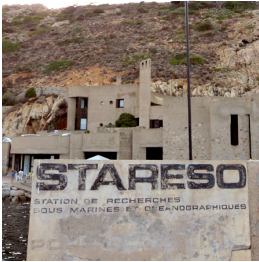
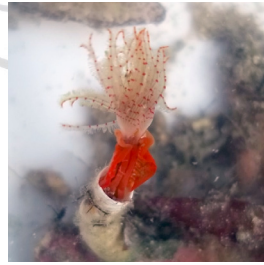
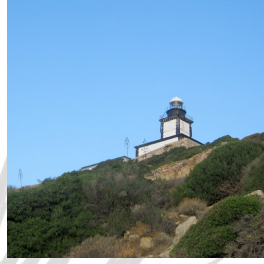
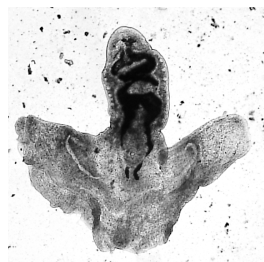
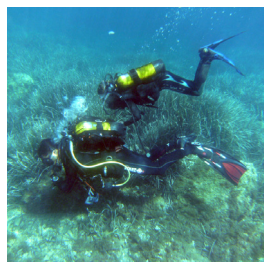


# Marine Biological Excursion



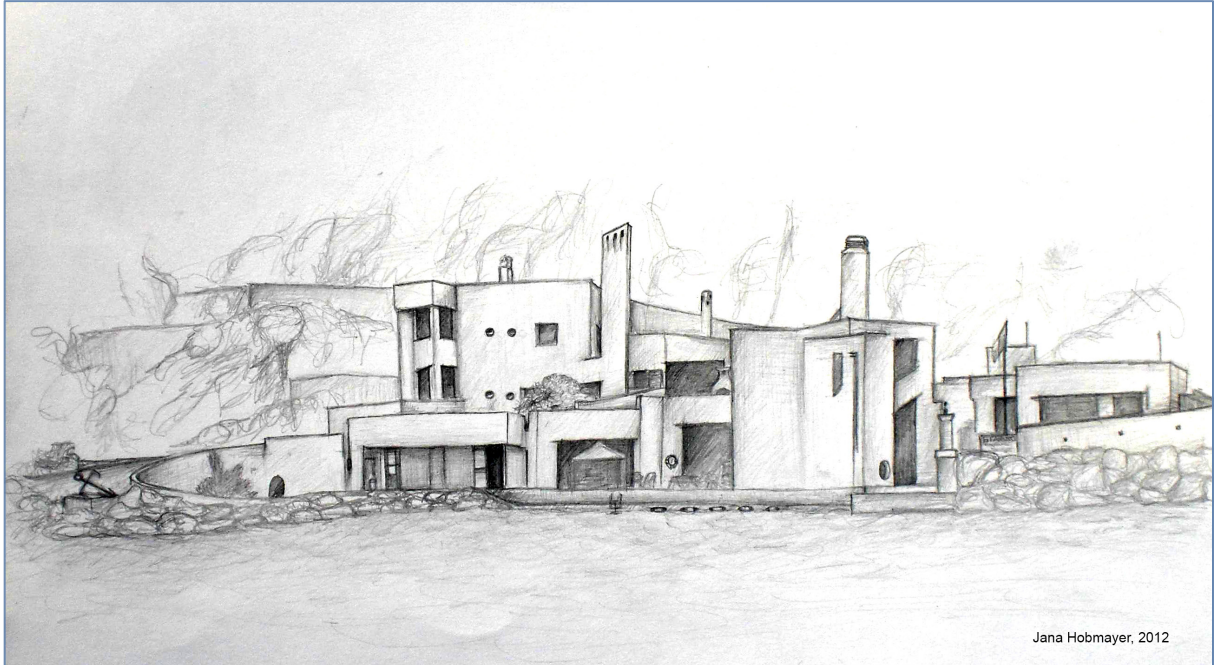
Calvi  
2012





# Marine Biological Excursion, Calvi - Corsica

August 25<sup>th</sup> - September 08<sup>th</sup>, 2012



STARESO, Station de Recherches Sous-Marine et Océanographique

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# Introduction

The marine biological research center “Station de Recherche Océanographiques et Sous Marines” (STARESO) in Corsica offers perfect research facilities by its direct access to the sea. Researchers and students may stay there for long periods to get insight into the different projects and various marine biological methods.

The combined excursion of the University of Innsbruck and Kiel took place between August 25th and September 8th, 2012. The first week aimed at an overview of the local marine species, their habitats and their adaptations to different environmental conditions. Lectures and seminars were given to convey the necessary background information about the local marine animal groups and habitat structure.

During intensive snorkelling the students learned to distinguish and to catch different Mediterranean fish species and they collected algae, bivalves, snails, worms, octopuses and sea urchins. They became familiar with the morphological and physiological features of different animal groups, they practiced to determine the respective genus or species and got a general look into the four big projects of the second week. The students worked in groups and summarized each topic by a daily report.

