in the temperate area and important for costal habitats. Only 0.1 to 0.2 % of the Mediterranean Sea is a suitable place for *Posidonia oceanica*. It occurs in a depth up to 40 meters the upper limit is the low water line or the movement of the water and the lower limit is dependent on the amount of light and the transparency of the water. The main growth is in autumn and winter. In spring the plant produces reserves due to rising temperatures and more light. In summer there is the highest amount of the Epiphytes on the leaves.

Figure 1- Seagrass Posidonia oceanica in the bay of STARESO

Fauna of the seagrass

In one hectar *Posidonia oceanica* can live up to 15 tons of animal biomass. The animals live on the leaves, between the leaves and on the roots of *Posidonia oceanica*. There are specialists, which adapted their body shape, colour, movement and life cycle to the plant, like sea horses. *Posidonia oceanica* offers protection and food. Some of the organisms are not specialized on this habitat like fish. Only Chelonia mydas and sea cows feed on *Posidonia oceanica*.

Figure 2- Animals living in Posidonia oceanica in Calvi

Ecological issues

- Stabilisation of the sea ground.
- Holds nutrients back, which are used by Porifera, Hydrozoans, Bryozoans, Polychaetes, etc.
- Slows down the water movement.
- Enlargement of the area. Under perfect conditions on one square meter sediment ground up to 1000 Posidonia leaves can grow. The leave area index achieves values over 20, which means that on one square meter ground 20 square meter settlement for organisms arise.
- Change the stream.
- Seagrass as habitat with other ecological conditions: structured habitat, which offers organism protection, hiding places, spawning ground and food.
- Primary production, however one quarter of the production is made by epiphytic algae, which live on the seagrass.
- Oxygen production.

Figure 3- The seagrass Posidonia oceanica plays an important ecological role in the Mediterranean Sea

Endangered habitat

Posidonia meadows are ecologically important and highly threatened habitats in the Mediterranean Sea. Under constant environmental conditions they grow very slowly, but in the last decades a decline of the seagrass meadows has been noticed. *Posidonia oceanica* is very sensitive to environmental changes. There are anthropogenic effects, like changes in the flow conditions and sedimentation by construction activity and shipping traffic. Heavy eutrophication leads to strong growth of algae, which suffocate the seagrass and decline the amount of light available because of the water turbidity. Mechanical damage through the anchor and the trawl nets tear holes in the seagrass meadows. Storms and strong currents increase these holes. These "wounds" often cannot heal because of the slow accumulation rate of *Posidonia oceanica*. There is also damage caused by the higher erosion of the soil and therefore an increased possibility of an emersion of fresh water. Climate change and an increase of the temperature of the water may have an effect on the development of *Posidonia oceanica* (R. Hofrichter, 2001).

Materials

- Knife
- Plastic bag
- Snorkelling equipment
-

Methods

We collected the seagrass with a plastic bag at a depth of 3-4 meters. We use the seagrass meadow at the sandy soil of the harbor of the research station STARESO. We used the plastic bag for our sampling to achieve the highest diversity possible of different epiphytic organisms. When we had reached the seagrass, we opened the plastic bag and pulled it very carefully over one single plant. To perform a controlled sampling of the *Posidonia oceanica* we cut the roots with a knife. Following this, we pulled the plastic bag further over the roots of the plant and closed it under water with the sample inside. Back on land, we put our sample with all the surrounding water and remains of the soil in an empty bucket and brought it immediately to the lab. Then, we examinded it under a stereomicroscop and determined the biodiversity by using Riedl (1983).

Figure 4- A snorkelling student looking for animals in the seagrass

Results

The following list contains all organisms found in the seagrass *Posidonia oceanica* at three to four meters depth, but it does not give the abundance of each species. Additional to the list, we also found some Polychaeta larva. The list was made according to Riedl (1963) and the website: http://www.marinespecies.org/index.php.

A total of 35 different species belonging to ten phyla were found in the seagrass sample, but only 16 individuals were identified to the species level. Most individuals belong to the arthropods and all algae belong to the phylum Rhodophyta. Due to the little time available and the difficulties of identifying animals, some individuals could not be identified to the species level.

Table 2- Summary of all collected and classified animals in Posidonica oceanica at a depth of 3-4 meters at the marine station STARESO in Calvi (Corsica) on the 22 August 2016

Phylum	Division	Class	Family	Species
Annelida		Clitellata	Tubificidae	Aktedrilus monospermathecus
Annelida		Polychaeta		
Annelida		Polychaeta	Onuphidae	Hyalinoecia fauveli
Annelida		Polychaeta	Serpulidae	Spirorbis pagenstecheri
Annelida		Polychaeta	Serpulidae	Spirorbis sp.
Arthropoda	Chelicerata	Arachnida	Acari	
Arthropoda	Chelicerata	Arachnida	Pontarachnidae	Pontarachna punctulum
Arthropoda	Chelicerata	Pycnogonida	Pycnogonidae	
Arthropoda	Crustacea	Copepoda		
Arthropoda	Crustacea	Copepoda	Harpacticidae	
Arthropoda	Crustacea	Copepoda	Porcellidiidae	Porcellidium sp.
Arthropoda	Crustacea	Malacostraca	Amphipoda (Order)	
Arthropoda	Crustacea	Malacostraca	Decapoda (Order)	
Arthropoda	Crustacea	Malacostraca	Gammaridae	
Arthropoda	Crustacea	Malacostraca	Gammaridae	Gammarus sp.
Arthropoda	Crustacea	Malacostraca	Isopoda (Order)	
Arthropoda	Crustacea	Malacostraca	Pancarida/Peracarida (Superorder)	
Arthropoda	Crustacea	Ostracoda		
Bryozoa				
Bryozoa		Gymnolaemata	Bicellariidae	
Bryozoa		Gymnolaemata	Cryptosulidae	Cryptosula pallasiana
Bryozoa		Gymnolaemata	Membraniporidae	Membranipora sp.
Cnidaria		Hydrozoa	Sertulariidae	
Nematoda				
Platyhelminthes				
Platyhelminthes		Rhabditophora	Prosthiostomidae	Prosthiostomum siphunculus
Foraminifera				
Foraminifera		Globothalamea	Homotrematidae	Miniacina miniacea
Sipuncula		Sipunculidea	Golfingiidae	Golfingia margaritacea
Xenacoelomorpha		Acoela	Sagittiferidae	Symsagittifera sp.

Table 3- Summary of all collected and classified algae in Posidonica oceanica at a depth of 3-4 meters at the marine station STARESO in Calvi (Corsica) on the 22 August 2016

Phylum	Division	Class	Family	Species
Rhodophyta		Florideophyceae	Acrochaetiales (Order)	
Rhodophyta		Florideophyceae	Ceramiaceae	Antithamnion sp.
Rhodophyta		Florideophyceae	Corallinaceae	
Rhodophyta		Florideophyceae	Corallinaceae	Lithophyllum incrustans
Rhodophyta		Florideophyceae	Corallinaceae	Litophyllum sp.

The phyla of *Posidonia oceanica*

A total of ten phyla were found in *Posidonia oceanica*. Approximately two-thirds of the species belong to the arthropods. More than ten percent of the individuals belong to the Annelida, the Bryozoa and the Rhodophyta. The other phyla represent less than seven percent of our findings.

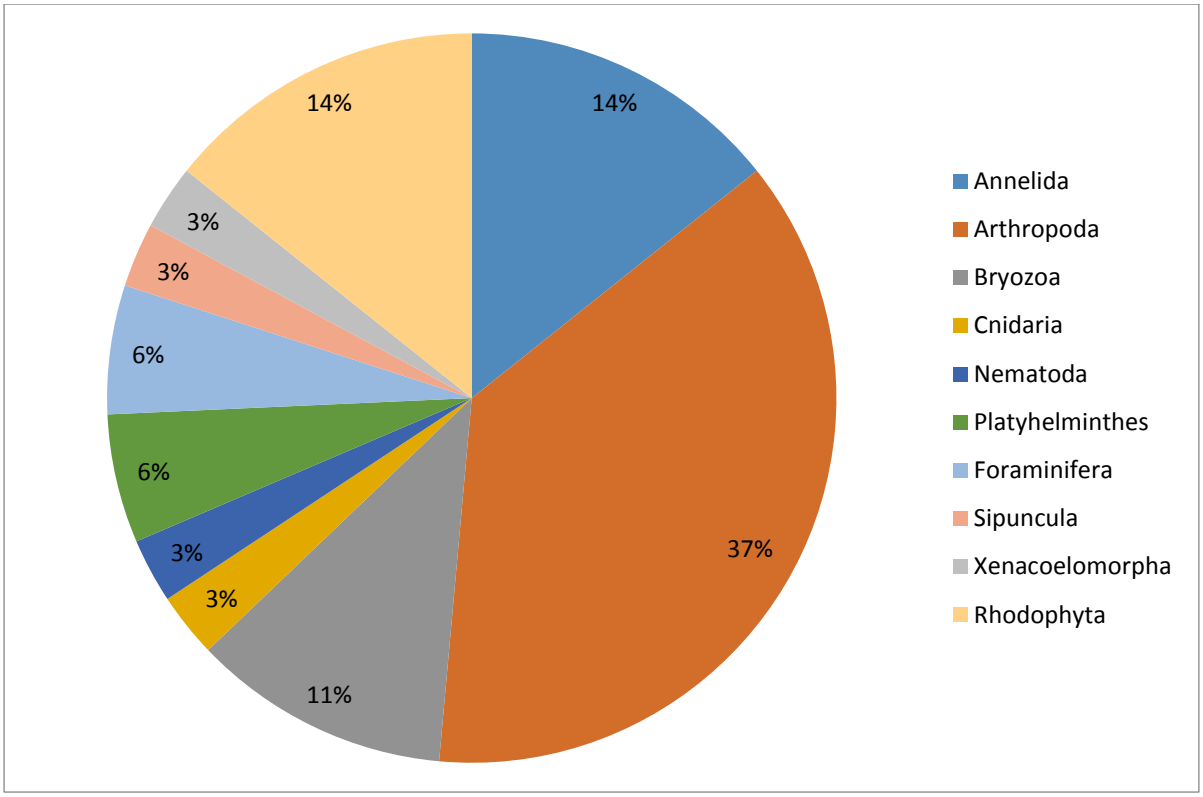

Figure 5- The different number of Phylum found in the Posidonia oceanica. Composition of the different Phyla found in Posidonia oceanica. The figure shows only the species diversity within phyla, but not the abundance of the species

The arthropods in *Posidonia oceanica*

We only found two divisions within the Arthropoda, three quarters belong to the Crustaceae and one quarter to the Chelicerata (Fig 6). Within the Chelicerata two-thirds of the species belong to the class Pycnogonida and one-third to the Arachnida (Fig 7). Within the Crustaceae there are three classes. 60 percent is made up by the Malacostraca followed by the Copepoda (30 percent) and only 10 percent consists of the Ostracoda (Fig 8).

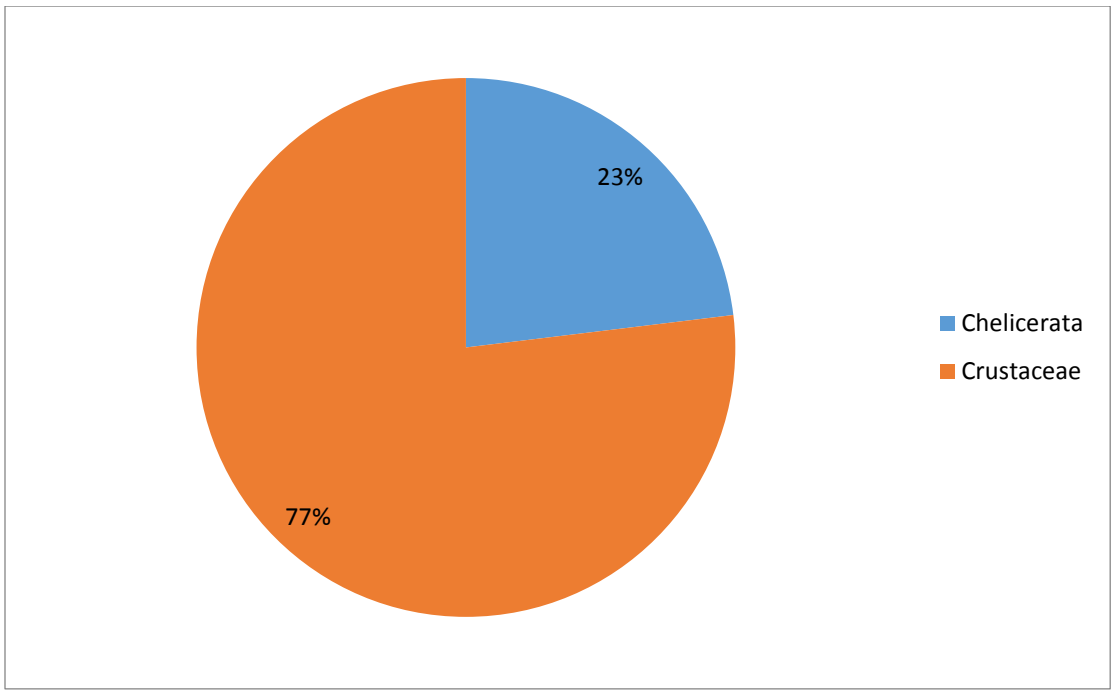

Figure 6- The different divisions of the Arthropods found in Posidonia ocanica. Two divisions of Arthropods found in Posidonia oceanica

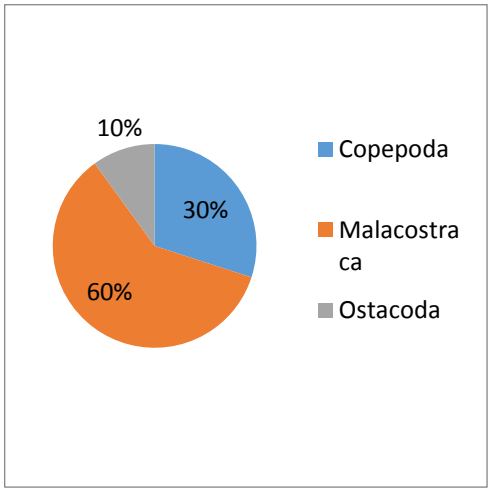

Figure 7- Two classes within the Chelicerata

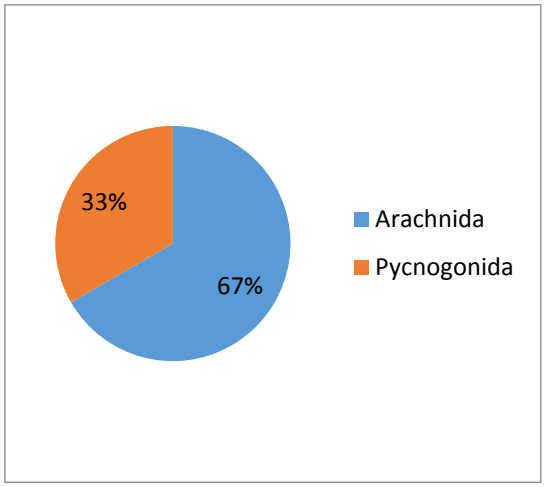

Figure 8- Three classes within the Crustaceae

The Species in *Posidonia oceanica*

Most of the identified species belong to the Annelida closely followed by the Arthropoda and the Rhodophyta. In some of the phyla the abundance of one species is high, but in contrast the variety is small.

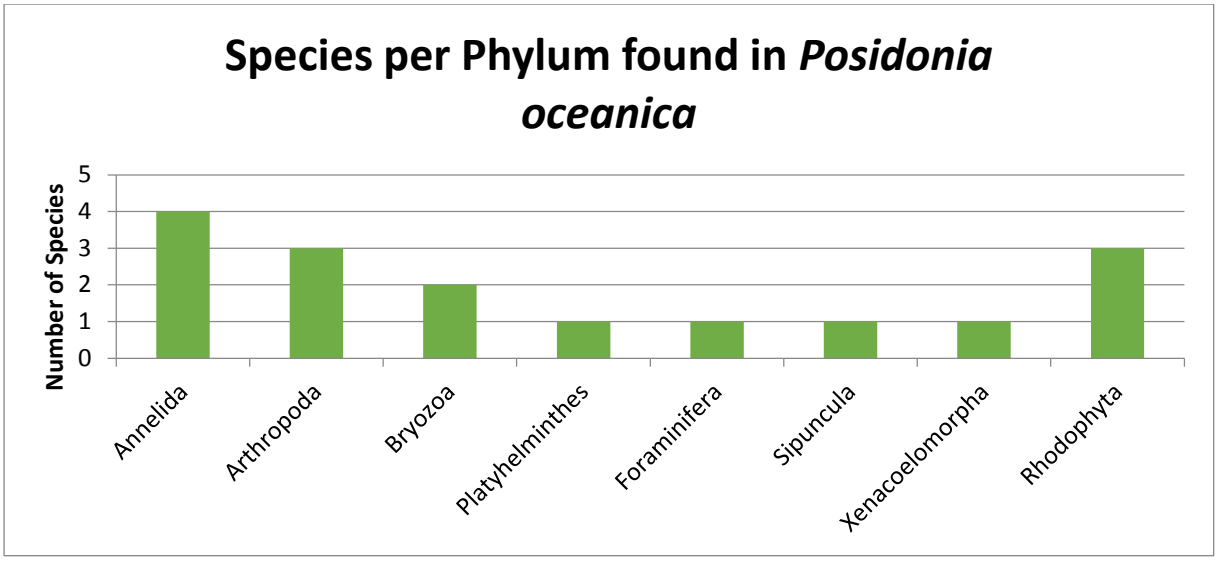

Figure 9- The different number of species of all Phyla that was found in Posidonia oceanica. Only one individual of a species was counted, thus the data not represent the abundance of each species

Discussion

In summary we found 30 animal species in our *Posidonia oceanica* samples, belonging to nine phyla. Additionally, we identified five different Algae, which were all classified as Rhodophyta. The phylum with the most identified species was Annelida, with four species identified, followed by Arthropoda with three species and Bryozoa with two species (see Tab.1, Fig. 5 and Fig. 9 and for the Algae Tab. 2). Within the Arthropoda, which represent approximately two-thirds of the species in all ten Phyla (including the Algaes), we found two divisions, were three quarter belong to the Crustaceae and the remains to the Chelicerata, which could be further divided in the classes Arachnida and Pycnogonida (see Fig 6 and Fig. 7). Whereas the Chelicerata could only be divided into two classes the Crustaceae could be separated in three: the Malacostraca (60%), the Copepoda (30%) and the Ostacoda (10%) (see Fig 8).

In comparison to 2014 less species could be found and identified. We speculate that this is primarily due to the small group of students of this year. In addition, the weather conditions were really bad. It was windy and we had to struggle with a heavy sea, which of course affected our sampling. It was really hard to collect the seagrass down at the meadow carefully, because we had to touch the plant and put it in the plastic bag with our hands, otherwise it would have been impossible to save the sample. Most probably, this led to loss of animals from the plant, enhanced the number of species found in the "open water", and after all resulted in a smaller amount of species in our collection.

In addition, the diversity of our *Posidonia oceanica* samples was dependent on our snorkelling skills. Because we were beginners and diving in difficult conditions (weather) was not our strength, we were not able to go deeper than 4 meters to sample the plants. This might well account for the decreased diversity of species compared with the excursion from the past years.

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Girolata & Fango

Baraldo Mattia, Bertemes Philip

Girolata

The region Girolata lies in the north west of Corsica and is famous for its sandy beaches. It is also part of the Regional Natural Park of Corsica and was added to the World Heritage List in 1983 (UNESCO, 2014). This reserve extends from the Scandola peninsula which is a relict of volcanic activity from the Permian era and shines in a bright red color. This red color is given by a complex of different minerals such as rhyolite porphyry and basalt (Michaela Hittorf, 2014). The marine ecosystem consists not only of sandy beaches but also of boulder fields, seagrass meadows and a wide system of underwater caves. Typical for the terrestrial part of the Natural Park is dense scrubland vegetation, often including herbs, grasses and geophytes.

The marine life in this part of the island is particular and in some aspects unique for the Mediterranean Sea. (Michaela Hittorf, 2014). Being more sheltered against incoming waves (see Figure 1), the Girolata beach is not a high energy beach like Golfe de Galéria (Fango Delta)

Figure 1- Map overview of the north-west of Corsica. ***A)*** *blue: The Gulf of Girolata.* ***B)*** *red: the Fango River*

Fango River

From the well which lays in the elevated plain of Paglia Orba the Fango River flows from east to west into the golf of Galèria. The Fango River has a total length of ca. 25 km and is situated in the south of Calvi. Since 1977 the Fango-valley is under the protection of the UNESCO program (UNESCO, 2014). However the Fango River is strongly influenced by weather and temperature, which can lead to a very muddy to dry arroyo or a dangerously high water level during the rainy season. This variation is visible at the mouth of the river. In the winter seasons (November 100 mm) (Climate-Data.org, 2016) the water level is so high that the river floods the entire beach. On the contrary to that in summer (July 9 mm) the river flows subterranean into the sea. The shape of the river is particular, because the water flow is interrupted by little pools which are situated along the river. The inner shore of the golf of Galèria next to the Fango-Delta is a so called high energy beach where the waves reduce the quantity of sediment present on the beach by carrying it out to bars under the sea (geography, 2016).

Material & Methods

The sampling was performed in three different ways. Firstly, the living organisms were observed and determined in their natural habitat without interfering with them. Secondly, to catch some fish and mollusks a hand net was used. Thirdly, mussels and snail shells were collected along the beach by hand and placed in plastic zip-lock bags, small plastic containers with screw on lids or in big plastic bowls. All animals were treated with respect and were immediately released after the determination. The observation and data collection in both habitats was performed with snorkel and mask. In addition, wet suits and fins were used at the Girolata beach. During the excursion the water level of the Fango River was in the norm.

Results

The animals were classified in two different places. A) At the Girolata beach (saltwater) and at the B) Fango River (freshwater). Furthermore the results of the excursion 2014 were compared to the year 2016 to analyze if there are some important aberrations. The animals were classified by using the Riedl as classification book (Riedl, 1983).

Girolata 2016 (A)

Table 1- List of the Girolata beach 2016: There were found 14 different animal species

Phylum	Class	Genus	Species
Arthropoda	Malacostraca	*Stenorynchus*	*sp.*
Arthropoda	Malacostraca	*Percnon*	*gibbesi*
Mollusca	Bivialvia	*Pseudochama*	*sp.*
Mollusca	Bivialvia	*Pinna*	*nobilis*
Mollusca	Bivialvia	*Arca*	*noae*
Cephalopoda	Octopodidae	*Octopus*	*vulgaris*
Echinodermata	Echinoidea	*Arbatia*	*lixula*
Chordata	Dasyatidae	*Dasyatis*	*pastinaca*
Chordata	Sparidae	*Sarpa*	*salpa*
Chordata	Sparidae	*Diplodus*	*annularis*
Chordata	Sparidae	*Diplodus*	*sargus*
Chordata	Sparidae	*Diplodus*	*puntazzo*
Chordata	Sparidae	*Oblada*	*melanura*
Chordata	Sparidae	*Diplodus*	*vulgaris*
Chordata	*Ammodytidae*	*Gymnammodytes*	*cicerelus*
Chordata	Sphyraenidae	*Sphyraena*	*viridensis*
Chordata	Actinopteriygii	*Mullus*	*surmuletus*

Fango 2016 (B)

Table 2- List of the Fango River fauna 2016: There were found only three limnic species in the Fango River

Phylum	Class	Genus	Species
Plathelmintes	Tricladida	*Dugesia*	*sicula*
Chordata	Actinopteriygii	*Salaria*	*fluviatilis*
Chordata	Amphibia	*Hyla*	*sarda*

Girolata 2014 (A)

Table 3 Collected data of the Girolata excursion 2014. The excursion of 2014 to the Girolata beach was the first time. The years before the excursions were done to the Fango-Delta. In total 32 different species were found

Class	Family	Genus	Species
Maxillopoda	Balanidae	-	-
Bivalvia	Arcidae	*Barbatia*	*barbata*
Bivalvia	Pinnidae	*Pinna*	*nobilis*
Bivalvia	Chamidae	*Pseudochama*	*sp.*
Bivalvia	Lucinidae	*Ctena*	*sp.*
Bivalvia	Ostreidae	*Ostrea*	*edulis*
Gastropoda	Thaididae	*Thais*	*haemastoma*
Gastropoda	Patellidae	*Patella*	*sp.*
Gastropoda	Trochidae	*Monodonta*	*turbinata*
Cephalopoda	Octopodidae	*Octopus*	*vulgaris*
Echinoidea		*Paracentrotus*	*lividus*
Echinoidea	Arbaciidae	*Arbacia*	*lixula*
Chondrichthyes	Dasyatidae	*Dasyatis*	*pastinaca*
Actinopterygii	Carangidae	*Seriola*	*dumerili*
Actinopterygii	Carangidae	*Trachurus*	*mediterraneus*
Actinopterygii	Centracanthidae	*Spicara*	*smaris*
Actinopterygii	Sparidae	*Pagrus*	*pagrus*
Actinopterygii	Sparidae	*Lithognathus*	*mormyrus*
Actinopterygii	Sparidae	*Pagellus*	*erythrinus*
Actinopterygii	Sparidae	*Diplodus*	*puntazzo*
Actinopterygii	Sparidae	*Diplodus*	*vulgaris*
Actinopterygii	Sparidae	*Diplodus*	*annularis*
Actinopterygii	Sparidae	*Oblada*	*melanura*
Actinopterygii	Sparidae	*Sarpa*	*salpa*
Actinopterygii	Labridae	*Coris*	*julis*
Actinopterygii	Pomacentridae	*Chromis*	*chromis*
Actinopterygii	Trachinidae	*Trachinus*	*radiatus*
Actinopterygii	Mullidae	*Mullus*	*surmuletus*
Actinopterygii	Serranidae	*Serranus*	*scriba*
Actinopterygii	Atherinidae	*Atherina*	*hepsetus*

Fango 2014 (B)

Table 4 Collected data of the Fango River excursion 2014. The excursion of 2014 to the Fango River reported only two insect species in the whole area

Class	Family	Genus	Species
Insecta	Cerambycidae	-	-
Insecta	Nymphalidae	*Caraxes*	*jasius*

Discussion

The collected data of the **Girolata** sandy beach shows a bright abundance of species. Lot of different fish species could be classified. Some species such as stingrays and barracudas were only found in this area. In total 14 different species were described which are less than in 2014 where 32 species were found. *Stenorychus sp, arca noae, Sphyranea viridensis,* and *Arbatia lixula* were found first this year.

At the **Fango River** we put our focus only on the limnic meso- and macrofauna and avoided classifying the terrestrial fauna. In the excursion of 2014 some terrestrial species were analysed, in particular insects. Sampling of the probes was executed in the afternoon. A reason for such a poor result could be that the extreme warm temperatures force animals to hide in deeper water or under massive rocks which are found all over the river. The main target of this excursion was to find the freshwater *Blenniidae Salaria fluvitalis,* which was quite abundant in the area.

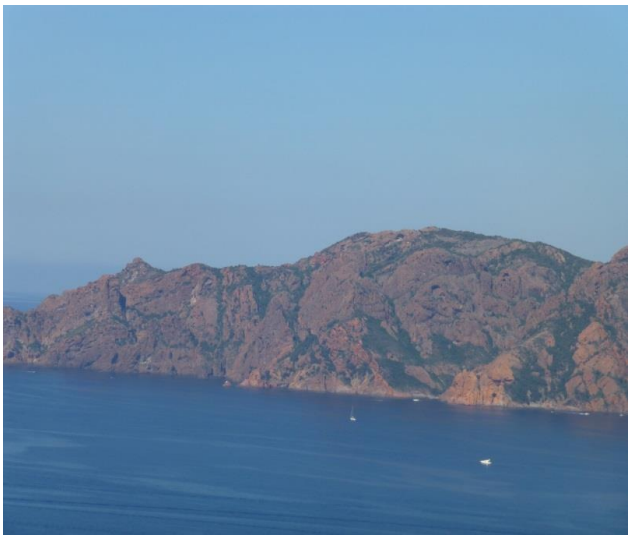
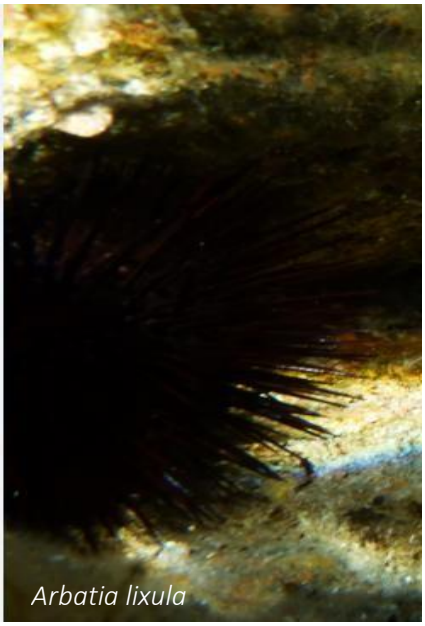


Figure 2- Overview of the Girolata bay and the typical red mountains of this zone. The red color is given by the minerals rhyolite porphyry, and basalt



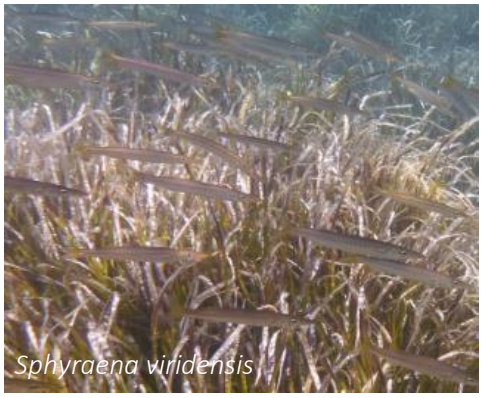


Figure 3– Species found in the Girolata Bay

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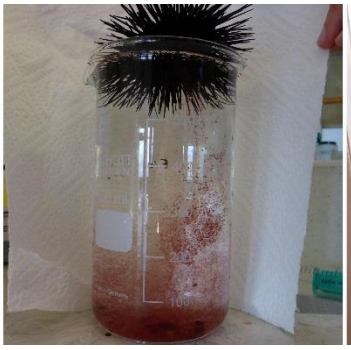
Projects


SEA URCHIN DEVELOPMENT

I) BODY AXIS MANIPULATION OF *ARBACIA LIXULA*

Laura-Sophie Frommelt, Stefanie Pfeifenberger, Stephanie Waich

INTRODUCTION

During the early development of an organism, different intercellular signal molecules work together to enable the establishment of a functioning body plan. The signal molecules of different families (including Wnt) act by binding to receptors on target cells in order to convert a signal and pass it on to other cells. These interactions proceed alongside intracellular pathways that are often highly complex and trigger cell responses like changes of gene expression or changes of the cytoskeleton, which lead to changes in the shape of a cell or its motility. The complexity of these pathways makes them vulnerable to all kind of influences and disorders initiated by chemical substances (Wolpert 2011).

Among other experiments conducted in the course, like reaggregation experiments and phalloidin-staining, we also dealt with the formation of the primary body axis in early developmental stages of sea urchin embryos of *Arbacia lixula*. In doing so, we turned our focus on the wnt-β-catenin pathway that is essential for the establishment of a polarization in the developing organisms. Beta-catenin is a protein that accumulates in the nuclei of early blastomers, as a result of the partial activation of the canonical Wnt-pathway. β-catenin accumulation happens to be mainly in the micromeres defining the vegetal pole. In the animal region however, it is almost absent creating a gradient alongside the animal-vegetal axis (see Fig 1).

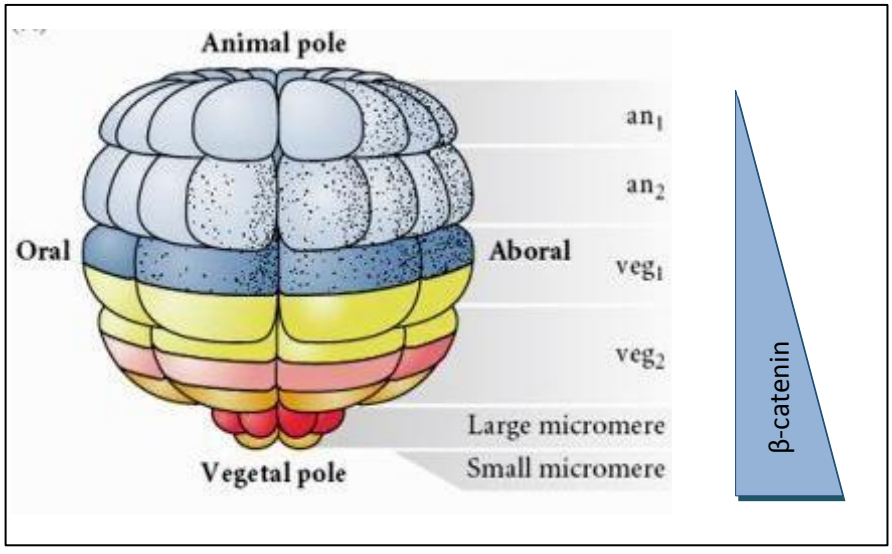


*Figure 1- **Polarization of the sea urchin embryo and formation of a β-catenin gradient (modified after Gilbert (2000)***

Beta-catenin is constantly degraded by cellular proteases, which are activated by the GSK-3 (glycogen synthase kinase-3) complex. If the GSK-3 complex is inactive, β-catenin remains in the cell where it is able to activate the transcription of different genes that are important for the maintenance aactivity of the vegetal pole by binding to TCF and LEF, families of transcription factors. The canonical Wnt-pathway stabilizes β-catenin by inhibition of GSK-3. Treatments that inhibit the β-catenin degradation, such as lithium chloride (LiCl) that is known to block GSK-3 activity (Fig 2), result in a vegetalization of early developing embryos, as the β-catenin accumulation expands beyond the normal vegetal region. This occurrence causes morphologically changes, so that embryos treated with LiCl exhibit both, a reduced ectoderm and an enlarged gut (Wolpert 2011).

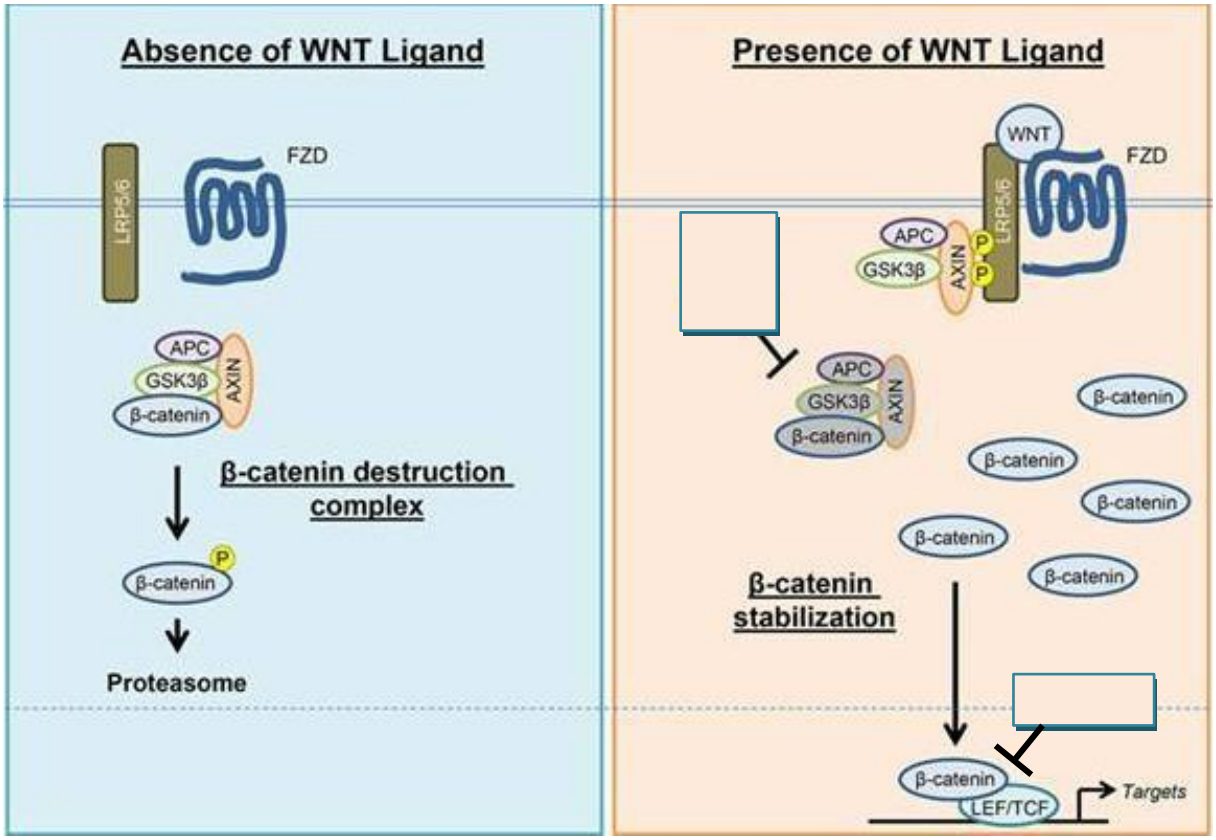


Figure 2- Simplified representation of the canonical Wnt-β-catenin pathway and the impact of three activators (Alsterpaullone, Bio and LiCL) and one inhibitor (iCRT-14) on it (modified after Suzuki et al. (2015)

Since β-catenin plays a key role in the Wnt-β-catenin pathway, it shows different effects on the development by down- or upregulation. In our experiments, sea urchin embryos were treated with four different substances including Alsterpaullone (Alp), BIO, LiCl and iCRT-14. Alsterpaullone is known as β-catenin activator that leads to a vegetalization of the embryo. BIO works in a similar way also causing vegetalization. Lithium chloride acts as a β-catenin stabilizer again leading to a vegetalization of the embryo. In contrary to these activators, iCRT-14 is a Wnt-ß-catenin pathway inhibitor. It down-regulates the effect of Wnt-signaling, by inhibiting the interaction between TCF/LEF and β –catenin resulting in a reduced vegetalization. Furthermore, iCRT-14 provokes a "Knock-out", which means that β-catenin gets into the nucleus but isn't able to bind to TCF/LEF and therefore causes an absolute Inhibition of gen regulation (Fig 2).

The aim of our experiments was to show how the activity of ß-catenin manipulates the development of early embryonic stages (Blastula to pluteus larvae) of *Arbacia lixula* when influenced by three activators (Alp, BIO, LiCl) and one inhibitor (iCRT-14). We expected to get more information about the formation of the animal-vegetal pole and its translation into anterior-posterior and dorsal-ventral polarities by observing the morphology of the embryonic stages. The second principal focus was laid upon the testing of the different substances available in the course and their influence on the embryonic development.

Material and Methods

For all manipulation experiments regarding the body axis formation we exclusively used embryos of the sea urchin species *Arbacia lixula*. The experiments were based on the excursion protocols (sea urchin part) of previous years whereby the experimental setup was modified and extended. Furthermore, also experimental experiences of body axis manipulation performed with the model organism *Hydra vulgaris* at the University of Innsbruck influenced our experimental design.

Based on information about the developmental speed of *Arbacia lixula* obtained from previous excursion protocols it was possible to establish a time table (see Tab 1.) to get an overview of the embryonic stages of *Arbacia lixula*.

Table 1- Time table for developmental stages of Arbacia lixula

Hours post fertilization (hpf)	Developmental stage
2,2	8 cell stage
2,5	16 cell stage
3,3	32 cell stage
4,1	blastula
14	gastrula
17	prism stage
20.7	pluteus larvae

Gathering of sea urchins

Adult sea urchins were collected in the harbor basin of the marine biological station and its near surroundings. The animals were detached from the substrate by means of a knife (fig.3) and put into a freezer bag full of seawater to transport them ashore. The sea urchins were then immediately taken to the laboratory in open plastic boxes filled with seawater to extract their gametes. A careful handling of the animals was essential to prevent stress-induced premature spawning.

Figure 3- Gathering of sea urchins by using a knife

Harvesting of gametes

To isolate the gametes of the sea urchins, the animals were placed with their aboral side (featuring gonopores) down on a beaker, which was filled to the brim with seawater (fig.4). Then, the sea urchins were dashed with seawater, which in some cases already triggered the release of gametes. If this was not stressful enough for the animals to spawn, 1 ml of 0.5 M KCl-Solution was injected into the soft tissue directly adjacent to the lantern of Aristotle using a syringe, followed by a second and if necessary also a third injection on the opposite side of the mouth opening. Within seconds up to one minute this treatment resulted in the release of gametes, either red eggs or milky white sperm, through the gonopores of the mature sea urchin. Female individuals were released into the sea after spawning, whereas male individuals were cut open immediately to extract the sperm containing gonads, which were transferred into an eppendorf tube. The undiluted sperm was stored at 4°C in the fridge and thus was useable for at least another two days. Female eggs however, can only be fertilized within a time period of 30 minutes after spawning. Therefore eggs had to be extracted anew daily from female individuals, whereas the stored gonads of two male sea urchins providedenough sperm for all experiments performed in the course. For detailed information of gamete harvesting see Banyuls (2001) – experimental embryology of marine evertebrates.

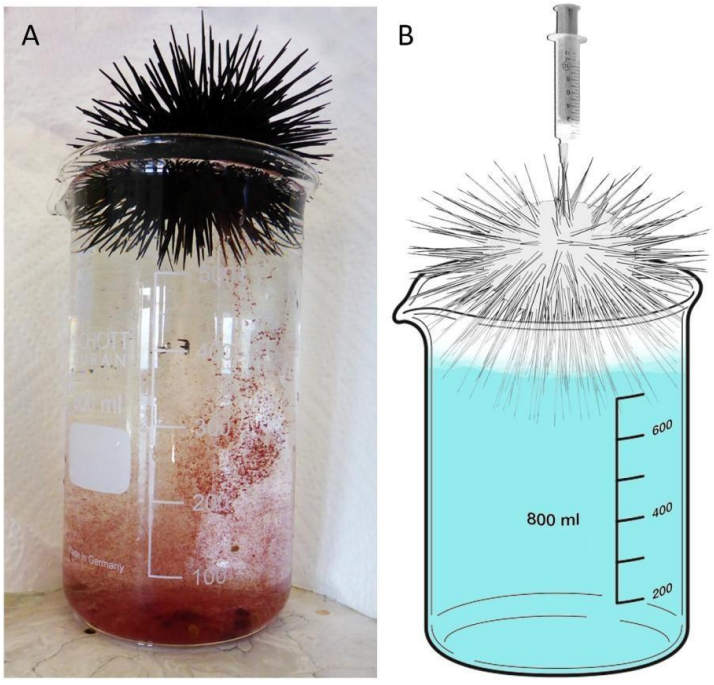


Figure 4- ***[A]*** *Spawning of a female individual (red eggs).* ***[B]*** *Shematic drawing of the KCl-injection into the soft tissue of the mouth region leading to the release of gametes.*

Fertilization

The stored sperm was activated by mixing a small amount of it (ca. 50 µl) with seawater in a 2 ml eppendorf tube. Subsequently, the activated sperm was used to fertilize the harvested eggs which accumulated at the bottom of the beaker. Therefore, surplus seawater was aspirated followed by the transfer of the activated sperm from the eppendorf tube to the beaker. After mixing the sperm-egg-solution, it was splitted between several petri dishes to provide optimal conditions for the development of the fertilized eggs.

Treatment

To manipulate the formation of the body axis, embryonic stages featuring 8 to 16 cells (approx. 2.5 hrs post fertilization (see table 1)) were treated with different concentration of activators (Alp, BIO, LiCl) and inhibitors (iCRT-14) of the wnt-β-catenin-signaling pathway. Table 2 shows the concentrations of the three activators and the inhibitor used in the "body axis manipulation experiments". Alsterpaullone, BIO and iCRT-14 stock solutions were diluted in DMSO (light-sensitive!), whereas LiCl powder was dissolved in seawater. Thus seawater was used as negative control for the experimental approaches with DMSO and LiCl whereas DMSO was used as negative control for the experimental approaches with Alp, BIO and iCRT-14.

Table 2- Concentrations of activating, inhibiting and control substances used in the three experiments of body axis manipulation

	Experiment 1 (6 hrs treatment)	**Experiment 2 (7.5 hrs treatment, LiCl**	**Experiment 3 (12 hrs treatment)**
DMSO	0.1%	0.1%	0.1%
	0.01%	-	-
	0.025%	-	-
Alsterpaullon	1 µM	0.1 µM	0.1 µM
	0.5 µM	0.05 µM	0.05 µM
BIO	-	1 µM	1 µM
	0.5 µM	0.5 µM	0.5 µM
	0.25 µM	0.25 µM	0.25 µM
LiCl	50 mM	50 mM	50 mM
	25 mM	25 mM	25 mM
iCRT-14	20 µM	20 µM	20 µM
	10 µM	10 µM	10 µM
	5 µM	5 µM	5 µM

To start the treatment, 1 ml of the egg solution containing mainly embryonic stages of 8 to 16 cells were transferred into each well of a 12-well plate. Subsequently, excess seawater was carefully aspirated and 3 ml of the respective activator/inhibitor/control solution was pipetted into the wells (fig 5). The 12-well plate was wrapped with aluminum foil for the duration of the treatment, since Alp and BIO are known to be light-sensitive. The 12-well plate was incubated at room temperature for at least 6 hrs, as embryos of *Arbacia lixula* reach the blastula/gastrula-transition after approx. 8 hrs post fertilization. The treatment was stopped by transferring the embryos from the 12-well plate to small petri dishes filled with fresh seawater. The seawater was changed two times per day to enable optimal conditions for the development of the embryos (oxygen and nutrients) and to prevent spreading of parasites and diseases.

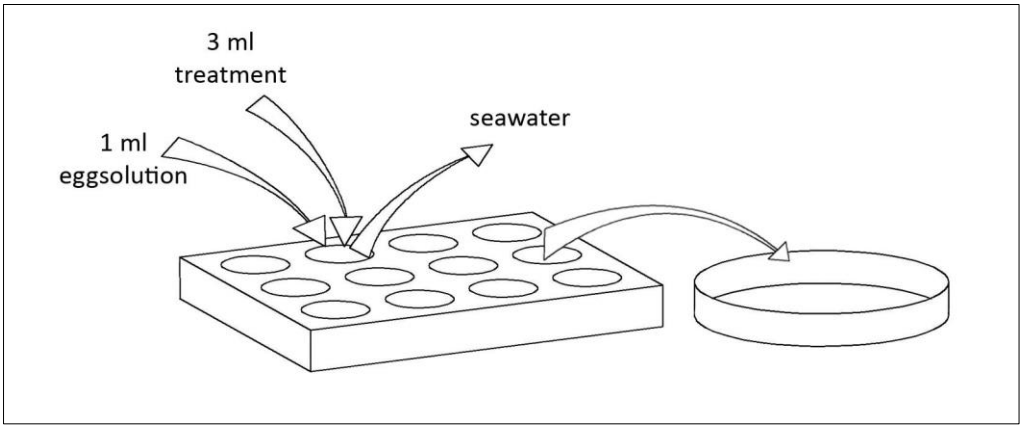


Figure 5- Schematic representation of the experimental set up for the treatment of the embryos with activators or inhibitors of the wnt-β-catenin pathway

Analysis and interpretation of the experimental outcome

After the treatments, embryos of all experimental approaches were examined and photographed under the stereo and light microscope at different time points. They were screened for axis-specific developmental defects like incorrect pigmentation of blastula/ gastrula stages, exogastrulae, flattened prism stages or deformed flattened pluteus larvae with missing arms as a result of vegetalisation or animalization. Furthermore, we also looked for defects linked to the non-canonical wnt-signaling pathway like deficient arrangement of cilia and associated circular motion due to polarization defects of the cells.

Results and Discussion

Alsterpaullone

Figure 6 [A] and [B] displays embryos treated with 0.05 µM Alsterpaullone 17 hrs post fertilization (hpf). This concentration resulted in the formation of many exogastrulae [A] and deformed prism stages [B]. The gastrulae stage looked more oval than round and some embryos showed circular motion. We also observed ca. 10% dead cells. At the same time point, the control embryos were mainly in the prism stage [C]. Thus, the development of the Alp-treated sea urchin embryos was partially decelerated.