The STARESO research center has two dry laboratories - including precision balances, mechanical dryers and a hood - and a wet lab containing aquaria with continuous sea water supply. The labs were used during the second week to work on the different projects which comprised mollusc species identification, sea urchin development, measurement of periodical differences of fish activity patterns as well as diet composition and weight length relationship of common wrasse species. Additionally to the on-site lab facility of the research center a fluorescence microscope, binoculars and marine identification books were brought from Innsbruck and ensured accurate work at a high standard.

Finally, the results were presented by the students during a mini-symposium at the end of the excursion.

STARESO serves as starting point and offers infrastructure for diverse scientific research e.g. inventories of plants and animals, fish counts, quality analyses of *Posidonia oceanica* and sea water, and measurements of animal diversity.

Longtime analyses summarize chemical, biological, physical and climate data of the Calvi Bay and indicate a change of the conditions resulting from the observed climate change during the last three decades. To gain a better understanding of the different environmental correlations further biological research is necessary. Therefore STARESO research center is and remains an important attraction for international research teams and students from all over the world.





# Algae

Maria Danelli, Ulrike Hanz and Balsam Al-Janabi

## Introduction

In this course we collected different types of algae, which are native near to the STARESO Station in Calvi, Corsica. Algae are a diverse group of eukaryotic organisms, which can be simply unicellular or multicellular. They use photosynthesis for energy absorption. As primary producers they are the main food source for many organisms to depth of 100 m or even more in exceptional cases. Occurring mainly in shallow and coastal areas they can be affected by human influence like eutrophication, which can change their natural distributions.

There are micro- and macroalgae, however we considered only the macroalgae in this course, because of the limited methods of accumulating material. Macroalgae can only attach to a substratum with a certain size through a sectorial organ. The sectorial organ can be discoidal or composed of many rhizoids. That's why they mostly occur on hard bottom substrates. The rhizoid passes into the cauloid, which is a trunk like structure, which again passes into the vegetative body of the plant, called thallus. The thallus can feature many different shapes, in which the photosynthesis is taking place. It is also likely to find algae overgrown by epiphytes. Along the coasts of Calvi at deeper water, algae can be found also in smaller forms (for example as a crust), same is true for the dark communities inside of caves.



Fig.1: (A) STARESO, Station de Recherches Sous-Marine et Océanographique; (B-D) different kinds of algae found in the area of STARESO.

Algae exhibit a wide range of reproductive strategies, from simple, asexual cell division to complex forms of sexual reproduction. Some algae have also a sexual and asexual phase. They can be annual or perennial, like terrestrial plants, though macroalgae .

Macroalgae can be divided into three groups, the Chlorophyta (green algae), the Rhodophyta (red algae) and the Phaeophyta (brown algae). The differences between these groups are mainly due to their photosynthetic pigments and result in specific depth distributions in depth (depending on light intensity). All algae contain Chlorophyll a (two absorption maxima) and  $\beta$ - Carotene (absorption max. at 350-550nm). Green algae additionally contain Chlorophyll b (max. at 400-500nm),  $\alpha$ - Carotene and Xanthophyll. Red algae additionally contain  $\alpha$ -Caroten, Zeaxanthin, Lutein, Phycoerythrin (max. at 500-600nm) and Phycocyanin (max. 550-650nm). Brown algae additionally contain Chlorophyll c, Fucoxanthin, Diadinoxanthin and Diatoxanthin. (fig.3a)

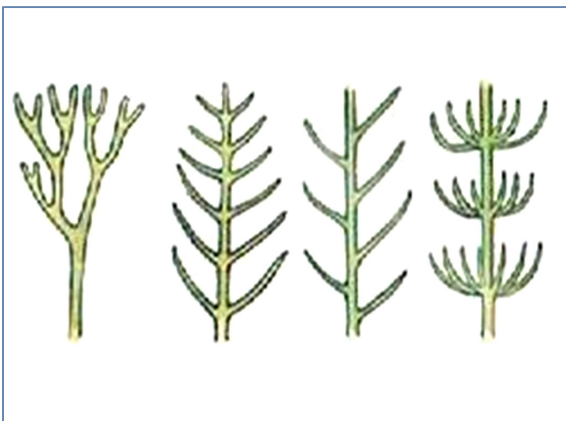


Fig.2: Different shapes of the thallus: dichotom, oppositely, alternate, verticillate. (Hayward/Nelson-Smith/Shields, Der neue Kosmos Strandführer p.16)

These different pigment profiles are the main reasons why, green algae are mostly found in shallower water, whereas brown algae can live until a depth of 35m and red algae can still live until a depth of 300m. The depth distribution is controlled by the use of different pigments, which have their maximal efficiency at different wavelengths. The wavelengths are characteristic for certain water depths (fig.3b). Nonetheless the three groups of algae do have an overlap in the same water depth, as we could observe in the communities.