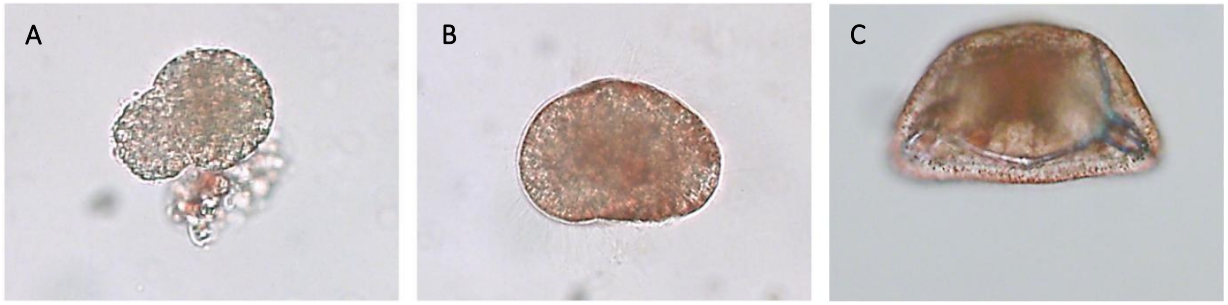


*Figure 6- **[A]** Exogastrulae, 0.05 µM Alp, **[B]** Deformed prism stage, 0.05 µM Alp, **[C]** Prism stage of a control individual*

Figure 7 shows blastula stages featuring different deformations. Sea urchin embryos treated with 0.1 µM Alp and higher concentration did not go further than the blastula stage [A, B, C]. In addition they also exhibited an incorrect pigmentation and hardly any movements. Under normal conditions, embryos should be in the gastrula stage 15 hpf (see tab.1) [D], but nearly none of the treated animals reached it. Furthermore, 25 hpf almost all embryos treated with 0.1 µM Alp were dead.

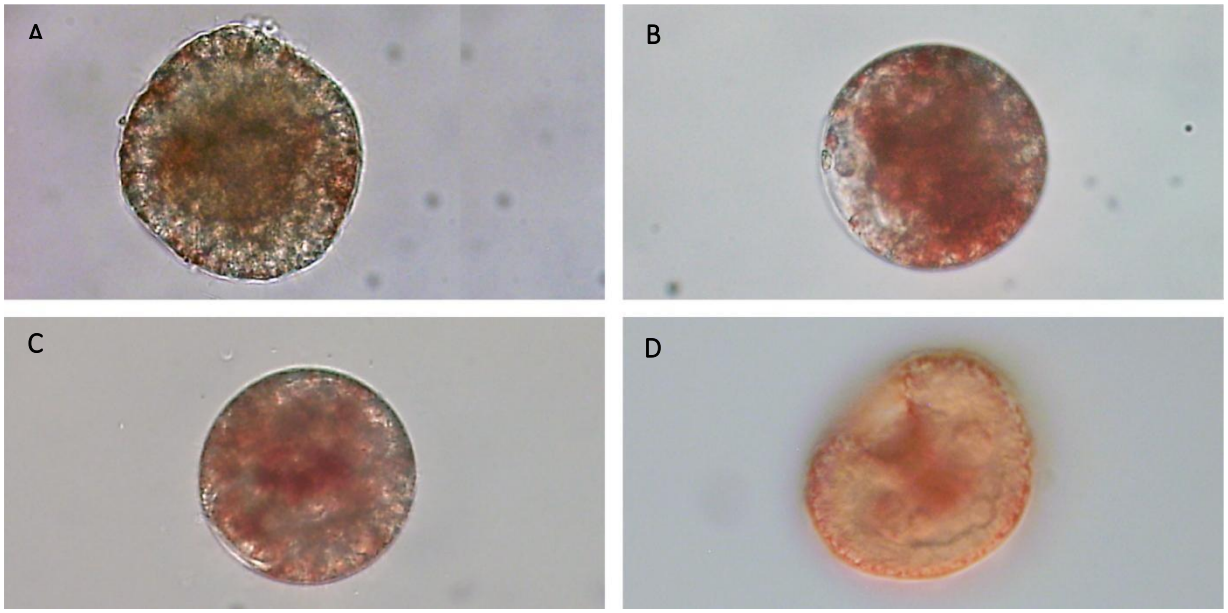


*Figure 7- **[A]** Blastula stage, 0.1 µM Alp, **[B]** Late blastula stage, 0.5 µM Alp, **[C]** Early blastula stage, 1 µM Alp, **[D]** Gastrula stage of a control individual.*

Alsterpaullone in high concentration prohibited a development beyond the blastula stage. It seemed that concentrations of 0.1 µM Alp and higher were toxic for the sea urchin embryos (tab.3). However, in the excursion protocols of 2014 they recommended an Alp-concentration of 5 µM to obtain morphologically visible effects on the body axis formation. In our case, at these concentrations no development was visible; only at the lowest concentrations (0.05-0.1 µM Alp) some of the embryos reached the prism stage. This indicates that this year embryos of *Arbacia lixula* were particularly sensitive for Alsterpaullone treatment.

Table 3- Alsterpaullone concentrations and their effects on the development of Arbacia lixula

Alsterpaullone	**0.05 µM**	**0.1 µM**
17 hpf	10% dead 5 % exogastrulae deformed prism	80 % dead 10% deformed blastula incorrect pigmentation
25 hpf	25% dead 50% oval gastrula stage 50 %deformed prism stage	80% dead 20% with bubbles of the fertilization membran
40 hpf	many blastulae a few pluteus larvae	100% dead

BIO

Figure 8 displays several deformed gastrulae stages at 17 hpf. Many individuals rotated quickly around their own axis in the blastula and gastrulae stage due to deficient arrangement of the cilia. This indicates that BIO also influences the non-canonical wnt-pathway, which is important for the establishment of a correct cell polarization and co-ordinated ciliation. Some of the treated embryos featured bubbles of their fertilization membrane, which is present from the fertilization up to the blastula stage [A]. This could be a sign of unhealthiness. In addition, some embryos seemed burst, because their cell content oozed out into the medium [B]. At a concentration of 0.5 µM BIO 80% of the embryos were dead, 10% of them showed exogastrulae [C] and another 10% displayed bubbles of the fertilization membrane. Overall, the development was slowed down what is apparent from the comparison with the control individuals [D].

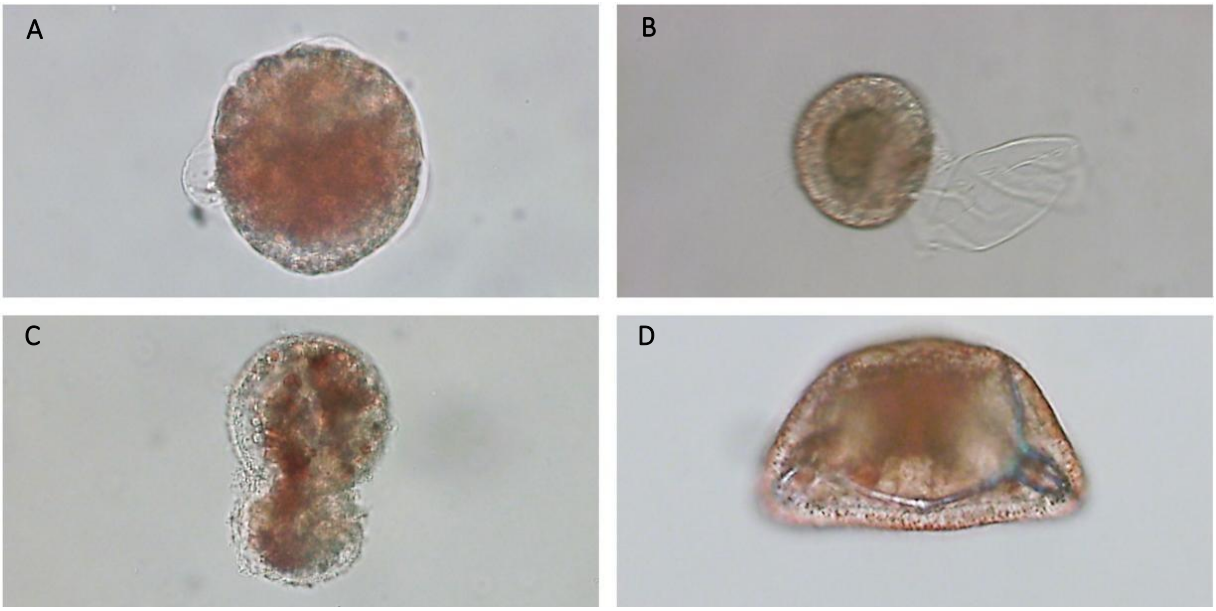


Figure 8- ***[A]*** *Gastrula stage with a bubble, 0.5 µM, BIO* ***[B]*** *Degenerated gastrula, 0.5 µM BIO,* ***[C]*** *Exogastrula, 1 µM BIO,* ***[D]*** *Prism stage of a wildtype*

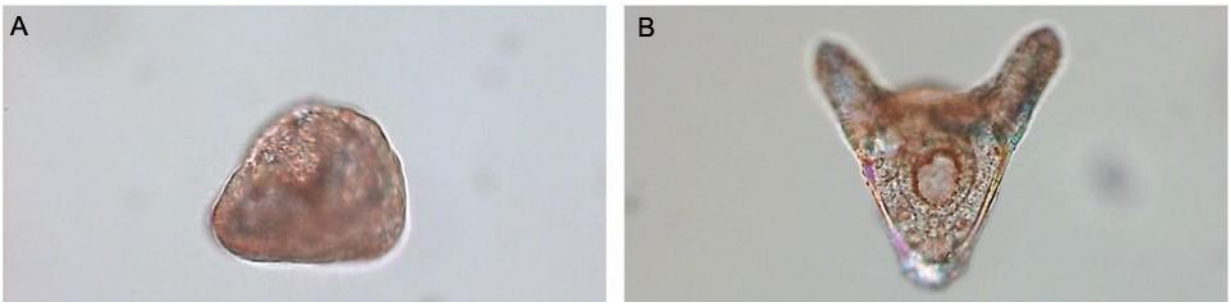


*Figure 9****: [A]*** *flattened Prism stage, 0.25 µM BIO,* ***[B]*** *Pluteus larva of the control group*

Figure 9 shows that a low concentration of 0.25 µM BIO led to a development with almost the same velocity as the development of the control group [B]. Whereby, it is also visible that the prism stage is flattened [A]. Furthermore, 25 hours post fertilization half of the BIO-treated embryos were dead.

Approximately 35 hpf some of the embryos reached the pluteus stage, but many of them were deformed with degenerated or missing arms (Fig 10). A large number were still in the blastula/gastrula stages. There was no significant difference in the deformation of the body axis between embryos treated with 0.25 µM BIO [A, B, C] and embryos treated with 0.5 µM BIO [D, E]. In both cases most of them were lacking whole body parts. At the higher concentration (0.5 µM BIO) many individuals were dead at 35 hpf and almost all were dead at 67 hpf.

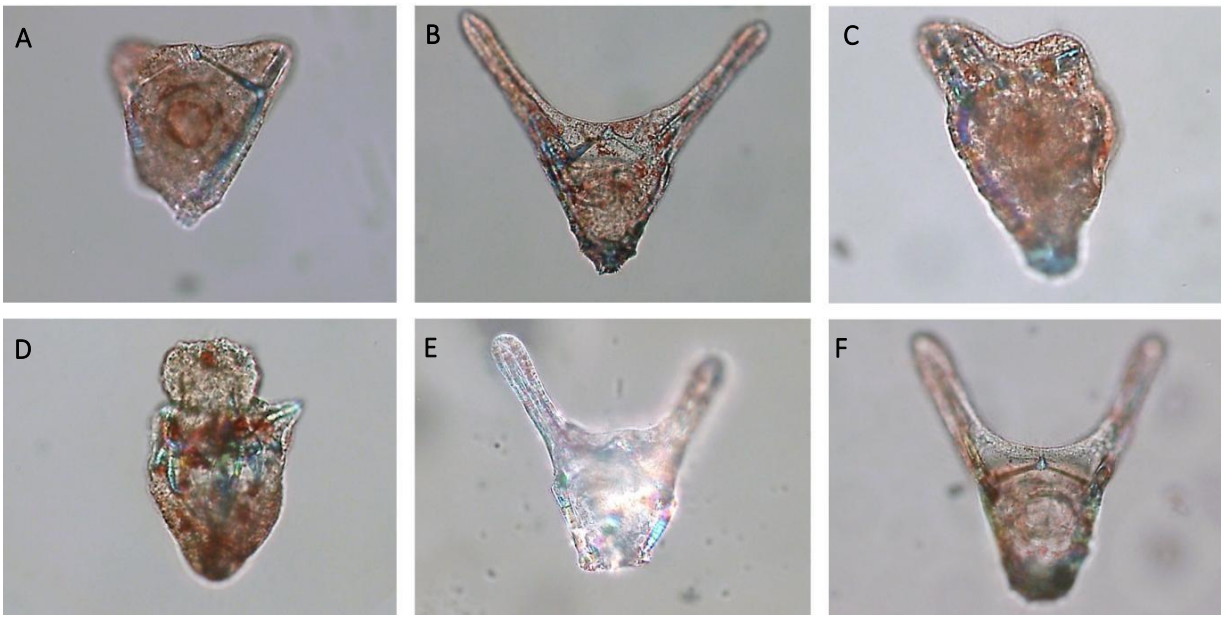


Figure 10- ***[A]*** *Pluteus larva, 0.25 µM BIO,* ***[B]*** *Late Pluteus larva,0.25 µM BIO,* ***[C]*** *Deformed Pluteus larva 0.25 µM BIO,* ***[D]*** *Deformed Pluteus larva, 0.5 µM BIO,* ***[E]*** *Pluteus larva, 0.5 µM BIO,* ***[F]*** *Pluteus larva of the control group*

The treatment with BIO had more impact on the sea urchin embryos as the treatment with ALP (compare tab3 and tab.4). On the contrary to embryos treated with ALP we could see a development beyond the blastula stage at all BIO concentrations. Most embryos reached the Pluteus stage, except for embryos treated with the highest BIO-concentration (1.0 µM). Here, 17 hpf ca. 80% of the animals were already dead. However, many different deformations were visible in all developmental stages. The excursion group of 2014 conceived nearly the same results as we did, but for the sea urchin *Paracentrotus lividus*.

Table 4- BIO concentrations and their effects on the development of Arbacia lixula

BIO	0,25 µM	0,5 µM	1,0 µM
17 hpf	30% dead 60% gastrula circular motion	80% dead 10%exogastrulae 10% blastulae incorrect pigmentation	80% dead 10%exogastrulae 10% blastulae incorrect pigmentation
25 hpf	50% dead 95% flattened prism stage 5% deformed pluteus larvae 85% circular motion	90% Exogastrulae 10% with bubbles of the fertilization membran deformed	100% dead
40 hpf	5-10% vegetalization animals were flattened	25% reduced Pluteus larvae 50% vegetalization	100% dead
60 hpf	90% dead 10% with deformations	100% dead	100% dead

LiCl

Figure 11 displays sea urchin embryos treated with 50 mM LiCl. Seventeen hours post fertilization 70% of the embryos featured deformation of the blastula/gastrula stages. For example, the fertilization membrane of embryos in the blastula stage partially formed bubbles [A]. In addition, some animals had exogastrulae [B] and also some incomplete gastrulation events were visible. Also LiCl caused rotation of the embryos around their own axis (30%). [C] displays an animal of the control group in late prism stage 17 hpf (normal development).

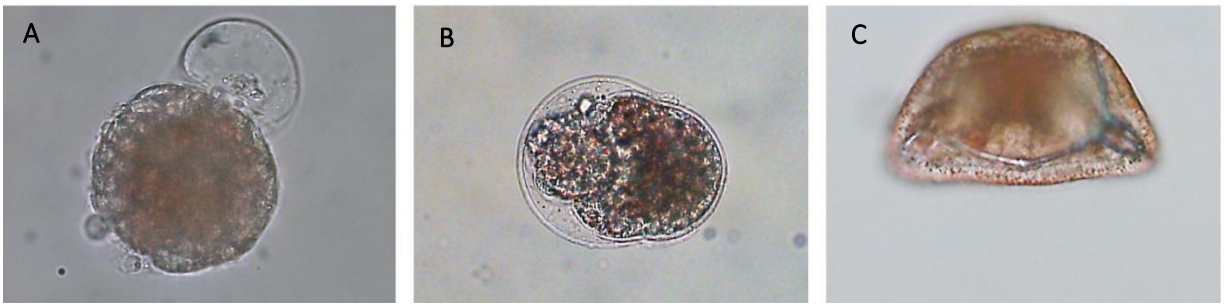


*Figure 11- **[A]** Gastrula stage with bubbles of the fertilization membrane, 50 mM LiCl, **[B]** Exogastrula, 50 mM LiCl, **(C)** Late prism stage of a control animal*

Many of the treatted individuals in the prism stage were flattened. Figure 12 shows Pluteus larvae approx. 37 hpf of both, LiCl [A, B] and the control group [C]. Only 5% of the LiCl treated embryos developed as far as the pluteus stage featuring great deformations (one to three arms) as they were missing their animal area [A]. Nevertheless also a few normally developed pluteus larvae were visible in the LiCl group [B] Most of the embryos however, stopped their development already in the gastrula stage.

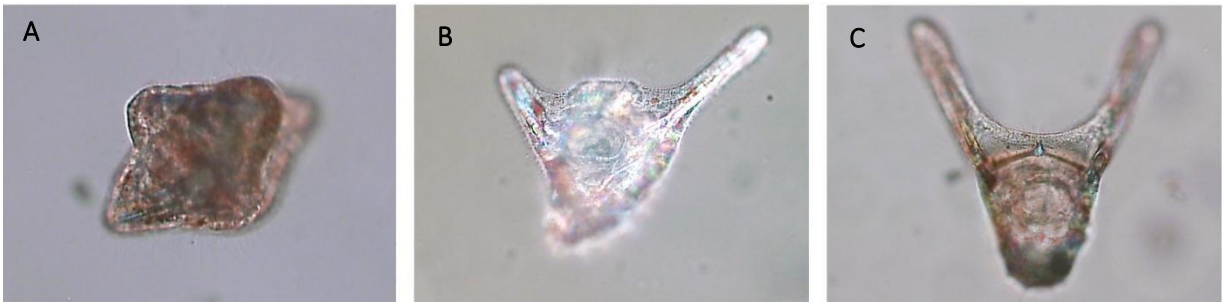


*Figure 12- **[A]** Deformed Pluteus larva, 50 mM LiCl, **[B]** Late Pluteus stage, 50 mM LiCl, **[C]** Pluteus larva of the control group*

The treatment with LiCl was less toxic than the treatment with the two other activating substances Alp and BIO, but nevertheless it also led to deformations of all developmental stages. It seems that some of the embryos were able to recover from the treatment and develop to a normal pluteus larva. Overall, embryos treated with LiCl showed a delayed development.

Table 5- LiCl concentrations and their effects on the development of Arbacia lixula

LiCl	50 mM
17 hpf	5% dead 70% oval gastrula stage 30% circular motion bubbles of the fertilization membrane
25 hpf	20% dead 15% flattened gastrula stage 25% exogastrulae 40% bubbles of the fertilization membrane animal pole reduced
40 hpf	Animal pole missing
60 hpf	5% vegetalized pluteus larvae animals with missing arms

Although Alp, BIO and LiCl are all known for acting as β-Catenin activators, the phenotypes of the embryonic stages varied in some aspects, which lead us to the conclusion that these substances might act in slightly different ways.

iCRT-14

iCRT-14 causes a knock-down of the canonical wnt-pathway, as it inhibits the interaction between TCF/LEF (transcription factor) and ß-catenin in the nucleus of the cell. This leads to an animalization of the embryo. In all performed experimental approaches the embryos were treated with the same concentrations of iCRT-14: 5, 10 and 20 µM. Since higher concentrations than 20 µM of iCRT-14 have toxic effects on Hydra, we chose not to treat our embryos with higher concentration than 20 µM iCRT-14. The three concentrations of iCRT-14 caused different phenotypes, indicating that they worked in different ways. In the first experiment we exposed the embryos for about 6 hours to iCRT-14. After that time the embryos in the control medium were in the blastula stage. Around 13 hours post fertilization the sea urchin embryos ought to be in the early gastrula stage (Fig. 13[B]). As there were no peculiar phenotypic changes in the treated embryos, we came to the conclusion that the exposure time should be longer in the second experiment. Around 16 hours post fertilization most of the embryos got stuck in the blastula, blastula/gastrula or gastrula stage. Individuals featuring invagination were hardly visible in the group treated with 5 µM iCRT14 (Fig. 13[A]).

We noticed a correlation between the concentration and the phenotypes as in higher concentration iCRT-14 causes less invagination (Fig. 13[C]. This would confirm our assumption that the inhibitor is able to delay the development of the sea urchin embryo.

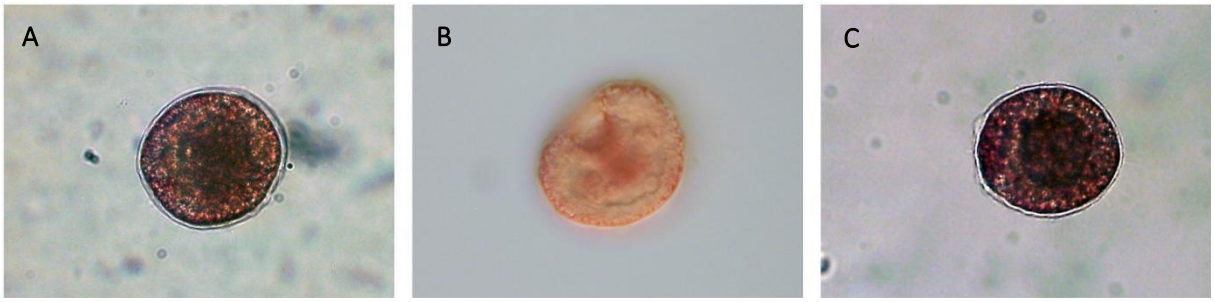


Figure 13- ***[A]*** *early gastrula stage, 16 hpf; 5µM iCRT-14* ***[B]*** *control; 13 hpf* ***[C]*** *blastula/gastrula, 17 hpf, 20 µM iCRT-14*

Around 21 hpf, up to 60% of the embryos treated with 5 µM iCRT-14 reached the prism stage (Fig 14[A], whereas 40% of the embryos showed normal gastrula stage. Some of the prisms were flattened. These changes showed that a rather low concentration of iCRT-14 causes only slight delays in the development. In the petri dish treated with 10 µM iCRT-14 no prism stages occurred, but the embryos happened to be all in an in-between stage of blastula and gastrula of which around 20% showed some kind of bubbles that looked like exogastrulae but were probably remains of the fertilization membrane, which is present till the blastula stage. The latter is more likely as exogastrulae are typical characteristics of embryos treated with activators of the wnt-β-catenin pathway (Fig 14[B]). Moreover, only a few embryos showed skeleton elements that might be evidence for early prism stages (Fig 14[C]) The highest concentration of iCRT-14 (20µM) caused bubbles and deformations on 90% of the embryos that all stopped their development in the blastula stage. Their fertilization membrane also formed bubbles (Fig 14[D]).

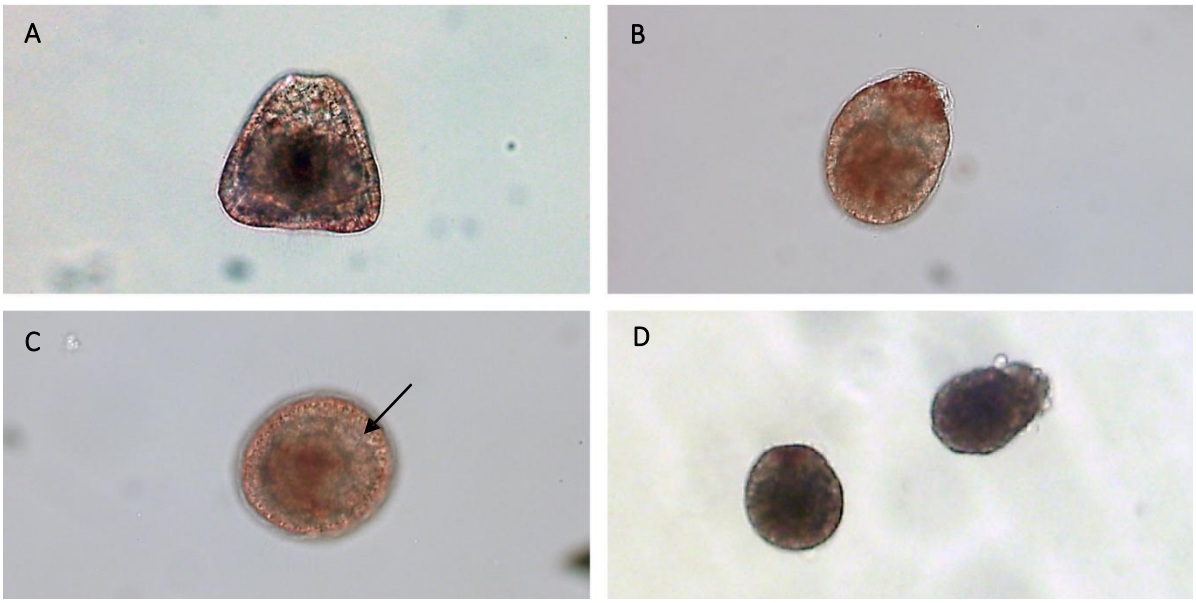


Figure 14- ***[A]*** *prism stage, 21 hpf, 5 µM iCRT-14;* ***[B]*** *blastula/gastrula stage with bubbles, 21 hpf, 10 µM iCRT-14;* ***[C]*** *skeleton elements (see arrow) as possible evidence for a deformed prism stage, 21 hpf, 10 µM iCRT-14;* ***[D]*** *deformed blastula stages with bubbles, 21 hpf, 20µM iCRT-14*

Another observation took place 37 hours after the fertilization. In the petri dish with individuals treated with 5 µM iCRT-14 we could identify predominantly normal pluteus larvae with only few anomalies though their development was slightly delayed. This delay corresponds roughly to the period the embryos were exposed to the inhibitor. In a concentration of 10 µM iCRT-14 most of the embryos remained in the blastula/gastrula stage (around 60%) and only a few showed the characteristics of a pluteus larva. The reason, why some individuals were trapped in the blastula stage and others managed to get on with a normal development might be that the treatment influences each individual during a different phase of their Wnt-regulation. During a specific time-slot the embryos are in an autocatalytic up-regulation of the Wnt pathway. When treated with iCRT-14 in this specific phase the embryos stop their development at the blastula stage. If they are faster or slower in their development, they are able to evade this time slot and show a relatively normal development. The highest concentration (20µM) showed the most striking changes in the development of the sea urchin embryos as around 80% of the organisms still remained in the blastula/gastrula stage exhibiting remains of the fertilization membrane (Fig 15[A]) and the rest developed into deformed prism stages with skeleton elements (Fig 15[B]) or pluteus larvae with different morphologies (Fig 15[C] and [D]).

Observations of embryos treated with 20 µM iCRT-14 confirmed that the substance is able to delay or inhibit normal development. The blastula-arrest is caused by the blocked micromers that would initiate invagination in the further development.

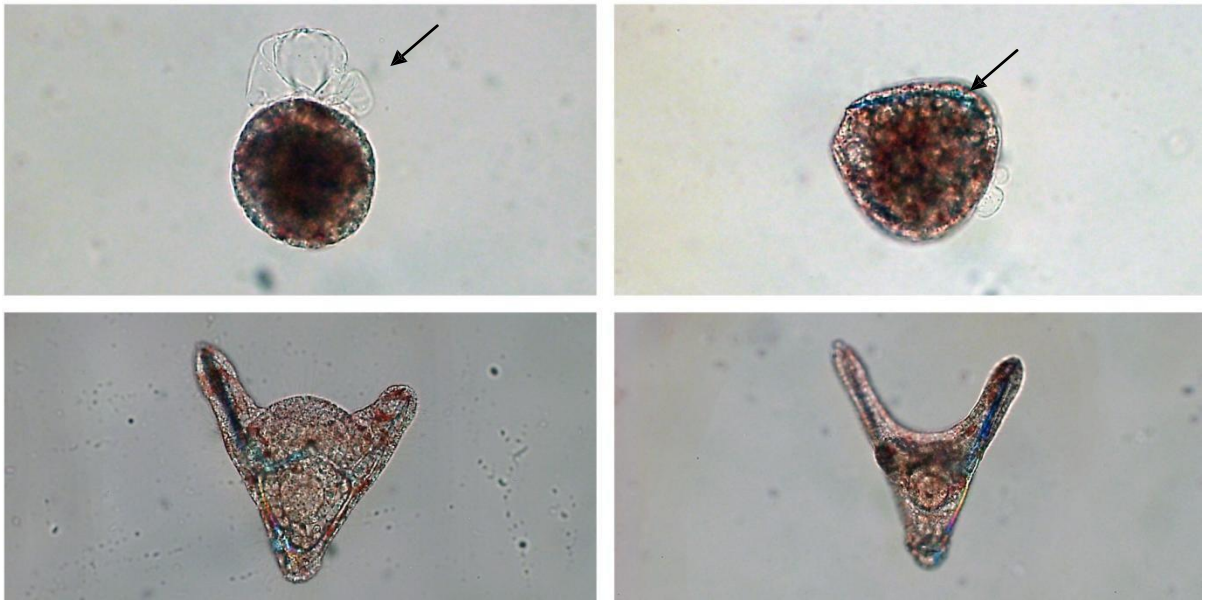


Figure 15- 37 hpf, 20 µM iCRT-14; ***[A]*** *blastula stage with remains of fertilization membrane (see arrow);* ***[B]*** *prism stage with skeleton elements (see arrow);* ***[C]*** *and* ***[D]*** *different phenotypes of pluteus larvae*

The second experiment turned out to be a bit complicated to interpret as there were scarcely eggs from the female sea urchins. We suppose that the storm the night before made the animals spawn as they do so when stressed. Nevertheless we took the eggs of at least two female individuals and fertilized them. This time the embryos were exposed to the same concentration of iCRT-14 (5, 10 and 20 µM) for about 7.5 hours to see if there are any differences in the developing phenotypes. During our first observation 17 hpf, we could identify around 20% of the embryos treated with 5 µM iCRT-14 to be in the shift from the gastrula to the prism stage. However, most of them showed deformations and bubbles (Fig 16[A]**.** Few showed abnormal pigmentation. According to Giudice (1986) the echinopluteus contains some heavily pigmented cells which already start to differentiate at mid gastrula, so the pigmentation we could observe (Fig 16[B]) might have derived from those early differentiating cells that are not affected by the iCRT-14. Some of the embryos were in the prism stage with visible skeleton elements (Fig 16[C]) or already in the early pluteus stage. Our observations demonstrated that iCRT-14 in low concentration is able to delay the development of the sea urchin embryos though the majority overcome this delay and show phenotypes similar to the control individuals that develop in accordance with the established timetable (tab.1).

The majority of the embryos (around 80%) treated with the highest concentration (20µM) were in the blastula/gastrula stage and showed abnormal characteristics in their morphology: some were ill- shaped (Fig 16[D]) and without a proper invagination. The few individuals that made it to the prism stage were flattened.

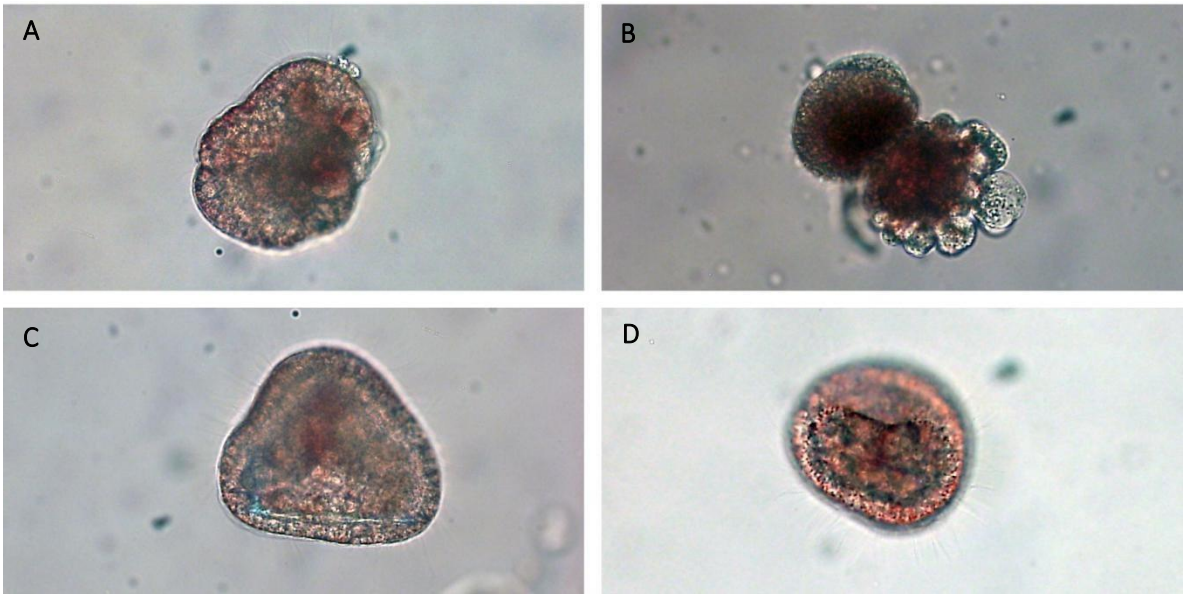


Figure 16- 17 hpf; ***[A]*** *deformed gastrula/prism stage with bubbles, 5 µM iCRT-14;* ***[B]*** *deformed embryo with unusual pigmentation, 5 µM iCRT-14;* ***[C]*** *prism stage with skeleton elements;* ***[D]*** *ill-shaped gastrula, 20µM iCRT-14*

At around 25 hpf, the embryos treated with 10 µM iCRT-14 exhibited a slight delay in their development as most of them were between the prism and pluteus stage. Unlike the embryos treated with 5 µM iCRT-14 most of the individuals in this petri dish were deformed and showed different anomalies such as exogastrulae-like protrusions, lattened and truncated prisms without the characteristic edges (Fig 17[A]) and rounded pluteus larvae. Some individuals showed characteristics of exogastrulae (Fig 17[B]) although this would not be a typical effect caused by the treatment with iCRT-14. Compared to the other two concentrations, embryos treated with the highest iCRT-14 concentration (20µM) were the slowest in their development as many of them were still in the gastrula/prism stage and even blastula stages could be found in a relatively huge amount. There were also some deformed individuals that exhibited pluteus characteristics but with shortened arms (Fig 17[C]). This phenotype supported our thesis that iCRT-14 cause animalization.

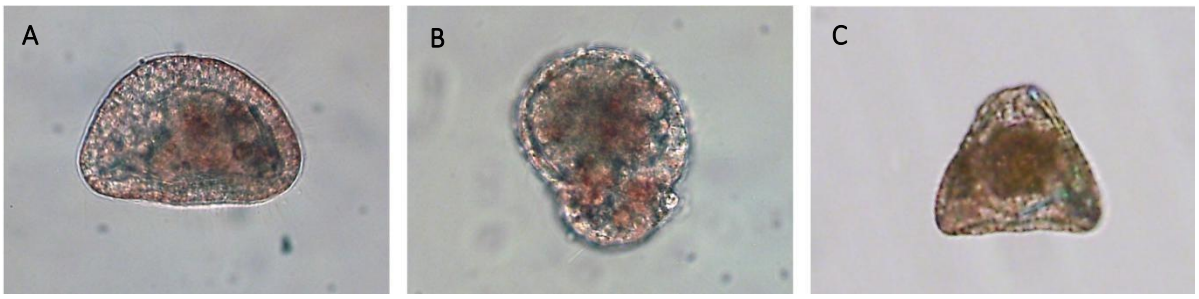


Figure 17- 25 hpf; ***[A]*** *flattened, truncated prism stage, 10 µM iCRT-14;* ***[B]*** *exogastrulae-like deformation, 10 µM iCRT-14;* ***[C]*** *deformed individual with pluteus characteristics (shortened arms), 20 µM iCRT-14*

Though there were some differences concerning the shape of the embryos, experiment 2 was very similar to experiment 1, which led to the conclusion that we had to extend the time of exposure once again but this time significantly to see striking differences. So, in the last experiment we used the same concentrations as we did in the previous two experiments (5, 10 and 20 µM), but this time we exposed the embryos for 12 hours to iCRT-14 expecting distinct phenotypes. Around 16 hpf the embryos treated with 5 µM iCRT-14 seemed very inactive and a large number of them (80-90%) were in the blastula stage exhibiting long fine cilia (Fig 18[A]) Some embryos (<10%) reached the gastrula stage and a few embryos died. The individuals showed a delayed development as, according to the time table (tab.1), they should have been at least in the gastrula or even prism stage. From the Individuals treated with 10 µM iCRT-14 around 80% died in order of rupturing. Some individuals exhibited incorrect pigmentation and remains of the fertilization membrane (Fig 18[B] and at least 20% remained in the blastula stage without any sign of activity. Finally, the combination of a rather high concentration (20µM) and long exposure time led to a mortality rate of 90% of the embryos. Some individuals still reached the blastula stage, exhibiting long cilia and also attempts ofinvagination could be observed in a few embryos that were about to enter the gastrula stage (Fig 18[C].

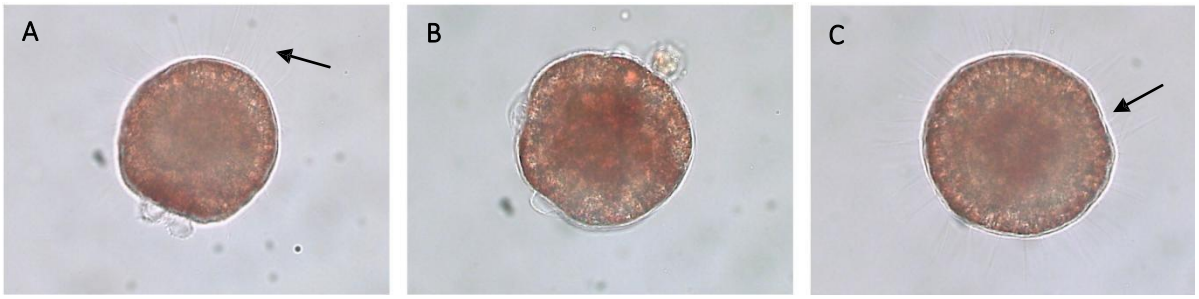


Figure 18- 16hpf; ***[A]*** *blastula stage with long, fine cilia (see arrow), 5µM iCRT-14;* ***[B]*** *deformed blastula stage with remains of the fertilization membrane;* ***[C]*** *blastula stage with suggested attempt of invagination (see arrow)*

In its capacity of inducing animalization, iCRT-14 is able to outweigh the effects of Alp, BIO and LiCl. For this was only tested on Hydra before, it would be interesting to conduct the same experiment with sea urchins in the next excursion course and see if the results are the same or at least comparable with those observed in other developmental model organisms.

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II) Reaggregation of sea urchin embryos

Jana Ribitsch, Daniela Spielmann, Julia Vorhauser

Introduction

Regular Sea urchins belong, as all representatives of the phylum Echinodermata, to the Bilateria. In contrast to most other classes which belong to this group, their bilateral symmetry can, however, usually only be seen in the embryonic stages; as soon as they have undergone metamorphosis and turn from a pluteus larvae into an adult sea urchin, they show a pentamere radial symmetry. (Westheide and Rieger, 2004).

Sea urchins have a highly determined early embryonic development, which means that the fate of every cell is determined from the very beginning, starting with the penetration of the sea urchin sperm into the sea urchin egg. By the process of fertilization a lot of downstream processes are set into operation and molecular signals are sent into different parts of the egg to regulate further development of the embryo. They immediately activate maternal factors, which are absolutely crucial for the embryonic development of sea urchins and determine every change the egg undergoes from now on, starting with the fixation of the animal-vegetal axis.

Two of the very first reactions that follow fertilization are the depolarisation and hardening of the egg surface and the creation of the fertilization membrane to avoid polyspermy (Giudice, 2012; Müller and Hassel, 2012). Since the egg will only later, in the late blastula stage, hatch from this fertilization membrane and become a free-swimming blastula, this membrane hinders experiments with early stages, and thus the membrane has to be removed artificially by Trypsinization (see Methodology section).

After developing the fertilization membrane, the egg undergoes several cleavages, which are already strictly controlled maternal factors in the animal pole. The first two cleavages are meridional and perpendicular to each other, so the cells divide along the animal-vegetal axis (Wolpert, 2011). This means that after these two cleavages all four cells have taken over all the information for further development from the zygote and can therefore, when separated, turn into complete and intact sea urchin embryos, even if a little smaller than usual. According to prior research, this is the last stage in which this separation of cells is possible (Müller and Hassel, 2012).

From the third cleavage on, the cells do not only divide along the animal-vegetal axis, but also equatorial. Therefore from this very stage the cells can't develop full embryos anymore. The embryo undergoes several further cleavages (Figure 1), in which the so-called macromeres and micromeres are formed. The latter ones are the cells that comprise the molecular information that eventually determine the fate of all the other cells of the embryo. By means of the Wnt-/beta-catenin-signalling pathway, the micromeres give signal to their adjacent cells, which then transfer their signal to their respective neighbouring cells and so on; the micromeres are therefore the organising centre of the whole embryo. (Wolpert, 2011; Gilbert 2000).

After the seventh cleavage the early blastula stage is reached (Gilbert, 2000). While constantly undergoing further cell divisions, the cell cluster continues to develop into a hollow sphere. Through creating tight junctions, the cells begin to form an epithelial monolayer that surrounds the blastocoel. On the outer surface of this cell-layer cilia come into being and the blastula starts to rotate within its fertilization envelope (Müller and Hassel, 2012). Secreting a hatching enzyme, the fertilization envelope is digested and the stage of a free-swimming blastula is reached. On the animal pole, the cell layer becomes thicker and forms the vegetal plate. This is the area where the cell wall begins to invaginate – the gastrula stage is reached. The invagination becomes larger, forming the archenteron, which eventually fuses with the cell wall on the other side of the hollow sphere, however not directly opposite to the animal pole, but slightly shifted to one side. The point of fusion turns into the oral pole. Since this new opening turns into the mouth and the original mouth will develop into the after, sea urchins clearly belong to the group of Deuterostomia. (Gilbert, 2000; Wolpert 2011).

At the same time as the gut comes into being, the differentiation of endoderm, mesoderm and ectoderm takes place and consequently skeletal and muscle tissue is formed (Wolpert, 2011). Later the embryo begins to develop small bulges, and therefore has a triangular shape; this stage is called prisma stage. These bulges then extend to become arms and the development of the pluteus larvae is completed. (Giudice, 2012)

In research and laboratory work, only working with embryonic stages up to this larvae stage is possible. After that the sea urchins are not able to undergo metamorphosis under artificial conditions and die, unless a lot of effort is made to ensure their survival. (Müller and Hassel, 2012)

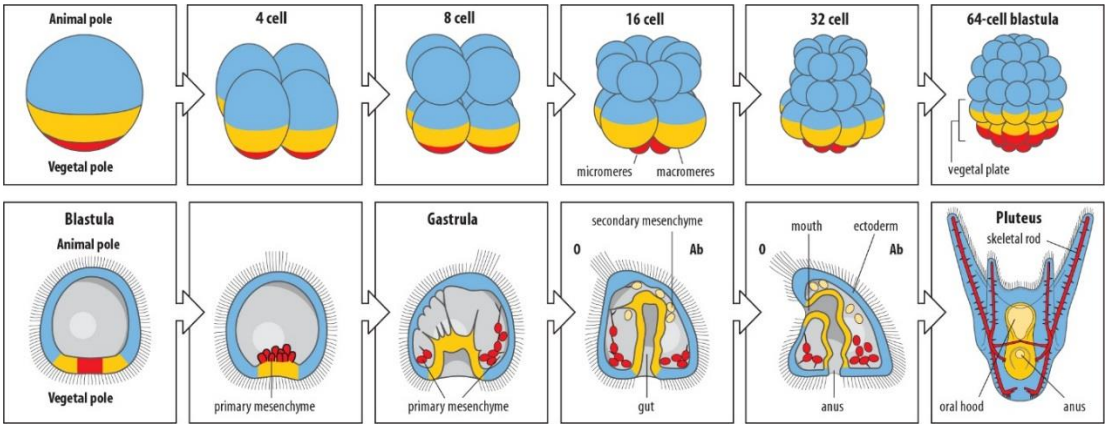

Figure 1- Embryonic development of sea urchins

The whole development of the sea urchin embryo is highly determined from the very beginning by maternal factors and the fate of the cells is already pre-determined at a very early stage. Question arise here: In how far is the cell's fate really determined? Can it be changed at some point?

In the following experiments, we want to find out whether the fate of the cells can be manipulated and whether they still have some plasticity, which means that they can change their fate and take over other characteristics of other cells in the embryo. We approached that by means of reaggregation experiments.

Material and Methods

Table 1- Required material

For experiments with gastrula stages to pluteus larvae:	For experiments with 16-32 cell stages to blastula stages:
Fertilized sea urchin eggs	Use the same materials as in the list on the left-hand side, plus:
Petri dishes (10 ml and 50 ml)	
Medium (seawater)	0,08 g Trypsin solved in 100 ml seawater
Stereo microscop	Aluminum foil
Plastic pipettes (1 ml and 2,5 ml)	Small spatula
Hyalin extraction media (HEM)	Laboratory scale
Erlenmeyer flask (1000 ml)	Graduated flask (100 ml)
Aluminium foil	
Eppendorf tubes (1 ml)	
Hand centrifuge with 4x 15 ml falcon tubes	
Syringe	
Light microscop	
Microscop slides	
Scalpel	
Pincette	

Our experiments were based on the protocols of the excursion in 2014 and the paper "Experimentelle Embyologie von marinen Evertebraten" (Banyuls, 2001), however slightly modified. We worked with embryonic stages, from the 16-32 cell stage to the pluteus larvae, of the sea urchin species *Arbacia lixula.* To become accustomed to the handling of the experiment we conducted a pilot project with the same species.

Breeding

To get the embryos required for our experiments, we first of all had to mate sea urchins. For this purpose, one of the animals was placed with the oral side up on the rim of a glass flask, which was filled to the top with seawater. To stress the animal, which leads to a release of the gametes, we first poured seawater onto the sea urchin. In case this was insufficient we injected 1 ml KCl in the mouth region of the animal one or two times. Within a few seconds the animal would release female gametes, i.e. eggs (red to violet), or male gametes, i.e. sperm (white). If the animals were female, we would wait for them to release the amount of eggs we needed for the experiment, after which they were set free into the sea; however, if they were male, we cut them open immediately, extracted the gametes containing the sperm and collected them in a tube. The sperm, when stored in the fridge, can be kept for two days after the extraction, which is why not a lot of males were required. Just before we started our experiments, we activated sperm from the refrigerator by mixing it with sea water and brought it together with the eggs. Then we distributed the fertilized eggs into some 50 ml petri dishes.

Preparation of the solutions

Hyalin extraction media (HEM)

Table 2- HEM components (cf. excursion protocol 2014)

HEM	1000 ml
NaCl	17,5 g
KCl	0,75 g
$MgSO_47H_2O$	2.5 g
Glycine	22.5 g
TRIS	1,21 g
EGTA	0,76 g
pH 8,2; salinity 34-36 ppt	

The HEM should be covered with an aluminium foil and stored in the fridge. Since it is a buffered solution consisting mainly of salty components, which are stable, it can be used for a period of a couple of days.

Trypsin solution

For the Trypsin solution, 0,08 g Trypsin were solved in 100 ml seawater.

Dissociation

After fertilisation of the eggs, the embryos were left to their natural development. After a certain time, depending on which embryonic stage was needed for an experiment[1], the petri dishes were checked for whether they actually contained the required embryonic stages (16-32 cells, blastula, gastrula, prism or pluteus larvae). The embryos of all the petri dishes with the correct stages were transferred into one or two new 50 ml petri dishes.

16-32 cells and blastula stages

Since those stages still have a fertilisation membrane (FM), which would hinder the dissociation step, they had to undergo one further procedure as compared with normal development – the digestion of the FM. To do so, we incubated the embryos within a Trypsin solution for 15 minutes. Then we washed the embryos three times with seawater. After this, we continued our dissociation process as seen below.

All stages

A calcium-free solution called hyalin extraction media (HEM) was added to the petri dishes with the embryos; in this solution the embryos were then incubated for 30 minutes. The absence of calcium leads to the dissolution of the adherens junctions, which are responsible for the cell-cell contacts; therefore, in the incubation step the cells lost their affinity for their neighbours. After that the embryos were transferred into an Eppendorf tube and centrifuged for ca. 30 seconds. Most of the upper supernatant was then carefully removed with a pipette and the remaining pellet dissociated with a syringe, pipetting the embryos up and down 4 to 6 times. At last, we checked the success of the dissociation step by examining the supposed single-cell-suspension under the light microscope. We let the solution settle for a bit and then worked with the supernatant, as the single cells have a lower density than intact embryos (possibly still present in the solution) which would therefore settle much quicker.

[1] for the exact time course of sea urchin development cf. the Calvi protocols from 2012

Centrifugation

The dissociated cells from the supernatant were centrifuged by hand for three minutes, always attempting to maintain a consistent speed throughout this period of time.

Reaggregation

For the reaggregation the supernatant of the previously centrifuged cells was removed and replaced by normal seawater, which contains calcium and which in turn allows the rebuilding of the cell-cell contacts. The cells remained like that for 30 minutes. Afterwards the pellets were transferred into a new 10 ml petri dish with fresh seawater and under a light microscope they were cut into 0,1-0,3 mm pieces by means of a scalpel and a tweezer.

Observation and Classification

The aggregates obtained in the steps before were examined and photographed under the stereo and light microscope at different time points and in addition to that the developmental stages of the cell aggregates were classified. To make their survival more likely, the cell aggregates were washed every evening, meaning they were transferred into new petri dishes containing fresh sea water.

Results

The following results are classified based on the developmental stage, in which the embryos were dissociated.

16-32 cell stage aggregates

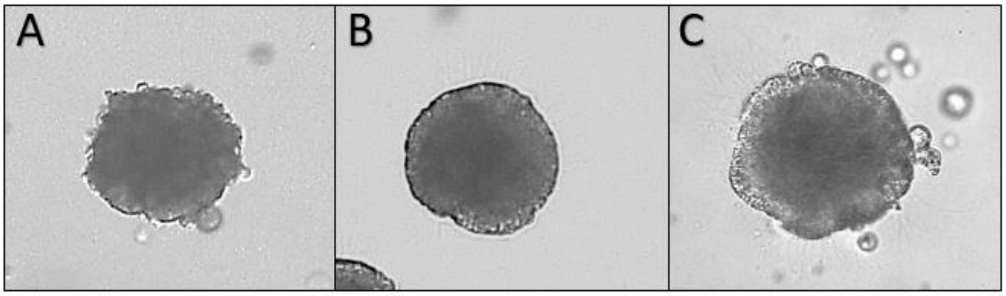

Figure 2- Former 16-32 cell stage aggregates at different time points. ***[A]*** *after 5 hours;* ***[B]*** *after 20 hours;* ***[C]*** *after 25hours of regeneration. All images were recorded at a 20xmagnification*

As shown in Figure 2, some of the 16-32 cell stage aggregates were able to regenerate from the disrupted developmental stage. After around 5 hours of recovery, the smoothening of the cell surface started, which can be seen at the bottom half of the aggregate (Figure 2A). In addition to that, certain cells of the initial aggregate were pulsed out of the cell mass, presumably because of their internal destruction and their consequent inability to fit in with the developing tissues. This is visible at the upper part of the aggregate. After 20 hours of regeneration, the whole surface appears smoothened and rounded-off (Figure 2B). Furthermore, some cilia, which in normal development are expected to form in the late blastula stage, were visible. With increasing time, the ciliation continued and the formation of the vegetal plate, which usually appears within the gastrula stage, was initiated, which can be perceived on the left part of the embryo (Figure 2C).

Blastula stage aggregates

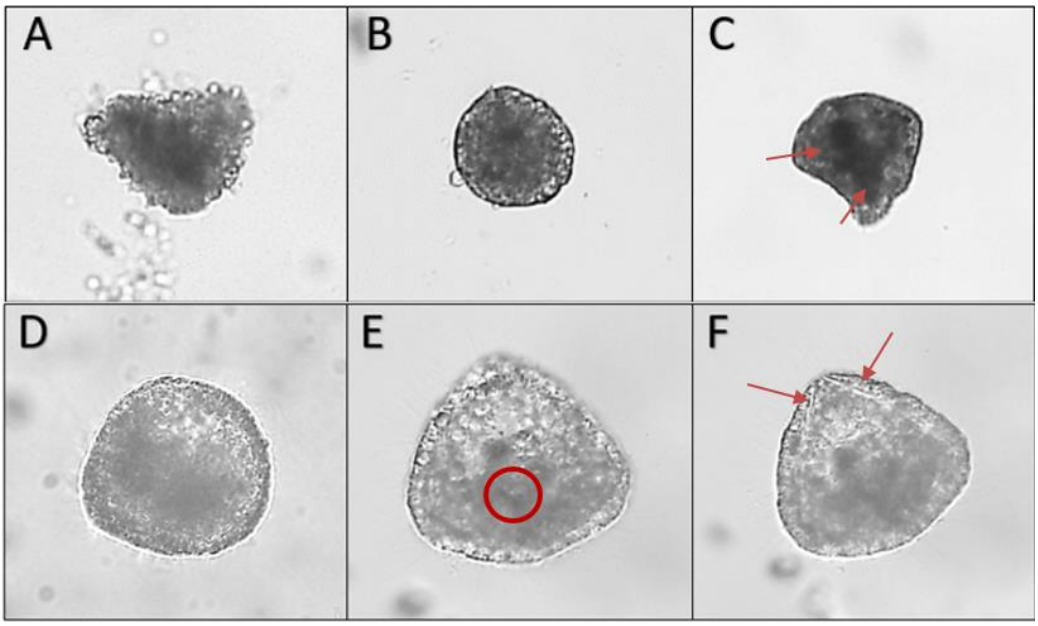

Figure 3- Blastula stage aggregates at different time points ***[A]****after 2hours;* ***[B]****after 16hours;* ***[C]*** *after 33 hours, the red arrows indicate the bulging of the arms;* ***[D]*** *after 46hours;* ***[E]*** *after 51hours, the circle indicates the blastoporus;* ***[F]*** *after 51hours, the red arrows indicate the skeletal elements. All images were recordered at a 20xmagnification*

The former blastula stages showed the best results, as cell aggregates derived from this stage could form the most advanced embryos. After an incubation time of two to 16 hours the cell mass surface smoothened and rounded off. A few hours later, the reaggregated embryos started to bulge (Figure 3C). This bulging would end up in arm formation, an event which can usually be perceived in the pluteus stage. Moreover, just like in the 16-32 cell stage aggregates, the formation of the vegetal plate became visible after around 46 hours (Figure 3D). One of the embryos, after a time span of 51 hours, showed formation of the gut and the blastoporus (Figure 3E and 3F), which indicates that the reaggregated cell mass was able to undergo gastrulation. The embryo even showed some skeletal elements, which usually appear in the late prisma and early pluteus stage.

Gastrula stage aggregates

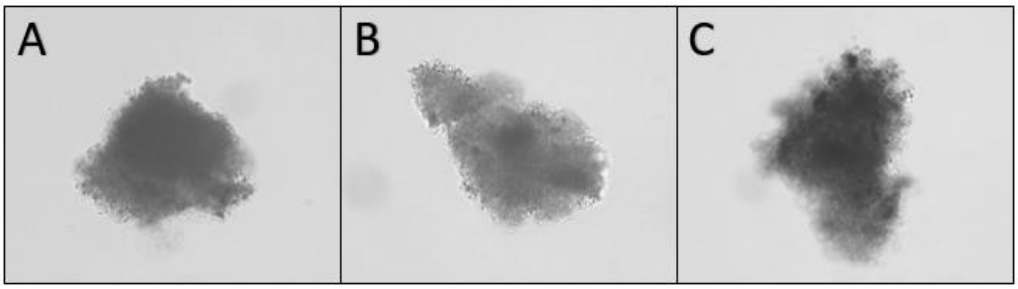

Figure 4- Gastrula stage aggregates at different time points. ***[A]*** *after 25hours;* ***[B]*** *after 29hours;* ***[C]*** *after 31hours. All images were recordered at a 20xmagnification*

The dissociated gastrula stages did not show any further development. They were not able to reorganize themselves, not even after a time span of 31 hours (Figure4). No smoothening or cell repulsion of the aggregates was observable indicating that the formation of a surface epithelium did not occur.

Prisma stage aggregates

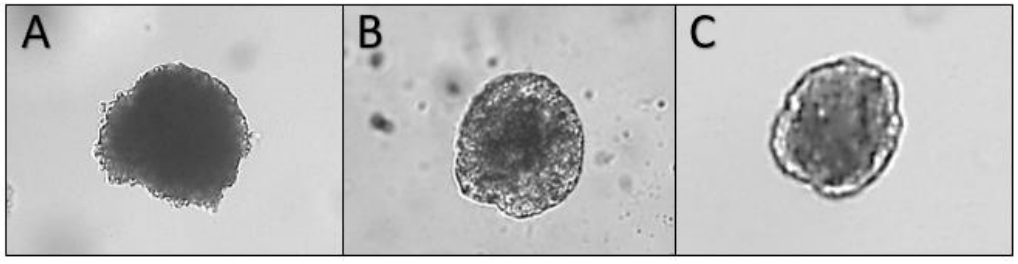

Figure 5- Prisma stage aggregates at different time points. ***[A]*** *after 6hours;* ***[B]*** *after 25hours;* ***[C]*** *after 55hours. All images were recordered at a 20xmagnification*

The prisma stages initially showed the same ability of reorganisation as the earlier stages. After 6 hours, their surface started to smoothen (Figure 5A) and after around 25 hours they seemed to be fully rounded-off and ciliated (Figure 5B). But despite being given a vast amount of time to recover, they were not able to develop any further (Figure 5C). From this observation it can be inferred that these rounded cell cluster represented the limitations of the reorganizational potential of the prisma aggregates.

Pluteus stage aggregates

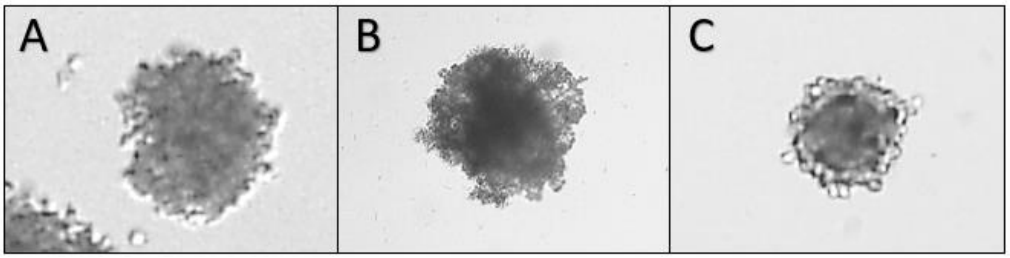

Figure 6-: Pluteus stage aggregates at different time points. ***[A]*** *after 3 hours;* ***[B]*** *after 23hours;* ***[C]*** *after 48 hours. All images were recordered at a 20xmagnification.*

The same observations as with the prisma aggregates were made with aggregates made at the pluteus stage (Figure 6) The initial aggregates started to smoothen and to repulse some cells, but, even after being given a long period for recovery (Figure 6C) no further developmental steps could be perceived.

Discussion

First of all, it can be said that the development of the embryos from the aggregates was temporally displaced in comparison to normal embryonic development. The aggregates needed up to 24 hours longer than the normal embryos to reach different developmental stages. In addition to that, neither embryos derived from different stages, nor embryos derived from one stage showed parallels in the pace of development. Therefore, no general statement about the timing of development in reaggregated sea urchin embryos can be made.

Aggregates of early developmental stages, such as 16-32 cell stages or blastula stages, exhibited smoothening of the surface, repulsion of cells, and formation of cilia and the vegetal plate, developmental events that can be found in the early gastrula stage of normal embryos. Blastula stages moreover were able to form the blastoporus, the gut, arms, and skeletal elements. These developmental structures are comparable with the structures that occur in late prisma or pluteus stages of embryos which undergo normal embryonic development. The ability to form the above-mentioned structures could be seen as evidence that the embryos also have the ability to form a body axis; consequently, the cells must have positional information. Later stages were only able to smoothen their surface and to develop ciliation, two events that appear in normal blastula stages. The critical step in the development here seems to be the invagination of the cell layer.

From these observations it can be inferred that early stages show a higher plasticity in comparison to late developmental stages. However, blastula stages seem to exhibit an even higher development capacity than 16-32 cell stages. The underlying reason for that could be the decreasing dominance of maternal components at the blastula stage. As mentioned before, in the development of Echinodermata the maternal components are known to be crucial for early cell determination. Consequently, a decrease of maternal components correlates with an increase of plasticity. However, this plasticity only seems to occur within a restricted time frame, as the determination and specification of the single cells of the embryo increase in later developmental stages. Therefore, according to our results, it could be inferred that development plasticity is highest in the blastula stage.

In our experiments, we came across several issues regarding the handling. First of all, some cell pellets were not firm enough, which could be led back to the fact that the centrifugation wasn't sufficient or in the case of the earlier stages that the trypsin treatment failed to digest the fertilization membrane. Secondly, some pellets didn't undergo any further development, the reason for which could be that the cell pellets didn't have the right size to enable an interaction between the cells within the aggregate. Another problem was that all the experiments on day 3 failed. A possible explanation could be that the HEM solution that was used throughout all three days had undergone an age-related modification, enhanced by the high temperature within the laboratory, or that our samples had been contaminated with bacteria, in consequence of the nonsterile working environment. Based on this problem the results for the experiments with the gastrula stages, which were conducted on this very day, are not representative. A further problem consistent in all the experiments were intact embryos found in the cell suspension.

They most likely resulted from either the dissociation of the embryos or the Trypsin and HEM treatment, being not sufficient. These intact embryos could therefore lead to a falsification of the results.

We tried to eliminate this problem by very careful visual inspection of the freshly dissociated cell suspensions. In conclusion, it can be said that our results may not be representative, as too few replicates have been done and most of the issues mentioned above haven't been solved yet, but that our first steps in this approach were basically very successful and that reaggregation is a valuable experimental approach to study developmental capacity and plasticity of embryonic cells.

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III) F-actin dynamics in the sea urchin *Arbacia lixula* from the fertilized egg to the pluteus larva

Mattia Baraldo, Philip Bertemes

Abstract

Sea urchins (= Echinoidea), one of the five classes of Echinodermata, are well-established model organisms for early embryogenesis. Their Pluteus larvae develops within one day after fertilization, they are transparent, very motile, and represent a part the zooplankton. In this work, we analyzed and photographically documented the embryogenesis and the formation of musculature from the fertilized egg to the early Pluteus larva by a Phalloidin staining. Since their locomotion is performed by a cilia-induced current, no real musculature system for motion was detected. However, the formation of cell-cell contacts by filamentous actin as well as the early gastral region was identified and characterized.

Introduction

Sea urchins have been subject to evolutionary and developmental studies for a long time. A search in the online library PubMed for "sea urchin embryo" results in over 5000 hits (date: October 2016). According to some authors, sea urchins were studied from the beginning of the microscopy in the 18th century until now to a point where the whole genome is well known and accessible (Cameron *et al.* 2009). The sea urchin embryo is a suitable model organism for a broad palette of studies because it is an easy accessible animal, its early development is rather basic, and the embryos can be simply manipulated (McClay 2011).

In this work, we make use of Phalloidin, a bicyclic heptapeptide toxin, which is produced by the dead cap mushroom *Amanita phalloides* (Lynen & Wieland 1938**)**. Two different forms of actin were previously described; one was termed active actin and the other was termed inactive actin (Straub 1943). The toxin, which is a rather small molecule, binds to the filamentous actin ("active actin") and stabilizes the filament, thus preventing its degradation into the monomeric globular G-actin (Cooper 1987).

The cell is unable to maintain the dynamics in the cytoskeleton and will eventually die. This strong affinity to F-actin renders Phalloidin the perfect substance to trace it. Scientific companies offer nowadays a lot of different fluorophore-conjugated Phalloidin to trace F-actin in cells or in tissues (e.g. a variety of 16 products are available from ThermoFisher, USA). We used Phalloidin to detect the dynamics of F-actin in the sea urchin *Arbacia lixula*, from the unfertilized egg to the Pluteus larva.

Material and Methods

Sampling, fertilization and preparing the fixation

Four to five adult *Arbacia lixula* were collected in the proximity of the harbour STARESO (N 42°34.80927', E 008°43.46232'). Two batches were collected on August 29th 2016, one at 10.00 and one at 20.00 (UTC+1), both in a depth ranging from 0 to 50 centimetres. Care was taken that the sampled animals would not spawn in the collection boxes. 0.5 M KCl (Potassium chloride) was injected with a needle through the soft tissue in proximity of the mouth on the ventral part of the animal, laying upside down on top of a Becker glass, filled with fresh sea water. Due to the osmotic shock of the KCl, the animals spawned. The sex of the animal was determined by looking at the colour and structure of the ejaculate: Sperm looks like a milky white fluid, eggs appear red-violet pigmented and granular. If the treated animal was male, the injection needle was dismissed to avoid premature fertilization in the following animal.

Prior to the fertilization, one male *Arbacia lixula* was cut open immediately after sex determination. The gonads of this animal were dissected and stored at 4 °C in a 1.5 mL Eppendorf tube. Collected eggs from one single female animal were mixed with a small part of the stored sperms, thus the timing of the fertilization could be determined. Note that this sperm is inactivated in the gonads of the males and gets activated by contact with sea water. The mixture of eggs and sperm was kept at room temperature and samples were taken from this developing stock accordingly to the time schedule in Figure 1. For each sample, fertilized eggs were transferred into a 1.5 mL Eppendorf tube. After most eggs have sediment, the sea water was removed and the fixative was applied.

Table 5- Fixation time schedule for a total of 17 different hpf (= hours post fertilization) stages. The numbers in brackets represent the dates of the fertilization of the batch, either 29. or 30. August

A (@ 10:00AM)	hpf	B (@ 08:00PM)	hpf
16:00 (29)	6	24:00 (30)	4
18:00 (29)	8	12:00 (30)	16
20:00 (29)	10	14:00 (30)	18
22:00 (29)	12	16:00 (30)	20
24:00 (30)	14	18:00 (30)	22
12:00 (30)	26	20:00 (30)	24
14:00 (30)	28		
16:00 (30)	30		
18:00 (30)	32		
20:00 (30)	34		
22:00 (30)	36		

Fixation, staining, visualization, and documentation

All samples were fixed in 1.5 mL Eppendorf tubes for 60 minutes in freshly prepared 4 % Paraformaldehyde (PFA) diluted in 1x phosphate-buffered saline (PBS). The samples were then rinsed five times over the course of 60 minutes in PBS-T_x (PBS + 0.1 % Triton-X100 as detergent), before getting stored in PBS-T_x at 4 °C. Staining was performed in 1.5 mL Eppendorf tubes. For that, fixed material was transferred into a new tube, PBS-T_x was removed, and the sample was incubated in darkness either one hour at room temperature or overnight at 4 °C in Alexa 488 conjugated Phalloidin, diluted either 1:100, 1:250, or 1:500 in PBS-T_x. The tubes were occasionally gently shaken to assure complete staining of the cell material. Next, the samples were washed several times with PBS during 12 hours before getting mounted in 25 µL PBS on a glass slide and were kept unsealed, since the first test with nail polish sealing significantly decreased the fluorescence of the stained animals, most probably due to the solvent components of the polish. Unstained and stained fixated material was stored in PBS at 4 °C for further experiments.

All pictures were taken on a Leitz Diaplan microscope, using a ImagingSource DFK 41F02 camera. Images were manipulated in GIMP v. 2.8. All figures in this work were composed in Inkscape v 0.91.

Results

A total of four individuals of *Arbacia lixula* were needed for this experiment, where one specimen was male and three were female, which were released after spawning their eggs. As a positive control, the Phalloidin-staining was done with flatworms from the family Lithophora (Platyhelminthes, Proseriata).

For the one-hour room temperature incubation, only the 1:250 concentration has brought an acceptable outcome. We therefore decided to take an insight look on the overnight incubations and found the 1:250 the most convenient. We used a flatworm as control since its muscular system is well studied and the predictable outcome could be used to determine whether all reactants as well as the microscope setup used in this work were fully suitable.

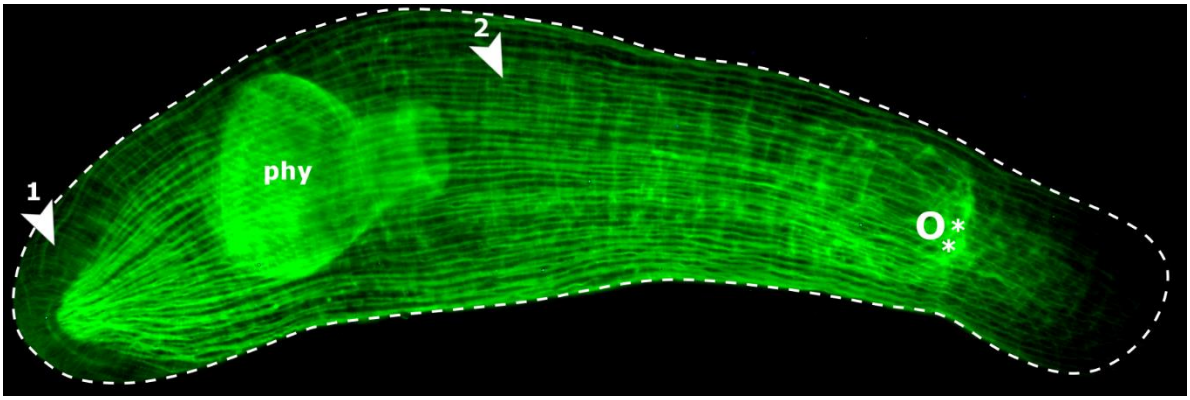

Figure 1- Phalloidin staining (1:250, overnight at 4 °C) of a Proseriata flatworm; pharynx (phy) is in the posterior part of the animal; tip of arrowhead (1) points on a circular muscle fibre; arrowhead (2) shows a longitudinal muscle fibre; O marks the most probable position of the statocyste (a sensory organ, the feature of the family Lithophora in Proseriata); the two asterisks mark the most probable position of the eyes; the animal is facing right, a scale bar could not be inserted due to a lack of camera-microscope calibration. Note that this animal (as most Proseriata) has a body length in the millimetre range

The obtained control staining, featured in figure 2, shows the expected structures of the specimen. Circular (arrowhead 1) and longitudinal (arrowhead 2) muscle fibres could be detected. The typical, well emasculated pharynx (phy) of the flatworm could be identified. The very muscular rich seminal vesicle (not shown in this picture) was also revealed. The position of the statocyste and the eyes could just be approximated by the usage of an image from a living animal, this is due to a lack of bright field images of the stained specimen.

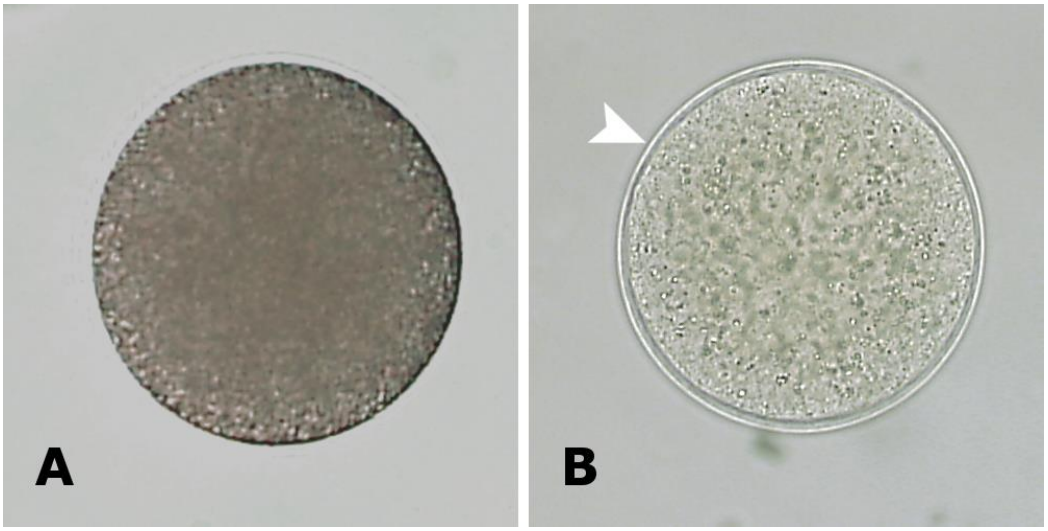

Figure 2- Eggs of Arbacia lixula, needed to ensure that the fertilization of the sample had worked; ***[A]*** *Image of a living, unfertilized egg;* ***[B]*** *fertilized egg, fixated in PFA; arrowhead shows the fertilization envelope, an emerging glycoprotein-network coating the egg seconds after the first sperm has entered, thus preventing polyspermy*

As an additional control in order to check whether the fertilization of a whole batch was successful, a sample was taken five to ten minutes after the fertilization. The eggs were microscopically analyzed and if more than 50 percent showed a fertilization envelope (figure 3 B), the batch was kept for further experiments. All our batches showed a fertilization success rate of more than 80 percent. The following panel (figure 4) shows the different stages of the embryogenesis, from fertilization to the early pluteus larvae, from *Arbacia lixula*. It is not a chronological timeline of one fertilization, but an assembly of three different fertilizations from three female specimens.

We choose to start this overview with the fertilized egg (Fig.3 A1). Seconds after the first sperm entered the egg, the fertilization membrane emerged. The arrowhead points on this membrane, also termed the fertilization envelope. It started to appear at the entrance point of the sperm and then spreads around the egg until complete coverage. In the differential interference contrast image (DIC), the fertilization envelope appears as a bright, narrow ring that encircles the egg. In the panel 3A2, a Phalloidin stained fertilized egg is shown. The border of the egg seems to be slightly brighter than the rest of the egg. However, our image quality is rather poor and has a very high background, so we would not take a final conclusion for this stage. 3B1 shows the two-cell-stage of the egg. The fertilization membrane encloses the two cells of the former egg. This stage could be observed four hours after the fertilization. We could observe a fluorescence highlight in 3B2 where the two cells are in contact with each other (arrowhead).

The fertilization envelope is slightly less stained than the actual cells. The four-cell-stage was observed after the second meridional cleavage in 3C1, which consists now of four well distinguishable identic blastomeres.

The fertilization envelope is still present, however not as visible as in the earlier stages. In the fluorescence staining in 3C2, the edges of each blastomere shows a stronger signal than the remaining cell body. However, the fertilization envelope is not detectable in this image. The third cleavage, which is called the equatorial cleavage, forms four animal and four vegetal blastomeres. The total of eight cells can be observed in panel 3D1. This stage can be identified by focussing through the cell cluster: a ring of six cells with one additional cell above and one underneath this ring can be seen. The envelope was not detectable in our images. For the first time, a strong fluorescence signal was detected on the edges of each blastomere (3D2). The 16-cell-stage (3E1), with micromeres and macromeres, firstly appeared 10 hpf. It is built by a ring consisting of eight cells with two clusters of four cells underneath the ring, and can thus be differentiated from the eight-cell-stage. In this picture, the fertilization envelope is clearly visible again. Unfortunately, the unspecific staining of the embryo renders the interpretation for this stage impossible (3E2). The fifth and sixth cleavages transform the cluster into the 64-cell-stage. The cells rearrange and form a hollow sphere, called “Blastula”, approximately 14 hpf (3F1). The fertilization envelope is still visible and protects the Blastula. The fluorescence staining in 3F2 revealed the first real cell-cell contact interaction. A mesh, looking like the penta- and hexagonal patches of a soccer ball, was detected.

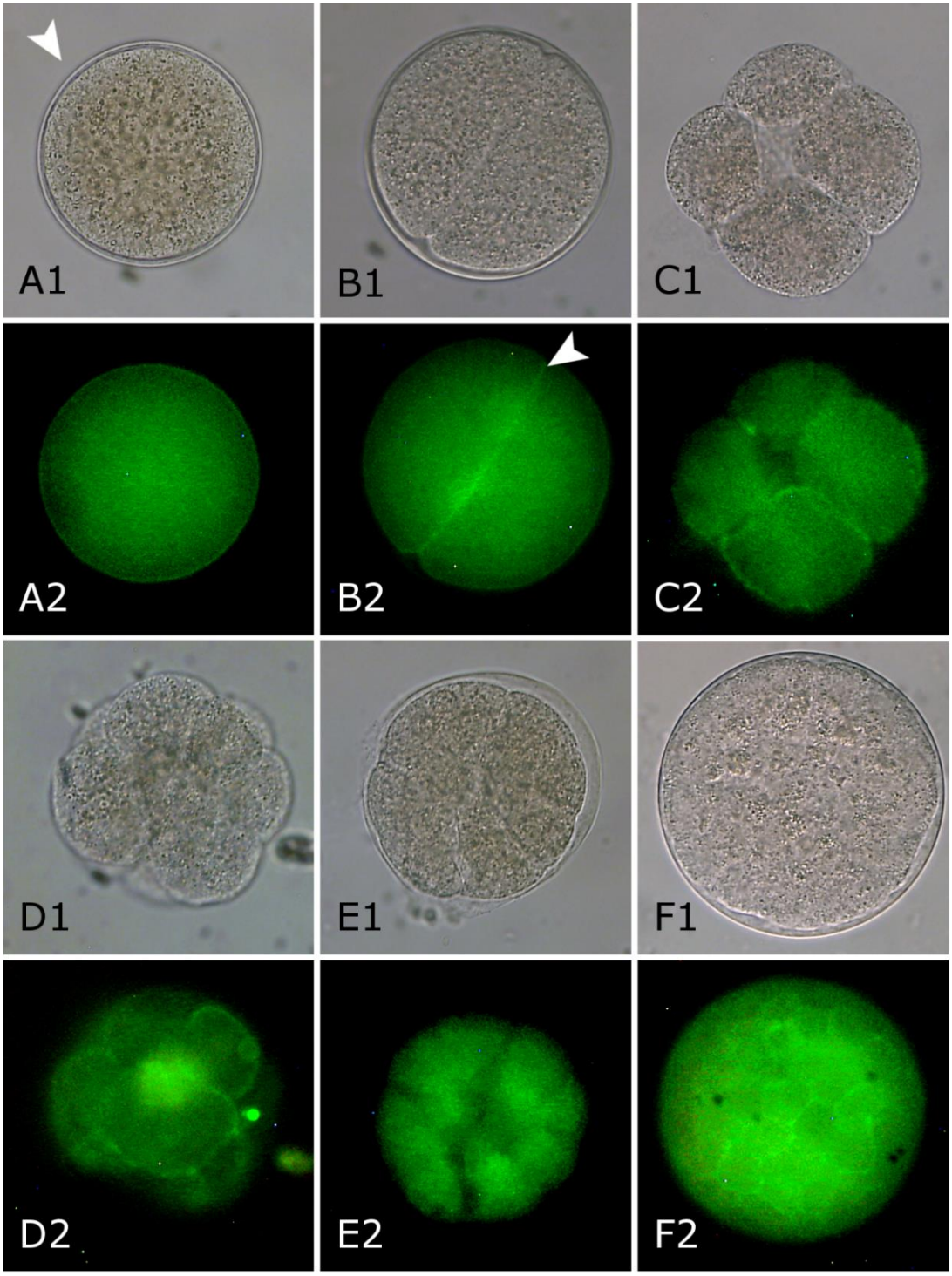

Figure 3- Panel with all stages from fertilization to the pluteus larvae of the sea urchin Arbacia lixula, documented in differential interference contrast and in fluorescence for visualizing filamentous actin (F-actin) by a Phalloidin Alexa-488 staining; ***[A1]*** *Fertilized egg with the fertilization envelope (arrowhead);* ***[A2]*** *No specific staining was detected (only background);* ***[B1]*** *Two-cell-stage at 4hpf;* ***[B2]*** *Edge of each cell detectable as a visualization artefact (arrowhead);* ***[C1]*** *Four-cell stage;* ***[C2]*** *Different edges visible as an artefact of the staining;* ***[D1]*** *Eight-cell stage as the third cleavage;* ***[D2]*** *F-actin visible on the edges of the cells due to an accumulation;* ***[E1]*** *16-cell stage with micromeres and macromeres;* ***[E2]*** *Unsuccessful staining of 16-cell stage;* ***[F1]*** *Blastula stage after 14hpf;* ***[F2]*** *Visible mesh-pattern of cell-cell contacts in the Blastula; no scale bars available.*

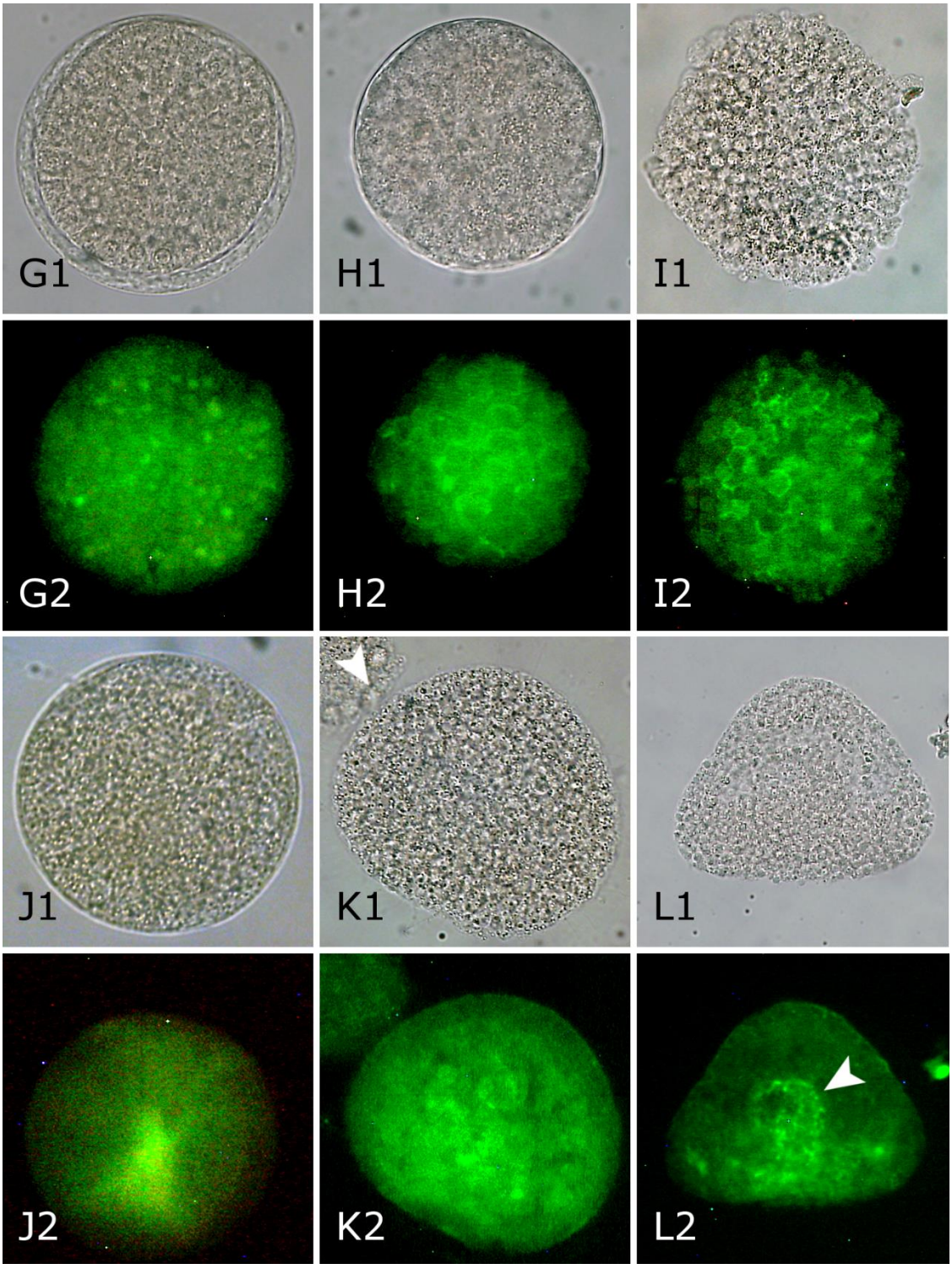

Figure 4- ***[G1-I1]*** *Developing Blastula, G2-I2 More cells with clearly visible F-actin cell-cell contacts;* ***[J1]*** *Early stage of the Gastrula, free from the fertilization envelope and with a ciliated epidermis (arrowhead),* ***[J2]*** *Brighter spot in one half of the Gastrula, probably induced by the formation of the Blastoporus; no scale bars available*

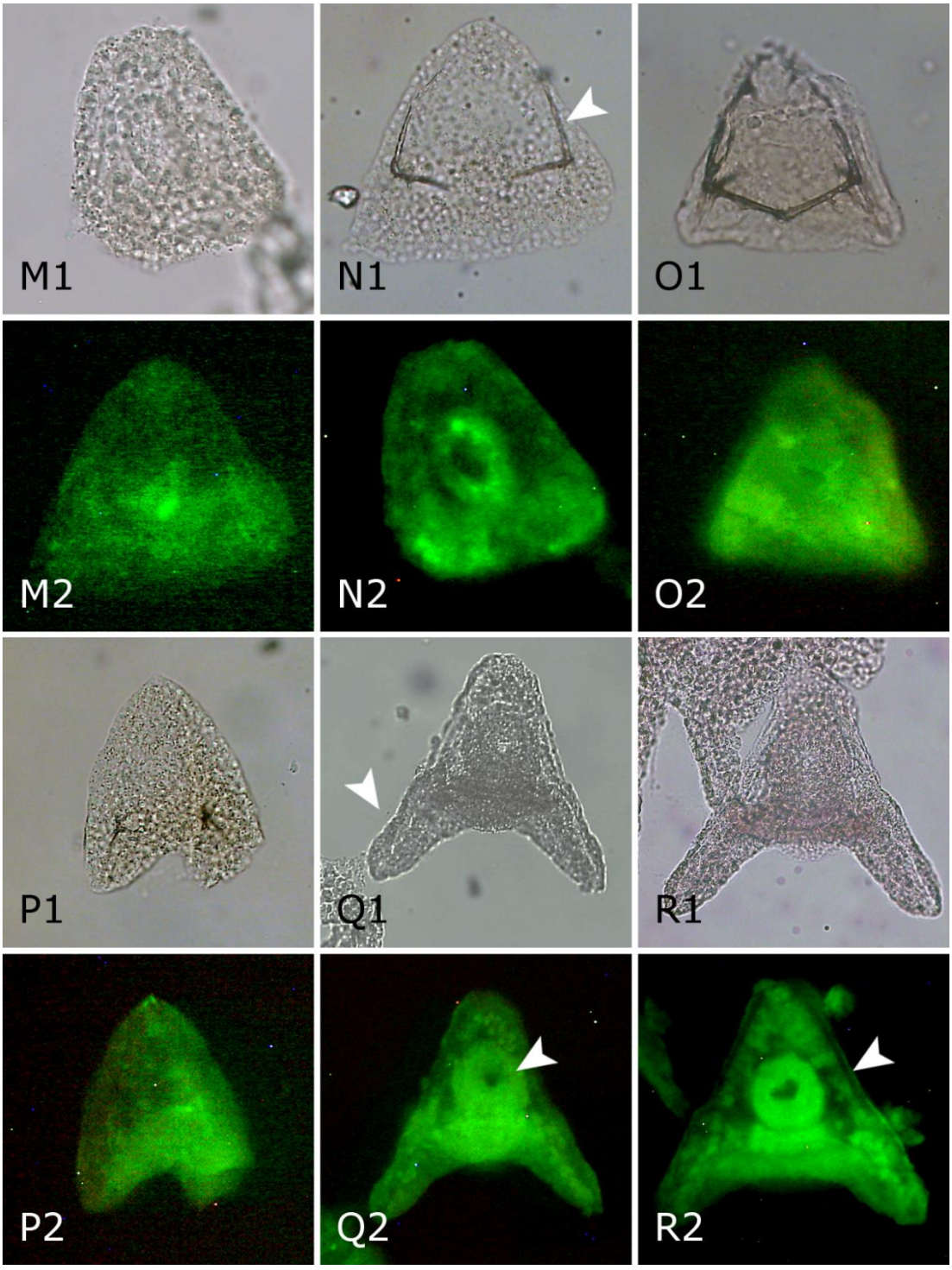

Figure 5: ***[M1]*** *Later stages of the Gastrula, begin of a morphological change from a round shape to a triangular one,* ***[K2-L2]*** *Inner real muscular structure visible (arrowhead) after 30 hpf;* ***[N1]*** *Early stage of the Prism stage with an emerging endoskeleton (arrowhead);* ***[N2]*** *Round shape visible in the middle of the larvae;* ***[O1-P1]*** *Developing Prism with a further growing of the skeleton;* ***[O2-P2]*** *Unsuccessful staining of the Prism stage;* ***[Q1-R1]*** *Early Pluteus larvae with long extensions (arrowhead),* ***[Q2]*** *Ring in the middle of the animal, most probably the musculature of the gut;* ***[R2]*** *Outer cell-cell contacts to seal the epidermis (arrowhead). Note that all transmitted-light images are computer adjusted (f.i. the brightness) differential interference contrast images; no scale bars available*

Over the next 10 hours, the cells undergo more cell divisions and the single cells begin to get undetectable with our microscopic setup (4G1-4I1). At the end of the Blastula stage, the latter is ball-shaped and has ciliated cells. Those cilia begin to swirl and the Blastula breaks out of the fertilization envelope (4I1). It is now able to swim in the sea water. The staining revealed a mesh that gets smaller since the cells get smaller. A strong F-actin signal was detected in the picture 4I2.

The early gastrula stage was observed in the picture 4J1. We determined this as a gastrula stage because it had an uncountable amount of cells. The bright surface in the lower part of the Gastrula in the panel 4J2 is most probably the invagination. The such formed hole in the Gastrula will be the anus of the animal, the terminal part of the gut. In the pictures 4K1-5M1, the Gastrula changes from a round shape into a more triangular one. The staining in the picture 4K2 has a very high background, however, 5L2 revealed a ring, positioned in the middle of the Gastrula, presumably the gut (arrowhead).

The Gastrula develops into a Prism larva, which is pyramid-like shaped. The Prism forms Spicule, which form the first calcareous endoskeleton of the larvae, highlighted with an arrowhead in 5N1. They are initiated in the inner part of the larvae, around the gut. The inner ring, most probably the emerging musculature of the gut, is best observable in the panel 5N2. In the picture 5O1, the Spicule have grown into the arms of the middle stage of the Prism larvae, which is reshaped into a more cornered form at this point. No further information could be acquired by the stained larvae (5O2). 5P1 shows a late Prism stage with long, well separated extensions or “arms”. The staining was unsuccessful (5P2). The four arms grow longer and the larvae is called early Pluteus from now on. It has a unique form, shown in 5Q1. The arrowhead points on one arm, containing one Spicule. The endoskeleton is completely assembled by now. The arrowhead on the stained animal in 5Q2 points onto a prominent ring-like structure in the gut region, containing F-actin. The picture 5R1 shows a late stage of a Pluteus. This is the last stage we can observe in vitro. The stained animal (5R2) shows a strong signal in the epidermis, this is due to the strong cell-cell contacts in the later.

From the stained animals, we propose the following figures for the presence of F-actin in cell-cell contacts in a developing embryo (Figure 6).

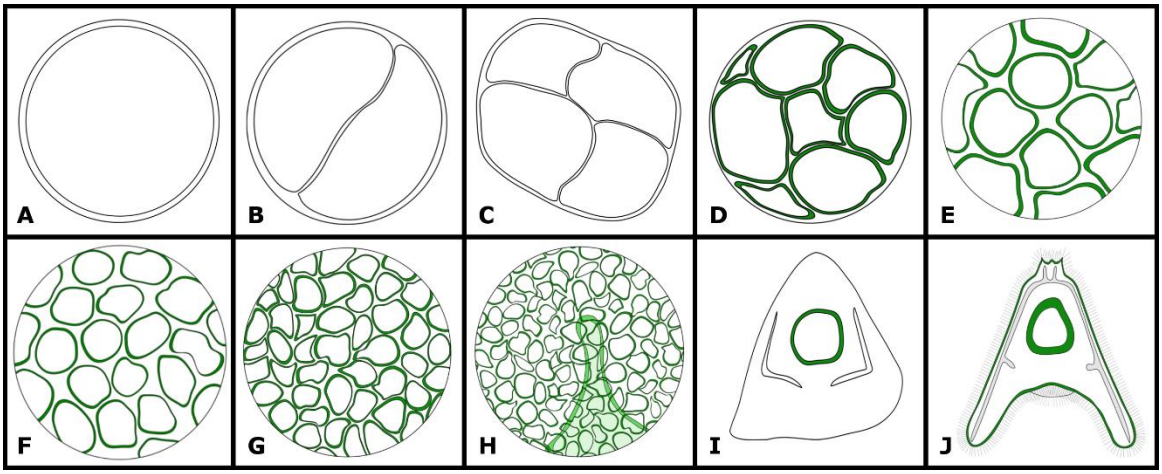

Figure 6-Figure based on the observed developed stages from the fertilization to the pluteus larvae, green shows the cell-cell contacts formed by F-actin, detected by the Phalloidin staining; ***[A]*** *Fertilized egg with the outer fertilization membrane, without any f-actin;* ***[B]*** *Two-cell stage without F-actin;* ***[C]****: Four-cell stage (second cleavage) with no F-actin;* ***[D]*** *eight-cell stage with F-actin on the boarder of the cells;* ***[E]*** *16-cell stages with a clearly visible cell-cell contact pattern;* ***[F]*** *32 to 64 cell stage with visible cell-cell contacts;* ***[G]*** *Blastula with tight cell-cell contacts;* ***[H]*** *Gastrula with a brighter spot in one half, this is due to the invagination and thus the formation of the Blastoporus;* ***[I]*** *Prism stage with particular triangle shape, formation of the endoskeleton (spicule), and with a emasculated ring in the middle of the animal;* ***[J]*** *Pluteus larvae with typical ciliated epidermis, long arms with spicule, a ring shaped gut in the middle of the animal, and outer tight cell-cell contacts on the epidermal level*

In the fertilized egg, only a very small amount of F-actin can be found on the edge of the egg, just underneath the fertilization envelope (6A). In the two-cell-stage, a signal was detected in the contact zone of the two cells. However, we think that this is an artefact and will be further discussed later in this work (6B). The four-cell-stage (6C) has a similar signal pattern than the previous stage. We could observe a signal on the edges of the four cells with a slightly stronger signal on the contact zones. The first strong signal appeared at the eight-cell-stage (6D). The green borders in the figure demonstrate the detectable Phalloidin-signal as well as the border of the single cells in this stage. For the 16-cell stage (6E) as well as the 32-cell-stage (6F) and the 64-cell-stages (6G), the cells get smaller and the signal gets stronger on the contact zones. Since the cell cluster gets hollow, more elaborate cell-cell contacts are needed. The F-actin clusters on the edges of each cell and interacts with the neighbouring cells. The obtained signal looks like a grid with penta- and hexagons. The site of the gastrulation can be identified by a brighter signal spot within the early Gastrula (6H). On this site, a total of four layers of cells and thus more stained F-actin get excited by the fluorescence light, therefore, the area appears brighter. In the Prism, the first real musculature gets visible (6I). A bright ring, the stomach, appears in the middle of the Prism in the stained animal. For the last observable stage, the Pluteus larvae (6J), the stomach gets bigger in diameter and in width. The epidermis of the larvae shows also a high amount of F-actin.

Discussion

Changes of the filamentous actin during embryogenesis in the echinoderm sea urchin *Arbacia lixula* was observed in this work. We found a shift from rather randomly dispersed F-actin in the first cell stages to a well-organized distribution in cell-cell contacts within only a few hours after the fertilization. The first respond of the egg after a sperm has entered is a change in the electronic potential of the cell membrane. A change in the electric potential (e.g. from -30 millivolts (mV) to - 20 mV) of the egg was detected, by Tyler (1956), immediately after penetration of a sperm cell in a star fish egg, another family member of the Echinoidea. This value was decreased during two minutes to its original value (Tyler *et al.* 1956). This is the beginning of the embryonic development of the sea urchin. Within two minutes, the cortical reaction takes place. Specialized organelles of the egg, called cortical granules, containing a mixture of different proteins, e.g. structure proteins and enzymes, forms the fertilization envelope (Haley & Wessel 2004). It emerges at the entrance point of the first sperm and proliferates over the whole egg within less than ten seconds. The fertilization membrane acts as an impenetrable barrier for the sperm, thus preventing lethal polyspermy of the egg. The fluorescent staining of the fertilized egg is not good enough for a convenient statement. However, a high amount of microfilaments in the cortical region of the egg was detected by Cline & Schatten (1986). Also, it is reported that the local amount of F-actin increases on the entrance point of the sperm (Terasaki 1996).

In the two-cell stage as well as the four-cell-stage, we observed a slightly higher amount of F-actin on the edges of the cells, however, this could be a microscopic artefact (Figure 6). Watched from above, it seems that the cell has a very bright highlight on the edge and is a rather low signal is detected in the middle of the cell.

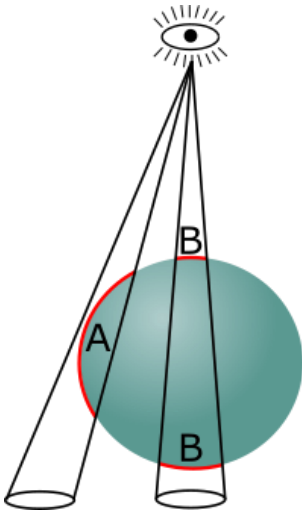

Figure 7- If viewed from above, more of the parallel edges (A) of the cell is detected than the upper and lower part
(B). The outer rim seems thus brighter than the centre of the cell

This problem could be eliminated by taking confocal stacks, in which only one layer is detected at a time. In our setup, the fluorophores of all layers were simultaneously excited and emitted a signal. We therefore have a very high background and the staining artefact. It is thus difficult to conclude final statements for the first embryogenesis stages, where the overall signal is very low. The first certain signal was detected at the Blastula stage. The Blastula stage ends when the approximately 1000 ciliated cells start to swirl and thus "hatch" out of the fertilization envelope (Wolpert *et al.* 2015). A few cells start to migrate on the inner wall of the hollow Blastula and form a cluster in the vegetal pole. Those cells form calcareous rods, which will form the skeleton in the later embryonic stages. The gastrulation starts with an invagination, an inward movement from the cell cluster in direction of the animal pole (Wolpert *et al.* 2015).

In the early Pluteus larvae, the animal has now a fully functional mouth, stomach, and anus (Cameron *et al.* 1987). They use cilia as well as peristaltic muscular contractions of the oesophagus to transport feeding particles from the mouth to the well emasculated stomach (Burke 1981). We therefore conclude that the central ring in the Pluteus larvae is a real muscular tissue. The fringe around the whole animal is no real muscular tissue, but tight cell-cell contacts in the epidermal layer.

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- http://www.devbio.biology.gatech.edu/?page_id=396

Fishes of Corsica

Fishes of Corsica – Who are they and what are they feeding on?

A survey in the bay of Revellata

Shaomin Chen, Serra Örey, Alica Ohnesorge, Rafael Meichßner, Fabian Wolf, Nora-Charlotte Pauli

Introduction

The Mediterranean Sea is a biodiversity hotspot (Coll *et al.*, 2010). According to the online database Fishbase (Froese and Pauly, 2016) 750 different fish species occur here. The bay of Revellata on Corsica near Calvi represents a typical Mediterranean coastal environment. Here, the coastal habitat is characterised by a rocky substrate, with an adjacent seagrass meadow. Both provide a three-dimensional habitat with many niches for a lot of different species. The area surrounding the headland of Revellata has been a marine protected area for more than 30 years.

In this study we were aiming to investigate the fish diversity, community composition, biomass and abundances in different habitats over time. Traditional fishery biology depends on data from net catches either from scientific cruises or commercial fisheries. This method however is not applicable in a shallow coastal rocky habitat. Therefore, coastal transect observations are important to estimate species richness, abundances and biomass in this area. The University of Innsbruck together with the Christian-Albrechts University Kiel and the GEOMAR Helmholtz-Centre for Marine Research have conducted biodiversity surveys in the bay of Revellata since 1992. Within this framework our data will contribute to a long-term biodiversity survey in this area.

Furthermore, we were interested in comparing the diet composition of a day and a night active predator sharing the same habitat. For this purpose, the following species were chosen: The black scorpionfish *Scorpaena porcus* and the painted comber *Serranus scriba*. *Scorpaena porcus* is a primarily night active predator living solitary on rocky bottoms (Froese and Pauly, 2016). It is known to feed on small fish, crustaceans and other invertebrates (Hureau and Litvinenko, 1986). Meanwhile *Serranus scriba* is a day active predator living on rocky bottoms and in seagrass meadows from 5 to 150 m (Froese and Pauly, 2016). It feeds on fishes and crustaceans (Tortonese, 1986)

We aimed to test the following hypotheses:

(I) Species diversity differs between different habitats and over time.

(II) Fish abundance differs between different habitats and over time.

(III) Different fish families contribute differently to the composition of total abundance.

(IV) Fish biomass differs between different habitats.

(V) Different fish families contribute differently to the composition of total biomass.

(VI) *Serranus scriba* and *Scorpaena porcus* mainly feed on crustaceans and fish. The diet composition of the day active *Serranus scriba* differs from the diet composition of the night active *Scorpaena porcus*.

Material and Methods

Transect

To estimate the biodiversity of fishes in the bay of Revellata we used transect swimming. We laid a 137.7 m long transect line out- and inside the harbour of the institute (Figure). It was divided into five sections with three different types of sediment (Figure 2 D-F). The end of each section was marked by a buoy (in form of a floating milk bottle) (Figure 2A). Along the transect line, marked stones were placed in order to show the width of the transect (Figure 2 B) It was 3 m wide on each side. Two persons swam with constant slow speed from one section to the next. At each milk bottles they stopped swimming and discussed which fish species they saw and how many. Abundance estimations were divided into the following categories: A: 1, B: 2-5, C: 6-30, D: >30 (Table 1). The line transect observation was repeated every three hours for approximately 20 minutes beginning at 12:00 on 29th August 2016 and ending at 12:00 on 30th August 2016. Within the whole observation period three pairs of two people each, conducted two surveys. In total 9 observations were done.

For several measurements, 40 fish of different species were caught with hand nets or speargun in the harbour of STARESO and in adjacent areas of the bay of Revellata. All fish were frozen directly after catch and stored for further analysis. For every single fish, length, weight and gutted weight were recorded. Furthermore, otoliths and genetic samples were taken for possible future analyses.

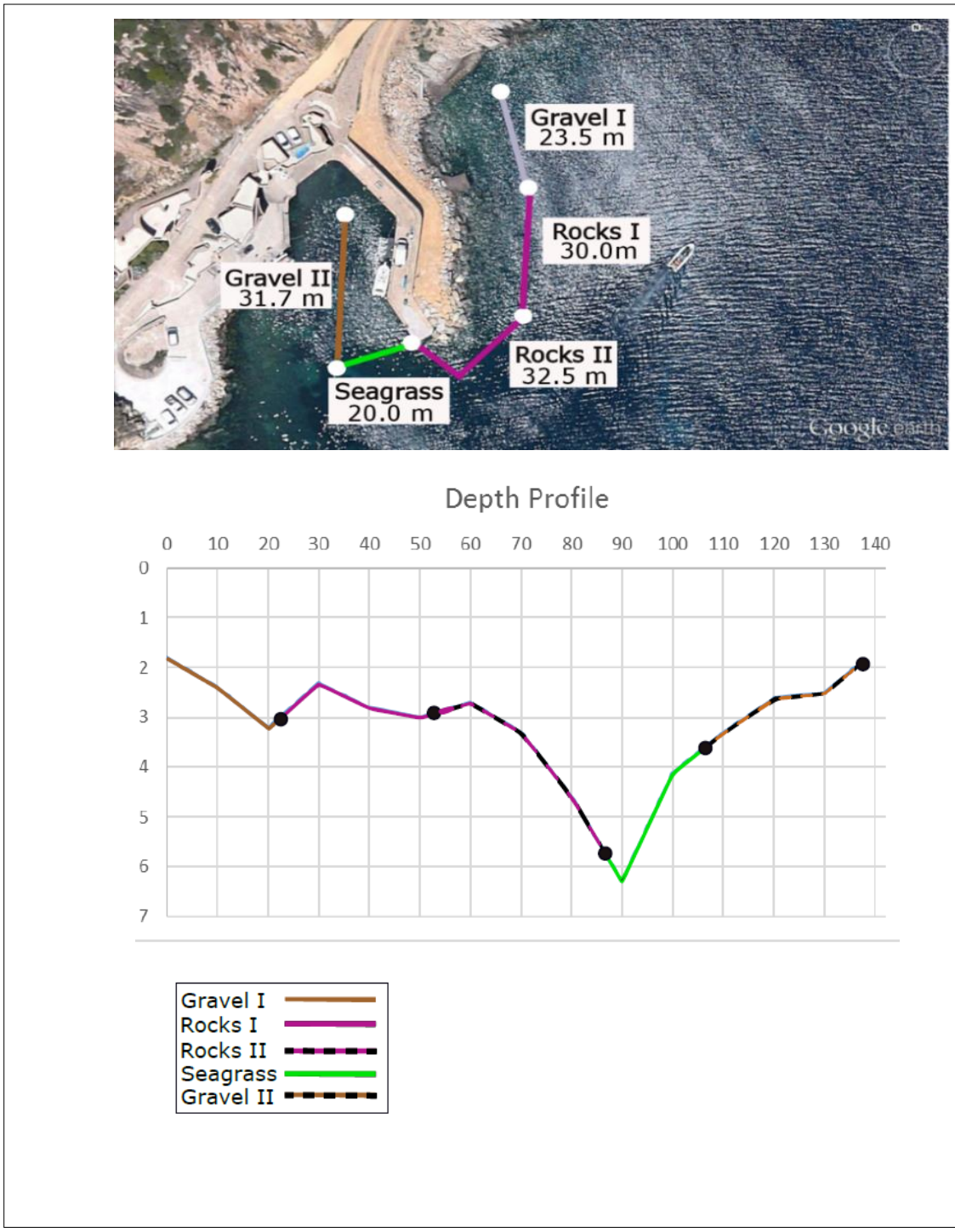

Figure 1- Satellite picture and depth profile of the transect

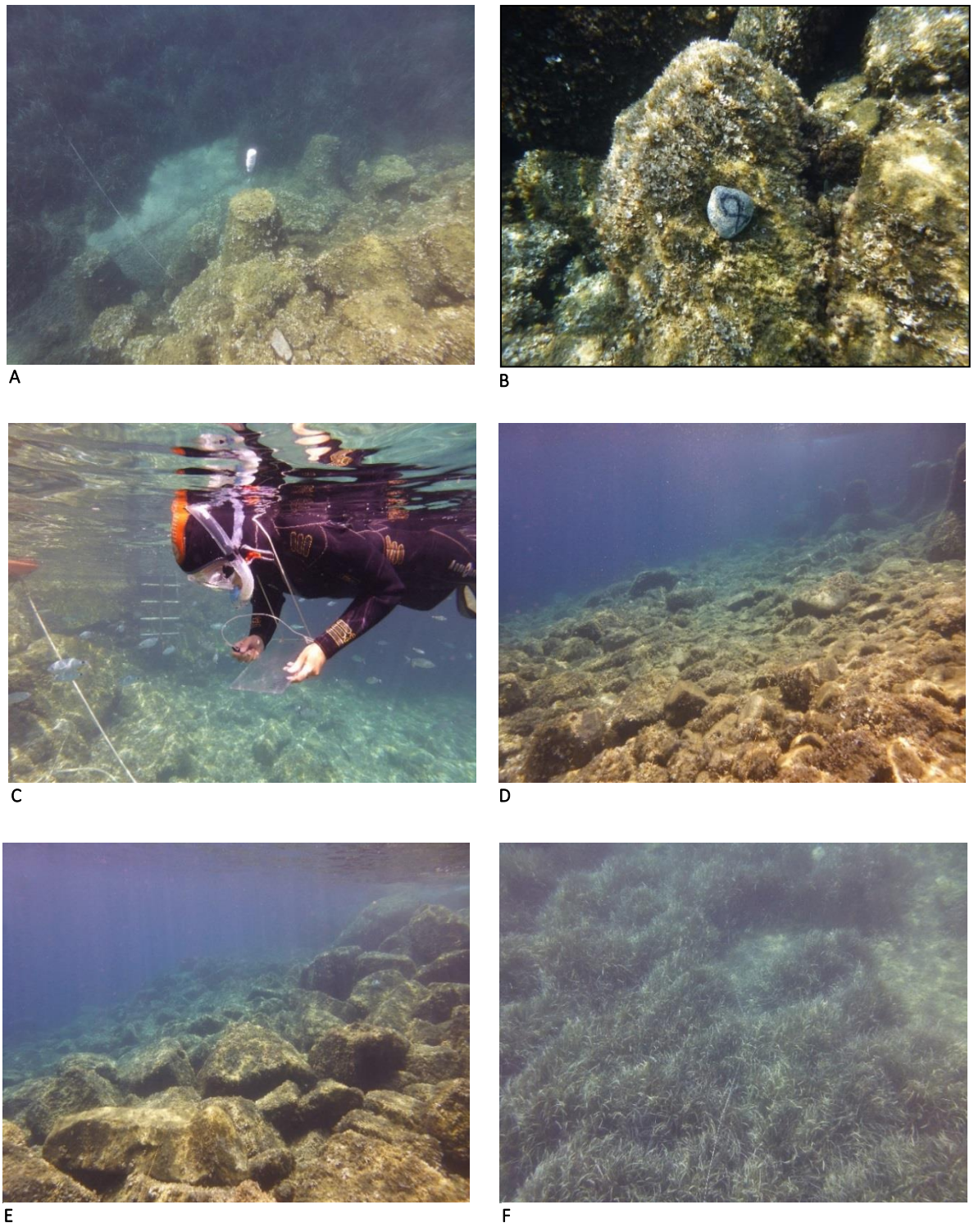

Figure 2- Transect in the Bay of Revellata. ***[A]*** *Each transect section was marked by a buoy (milk bottle).* ***[B]*** *The width of the transect was marked by stones three meters of each site of the transect line.* ***[C]*** *The abundances of each fish species was documented at the end of each transect section. [D] Gravel habitat in sections 1 and 5.* ***[E]*** *Rocky habitat in sections 2 and 3.* ***[F]*** *Seagrass habitat in section 4. Photos © Fabian Wolf*

Transect - Diversity

During the 24-hour survey, abundance of each observed fish species was recorded every 3 hours from 12:00 on August 29th to 12:00 on August 30th, 2016. All the observed species were categorised by families to look at changes in abundance of different families with time.

Transect - Abundance

As mentioned above, for practical reasons we decided to estimate abundances in categories during our observations in the transect. There were four categories from the least abundant to the most abundant in the following order (from lowest to highest): A, B, C, D. For the analysis it was necessary to connect a certain number to each category (A:1, B:3, C:20, D:60, Table1). The chosen numbers were the best representative values based on empirical evidence. Total abundances were calculated by summing up all sightings observed within the total observation period.

Table 1- Categories of abundances and chosen values

Noted	Count	Analysis
A	1	1
B	2-5	3
C	6-30	20
D	>30	60

Transect - Biomass

For each recorded species common length was taken from Fishbase (Froese and Pauly, 2016) and weight was calculated using the Fishbase length- weight- calculator tool. For some species the common length was corrected by an estimation based on the sizes observed during the study. Subsequently, the transect area was extrapolated to one hectare and the biomass was calculated for each species using the calculated weights of the species multiplied by their highest observed abundance among all repeated transects.

Gut content

We analysed the gut and stomach contents of 40 different fish belonging to 18 species. The fish were thawed, dissected and the content of stomach and gut identified to phylum level using a stereo microscope. The amounts of the different food items in terms of volume were estimated in percentages.

Condition index

To get a rough idea about the health of the fishes we caught, we calculated the expected weight from the total length measured. This expected weight was compared with the actual weight of the fish. This so called *condition index* is expressed in percent. The length-weight relationships were taken from different publications (Table 2) which reported length and weight based on large sample sizes per species. From these numbers, mathematical equations were derived, which were in the following used to calculate the condition indices. For *Tripterygion tripteronotus* no length-weight relationship could be found in the literature.

Table 2- Length-Weigh relationships for different species caught in the bay of Revellata, Corsica. W: Weight [g], L: Length [cm] except for Scorpaena notata L [mm]

Species	Length-Weight Relationship	Publication
Apogon imberbis	$W = 0.1135 * L^{2.117}$	Karakulak et *al*. (2006)
Boops boops	$W = 0.0119 * L^{2.8554}$	Morey et *al*. (2003)
Gobius geniporus	$W = 0.005 * L^{3.255}$	Altin et *al*. (2015)
Gobius paganellus	$W = 0.0033 * L^{3.5738}$	Morey et *al*. (2003)
Labrus merula	$W = 0.01047 * L^{3.076}$	Moutopoulos et *al*. (2003)
Labrus viridis	$W = 0.03595 * L^{2.669}$	Moutopoulos et *al*. (2002)
Salaria pavo	$W = 0.0110 * L^{2.977}$	Koutrakis et *al* (2003)
Sciaena umbra	$W = 0.0053 * L^{3.2542}$	Morey et *al*. (2003)
Scorpaena notata	$W = 0.000073 * L^{2.727}$	Petrakis et *al*. (1994)
Scorpaena porcus	$W = 0.0215 * L^{2.915}$	Karakulak et *al*. (2006)
Scorpaena scrofa	$W = 0.012 * L^{3.135}$	Altin et *al.* (2015)
Serranus cabrilla	$W = 0.012 * L^{2.908}$	Altin et *al*. (2015)
Serranus scriba	$W = 0.0044 * L^{3.409}$	Sangun et *al.* (2007)
Symphodus mediterraneus	$W = 0.0147 * L^{3.005}$	Ilhan et *al*. (2008)
Symphodus melanocercus	$W = 0.008 * L^{3.181}$	Altin et *al*. (2015)
Symphodus ocellatus	$W = 0.0131 * L^{2.9664}$	Morey et *al*. (2003)
Symphodus rostratus	$W = 0.0070 * L^{3.292}$	Ilhan et *al*. (2008)
Tripterygion tripteronotus	NA	NA

Results

Transect – Diversity

The family Labridae (wrasses) was observed in relatively high abundance (above 100 individuals) only during the daytime (between 9:00 and 18:00) and became absent during the night (between 21:00 and 6:00). The number of wrasses decreased to absence from noon to 21:00. The family Atherinidae was found throughout the whole monitoring period with a higher abundance during the night and the peak at midnight (0:00). Scorpaenaidae was found to be almost absent at all time except that a small number of individuals were spotted at 0:00 and 3:00. A few individuals of the family Serranidae and a large number of Sparidae were found throughout the whole 24-hour period. Overall, Labridae, Atherinidae and Sparidae had a greater abundance than that of Serranidae and Scorpaenaidae (Figure 3).

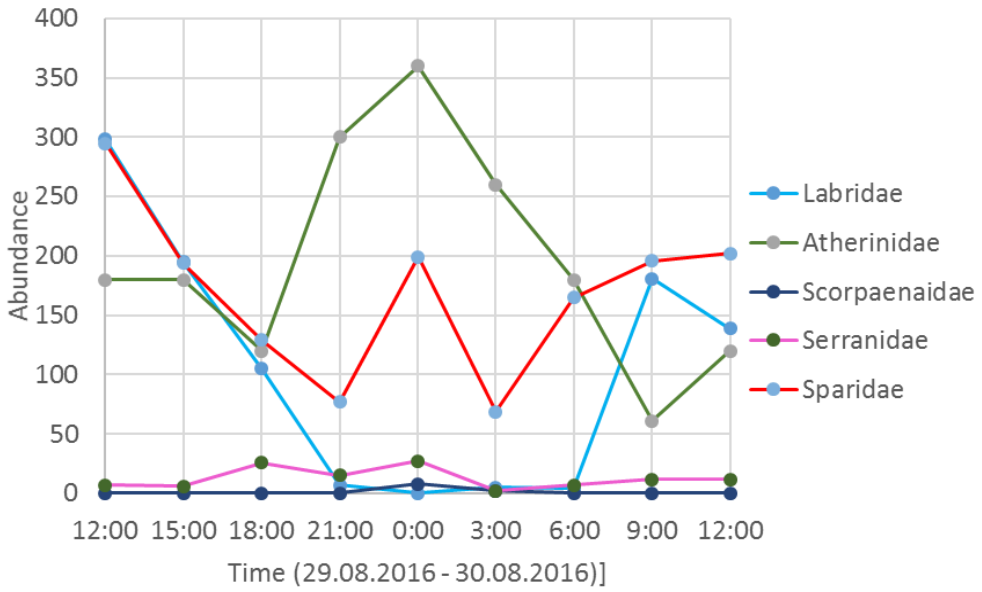

Figure 3- Change in abundance of different fish families with time. Time in hours is depicted on the x-axis. The abundance of the fish families are depicted on the y-axis

Our results show that the number of species was highest in the rock habitats (Rock I and Rock II) with 24 and 26 species respectively while it was slightly lower in the gravel habitats (Gravel I and Gravel II) with about 22 species. The species richness in the seagrass habitat was the lowest with only 16 species (Figure 4).

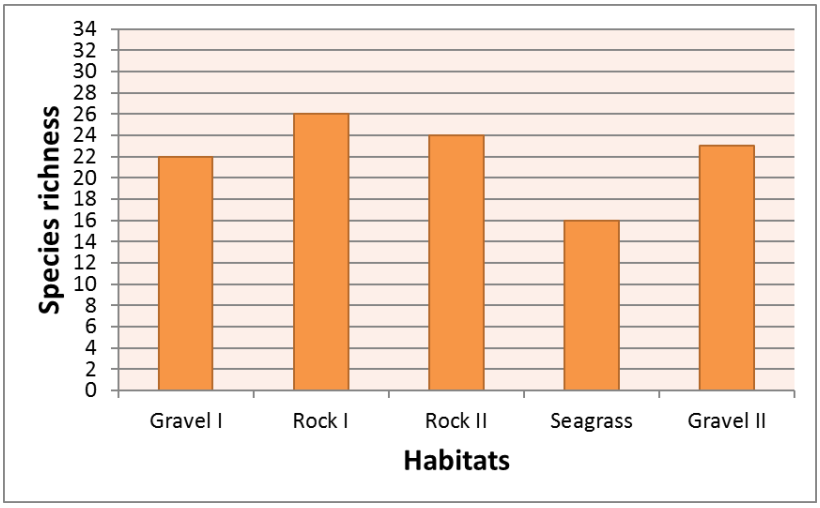

Figure 4- Total species richness at different habitats for all time. The different habitats are depicted on the x-axis. The species richness observed is depicted on the y-axis

Throughout the whole 24-hour period, the number of species was about 20 during daytime (between 9:00 and 18:00) and reduced during the night (between 21:00 and 6:00) with around 13 species (Figure 5).

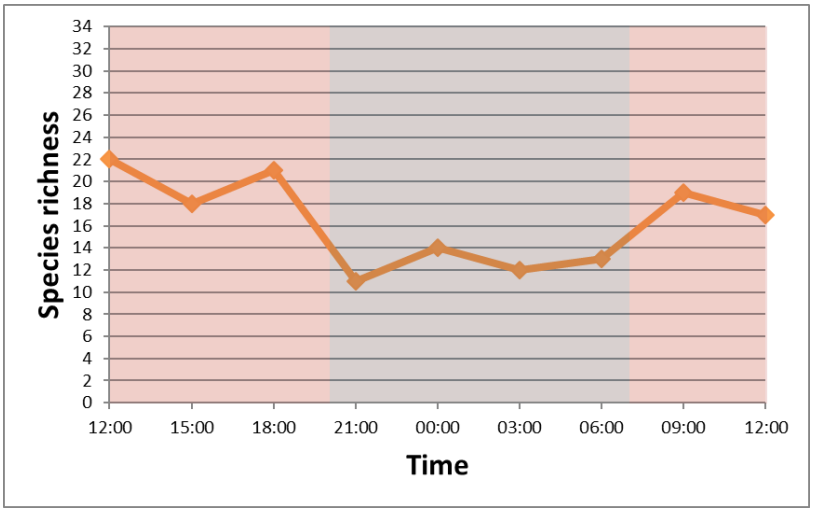

Figure 5- Species richness at different time for all habitats. Time is depicted on the x-axis. Sunset on August 29th 2016: 20:02; sunrise on August 30th 2016: 6:47. The species richness observed is depicted on the y-axis

Transect - Abundance

Abundance of fishes in the total transect survey is displayed on family level. In terms of percentage, the most abundant fish family in all habitats was Atherinidae with about 30%, followed by Sparidae with about 28%. The rarest families were Sphyraenidae and Muraenidae with each being observed only once in the transect (Figure 6).

Family composition was generally similar in all habitats with Atherinidae, Sparidae, Labridae and Pomacentridae being always the most abundant and present. However, the seagrass habitat was an exception as it had more Atherinidae and less Labridae (Figure 6).

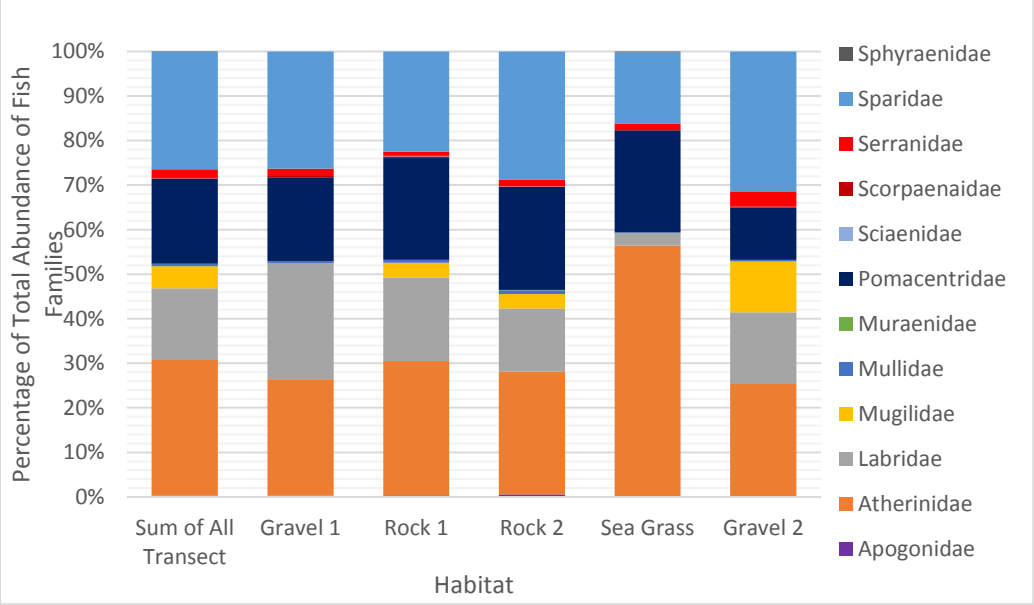

Figure 6- Percentages of the total abundances of all observed fish families. The different habitats are depicted on the x-axis. Each family is depicted by a colour in the stacked bar chart

When comparing different habitats in terms of total abundance (Figure 7) it turns out that least fish were found in Sea Grass having 638 individuals in 120 m^2 (5.3 individuals per square meter, see fig. 8) whereas the most fish were found in the Gravel 2 part with a total number of 1750 in 190 m^2 (9.2 individuals per square meter). Nevertheless, both Rocky 1 and Rocky 2 habitats had a similar number of fish with 1326 and 1307 individuals in 180 and 195 m^2 transect area (7.4 and 6.7 individuals per square meter subsequently). In contrast, the two gravel habitats differed respective to their total number of fish although habitats had similar morphological features (Gravel 1 habitat with total number of 765 in 141 m^2 (5.4 individuals per m^2)).

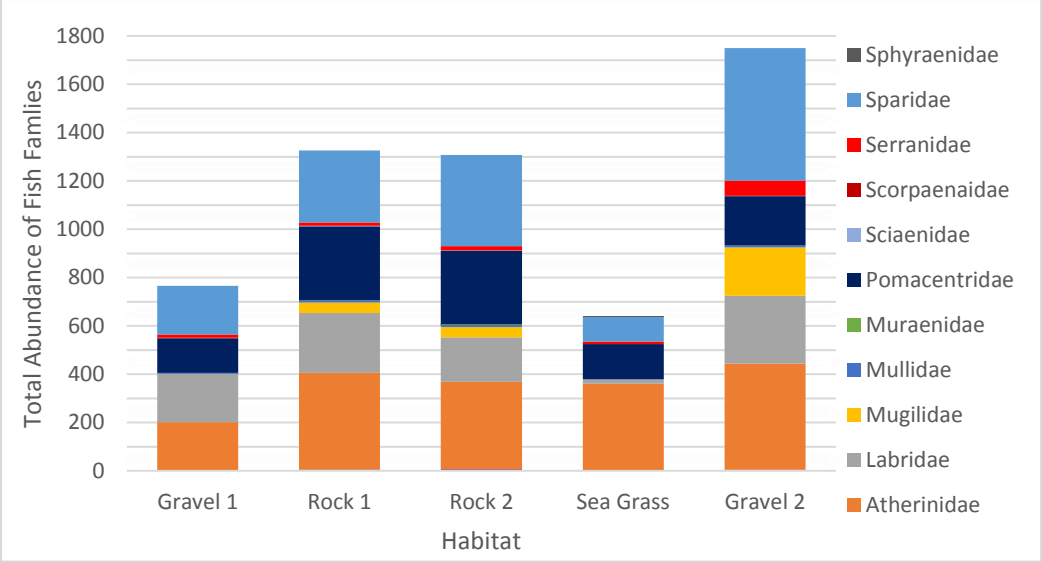

Figure 7 Total abundance of all observed fish families. The different habitats are depicted on the x-axis. Each family is depicted by a colour in the stacked bar chart

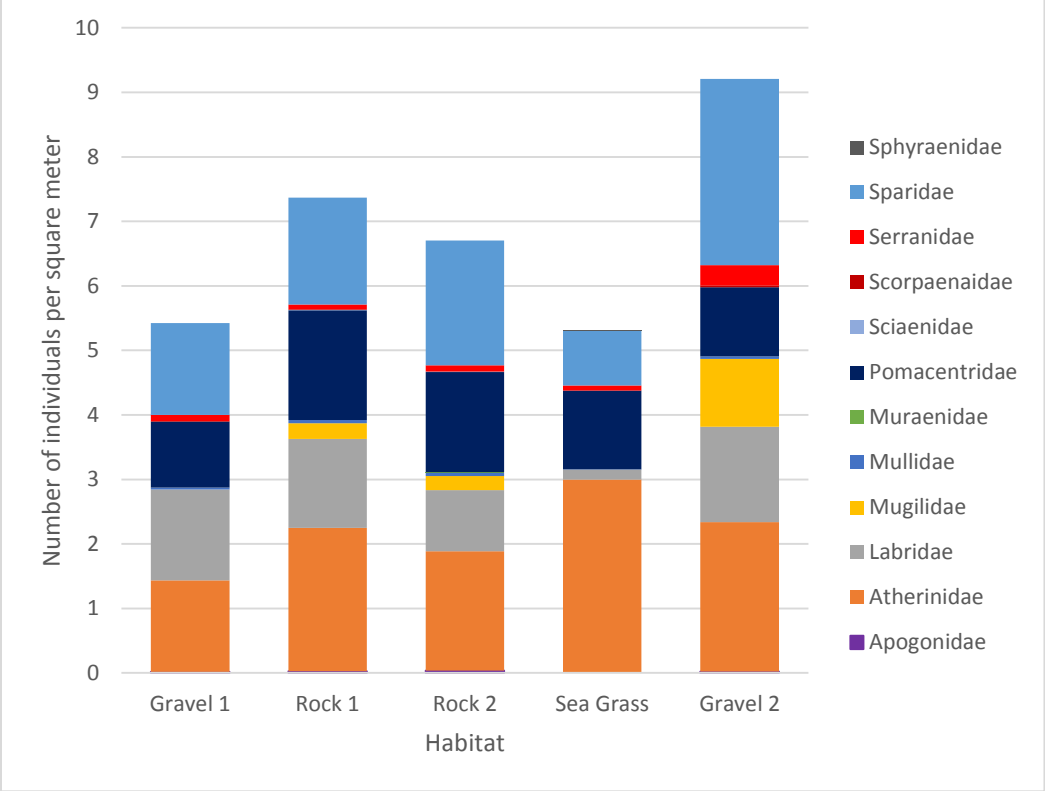

Figure 8- Number of individuals per square meter. The different habitats are depicted on the x-axis. Each family is depicted by a colour in the stacked bar chart

Transect- Biomass

Abundance data simply give the total fish count in an area. However, to better understand the composition of the local fish community, sizes of the species have to be considered. The fish biomass found in different habitats is shown in Figure 9 and 10. As seen in Figure 9 among all habitats, Gravel 2 had almost four times more fish biomass than the other examined habitats. Therefore, it was considered as being an outlier (see discussion) and excluded in graph Figure10 to make the differences between families more obvious among the other habitats.

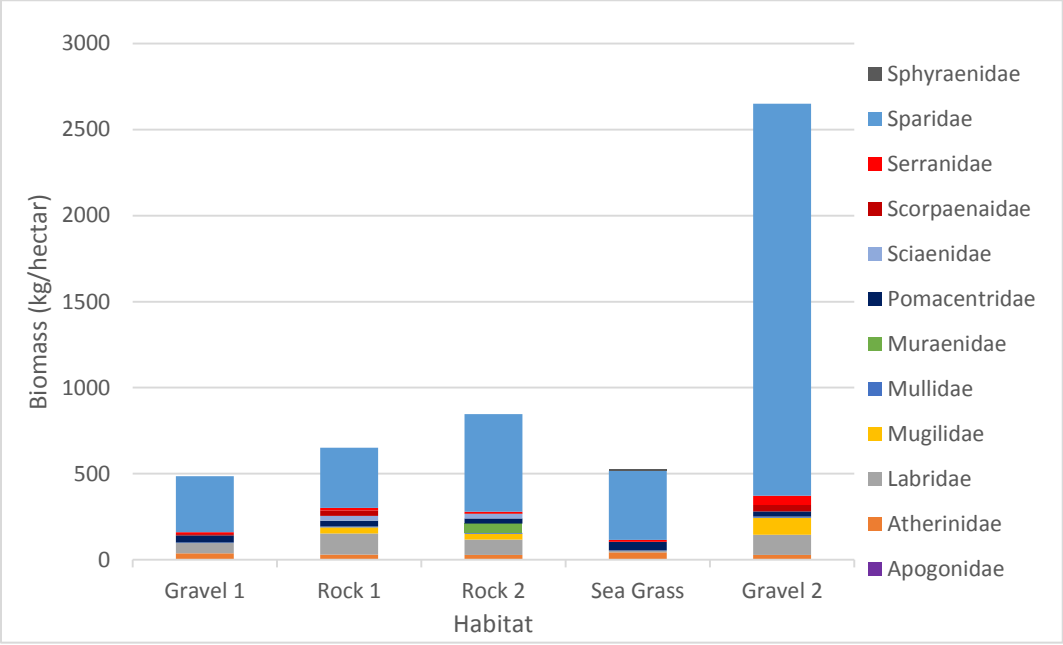

Figure 9- Biomass in kg per hectar of the fish families per habitat. Different habitats are depicted on the x-axis. The biomass is given on the y-axis

Species of the family Sparidae represent the highest proportion in terms of biomass in all habitats. The highest total biomass was found in habitat Rock 2. Gravel and Seagrass generally harboured about 30% less fish biomass (about 500 kg/hectar) than rocky habitats (ca. 600-900 kg/hectar). Labridae were found in high biomasses (ca. 50-100 g/hectar) on rock dominated areas whereas they were rather rare on sea grass.

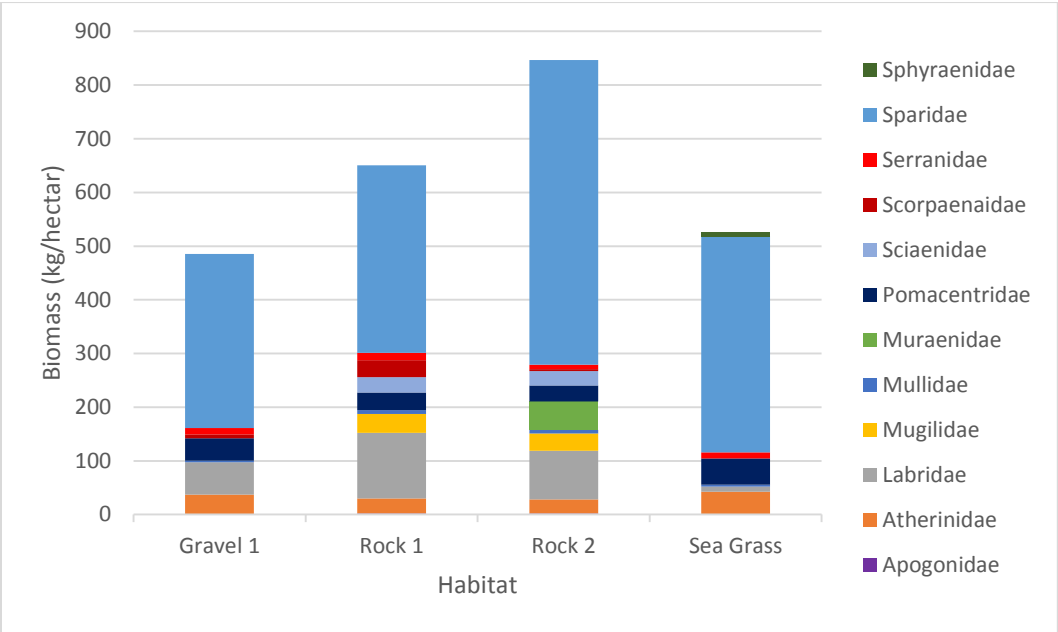

Figure10- Biomass in kg per hectar of the fish families per habitat. Different habitats are depicted on the x-axis. The biomass is given on the y-axis.

Gut content

The guts of eleven individuals of *Serranus scriba* and ten individuals of *Scorpaena porcus* were analysed. The gut of *S. scriba* contained 77% crustaceans and 23% fish (Figure 12). In three *S. porcus* individuals the guts were empty. Those were excluded from the analysis. The food items identified in the remaining individuals of *S. porcus* contained 100% crustaceans (Figure 11).

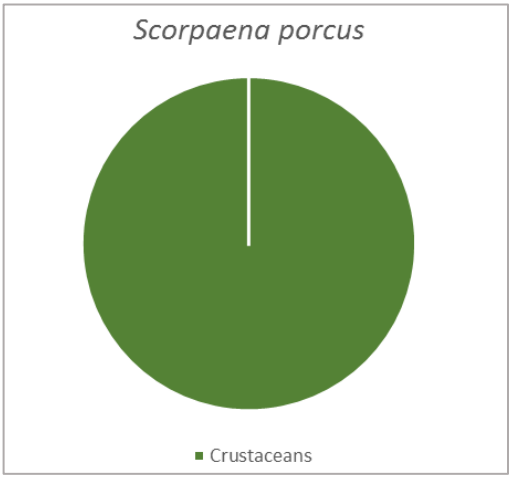

Figure 11- Gut content of Scorpaena porcus (N = 7)

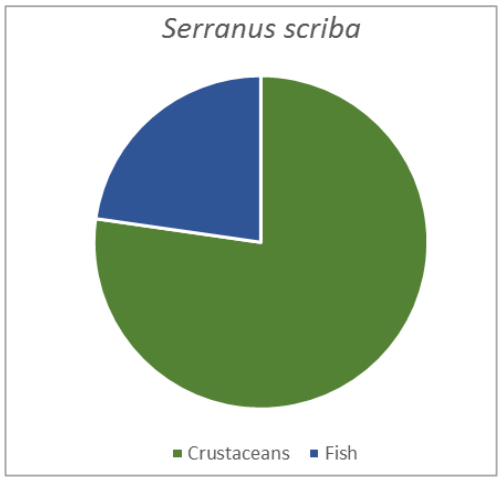

Figure 12- Gut content of Serranus scriba (N = 11)

In addition to *S. porcus* the guts of one specimen of *Scorpaena notata* and two of *Scorpaena scrofa* were analysed. Hence, considering all species from the genus *Scorpaena* their diet contained 62% crustaceans and 8% fish with 31% of empty guts. Overall, we analysed the gut content of 40 fish belonging to 18 species. Eight of those fish had empty guts. Considering all other fish there was a great variety of food items (Figure 13). In total 31 individuals of 13 species fed mainly on crustaceans (73%), followed by fish (18%). Furthermore, other arthropods, molluscs, foraminifera, polychaetes, seagrass and algae could be identified as food items.

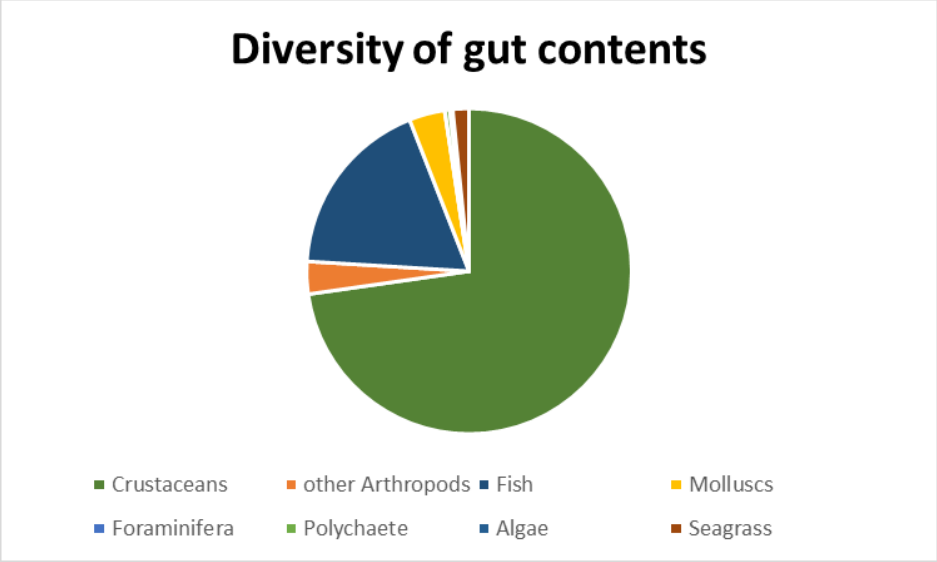

Figure 13- Diversity of food items of 13 species (total N = 31)

Condition index

From 39 individuals of 17 fish species the expected weight could be calculated. The fish species *Scorpaena porcus*, *Scorpaena scrofa*, *Sciaena umbra* and *Serranus cabrilla* had mainly higher actual than expected weights. All other 13 species had lower actual than expected weights. But 38 individuals had a condition index higher than 70%, 30 higher than 80% and 20 higher than 90%.

Discussion

We found that the species richness was highest in the rocky habitats and lowest in the seagrass beds which is similar with the findings by Giakoumi & Kokkoris (2012). Substrate type is a determining factor on the structure and composition of demersal fish communities. Species richness and abundance were always found higher on hard bottoms with higher complexity which provides numerous microhabitats and abundant prey (Bonaca & Lipej, 2005). On the other hand, seagrass bottom was less complex and dynamic, and hence was less diverse. Nevertheless, the species richness in the seagrass habitats might be underestimated due to the difficulties in spotting the fish that were hiding beneath the seagrass. Moreover, sea grass plays an important role for fish nursery.

The decrease of the total number of species from daytime to night- time was almost double. This could be explained by the absence of family Labridae during the night as they show hiding behaviour and also have the most diverse species composition in all families during the transect observation. Another reason for the lower observed species diversity at night might be the poor light conditions. As hand torches were used for observing the sea floor this might lead to missing some species or misidentification due to the limited field of view.

As our data show, fish abundance indeed differs between different habitats that we examined. There was a great difference between the total biomass of the gravel 1 and gravel 2. We think that this effect was caused by the fact that gravel 2 was also the port of the marine station. The habitat was heavily influenced by humans in many ways such as the light of the buildings at night as well as the regular feeding of the fish by the station staff. This led to an unnatural accumulation of several opportunistic fish species feeding on the supplied bread or on fish or invertebrates that are attracted by the food.

However, the similar abundance results between two similar rock habitats (Rock 1 and Rock 2) support that the method is consistent.

It is clearly visible that both rocky habitats have higher abundances than Gravel 1 and Sea grass. This may be due to the fact that the big rocks create more surface area for species to occupy. There are also many crevices to hide which do not exist in gravel and sea grass habitats. However, in the rock as well as in the sea grass habitat, the numbers may have been underestimated due to hidden and therefore unseen individuals.

The rocks are also a habitat for many algae which provide carbon source for higher trophic levels. From our observations, Gravel and Sea grass seemed to harbour fewer algae to support heterotrophic life. As sea grass primary production is harder to be transferred to higher trophic levels than algal primary production (Fry 1984), it is not surprising that sea grass has less fishes than rocks overgrown by algae.

Most families are found in similar proportions in all habitats. However, exceptions are Labridae, Apogonidae and Muraenidae. The Labridae are found in high abundances in all habitats except the sea grass. This can be explained by the fact, that only one species of this family (*Labrus viridis)* prefers sea grass while all other members of the family that we observed are found on rock habitats. Apogonidae and Muraenidae were only seen in the Rock habitats. This is because *Apogon imberbis* and *Muraena helena*, the only representatives of the first two families are really bound to rocks.

Our results demonstrated the change in abundance of different fish families over time which could be an indicator of day-night shifts in fish activity. The absence of Labridae can be explained by their behaviour of hiding in shelters such as rocks and seagrass beds at night (Nagelkerken et *al*., 2000). Nevertheless, the high abundance does not necessarily indicate fish activity in the community. Although Atherinidae was found in huge amount during the night, the swarms were disassembled and the fish were observed to rest still in the water column, showing no sign of any feeding activity. As nocturnal species, Scorpaenidae were expected to be found only during the night which matched our findings. The low abundance in Scorpaenidae was probably due to the difficulties in observing the fish from the surface. The three species we observed (*Scorpaena porcus, Scorpaena notata* and *Scorpaena scrofa*) were well-camouflaged and spent most of their time resting at the bottom, causing challenge in estimation of the abundance. Labridae, Atherinidae and Sparidae appeared to be the most abundant families in the investigated area.

As it is visible in Figure 9 and 10, there were significant differences in the fish biomass between the different habitats. While the two Rock habitats harboured 600- 900kg/hectar of living fish biomass, Gravel 1 and Sea grass harboured only about 500kg of fish biomass. The reasons may be the same as described in the abundance section, rocks provide more area and usable primary production than sea grass or gravel.

In terms of biomass, Sparidae was by far the most dominant family in all habitats, although it was outnumbered by Atherinidae in terms of abundance. The obvious reason is that species of the family Sparidae are usually much larger than species of the family Atherinidae. Since Sparidae was the second most abundant family it exceeded other larger sized fish families (e.g. Mugilidae) in terms of biomass, as well.

A reason for the general dominance of Sparidae in the ecosystem may be that this family has experienced a strong radiation in the Mediterranean and hence its members occupy many different ecological niches. Besides the Sparidae, Labridae was the most species rich family in the area. But even though Labridae were abundant as well, Sparidae were even more abundant and generally exceeded the Labridae species in size. The use of biomass diminishes the role of Pomacentridae and Atherinidae in the ecosystem. Both families were very numerous but contribute only little to the biomass due to their small sizes.

Harmelin- Vivien et *al.* (2008) examined the influence of marine protected areas (MPAs) on fish abundance and biomass in the western Mediterranean. Outside MPAs they found values of 56- 524 kg/hectar, within MPAs they found values of 360- 2256 kg/hectar. The lowest values were found in a sea grass habitat which resembles our results. The results of our study (400- 900 kg/hectar) also match the concrete numbers found by Harmelin- Vivien and colleagues because they are in between the results found inside and outside an MPA and our study site was located at the border of a MPA. It may be that biomass values increase towards the centre of the MPA close to Stareso. As Harmelin- Vivien et *al*. (2008) used the same method as this study, it is allowed to claim that our calculated biomasses are realistic.

Our results of the diet of *S. scriba* are in line with the findings by Tortonese (1986). In contrast to the results presented by Hureau and Litvinenko (1986), we only found crustacean remains in the gut of *S. porcus*. These differences may be caused by a total of 30% empty guts and a low sample size. Moreover, Arculeo *et al*. (1993) investigated the food preferences of *S. scriba* and *S. porcus* in the Tyrrhenian Sea. Their results coincide with ours as they found decapod crustaceans as main prey for *S. porcus* and a mixture of crustaceans and fish as food items in *S. scriba*. Hence, we can confirm our hypothesis regarding *S. scriba* having a higher portion of crustaceans than fish in its diet. However, the hypothesis concerning *S. porcus* feeding more on fish than *S. scriba* cannot be confirmed. This might be due to the high amount of empty guts, which do not provide any information about feeding preferences.

Almost all individuals had a condition index higher than 70%. This shows that the fishes are in good, but not in perfect condition. Although this was expected due to the nearby marine protected area, which should provide enough food for all species, a condition index exceeding 100% was only met by carnivorous species. *Scorpaena porcus* having a condition index above 100% seems to obtain an advantage over Serranus *scriba* having a condition index below 100%. But as we only had few or no replicates for all other species, these condition indices are probably not representative.

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