## Material and Methods

In order to understand how diversity changes in depth and light, algae were collected in 1 m, 1 – 3 m and more than 3 meter water depths. The collection took place on 30th of August 2012. The algae were collected by snorkelling, plucking them without a knife and putting them into two different plastic bags. One bag was for plants in

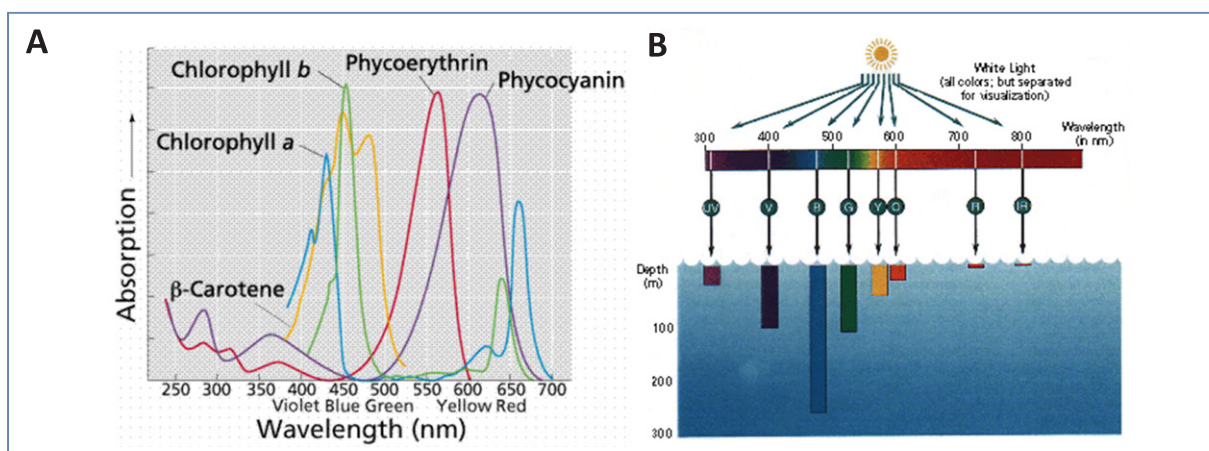


Fig.3: (A) Some absorption profiles from different pigments in different wavelengths (B) the light penetration of sunlight in water (www.emc.maricopa.edu/ www.disc.sci.gsfc.nasa.gov)

shadow habitats and one for plants exposed to more intensive sun light. Every person was responsible for one of the three depth zones. Afterwards the algae were put into different bowls for subsequent species identification, which was accomplished by the use of appropriate literature.

## Results

34 algae species were collected which belong to 19 families. The most abundant algae group were the Rhodophyta (red algae) (14 species) and Chlorophyta (green algae) (12 species), which represent 76% of all algae found (fig.4).

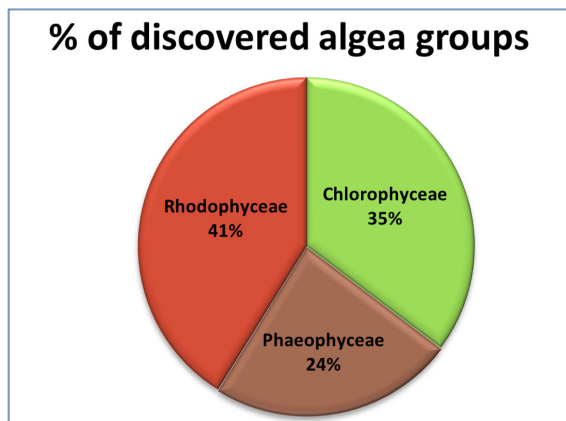


Fig. 4: The Percentages of the 3 families of algae.

The most abundant families were the Codiaceae withing the Chlorophyta, Dictyotaceae and Cystoseiraceae and within the Phaeophyta and the Corallinaceae within the Rhodophyta (species list).

Red algae were mainly found in shadow habitats and grater depths while green algae dominated at light exposed sites, species which classified to Chlorophyta were found. Brown algae were collected in in both habitats (fig.5).

As illustrated in figure 5, it could be observed that the green algae were found in highest abundant in greater depth of 3m under the sunlight influence, but simultaneously there were no green algae in depths 0 to 1 meters. Additionally, the majority of Rhodophyceae species (7 species) were detected in the shadow sites in 3+ meters although the

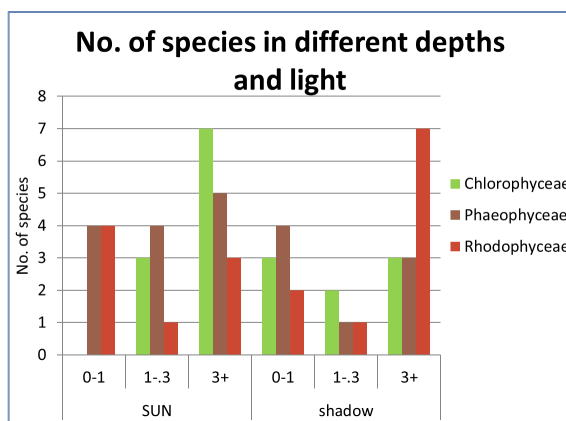


Fig. 6: Number of species in different depths and light

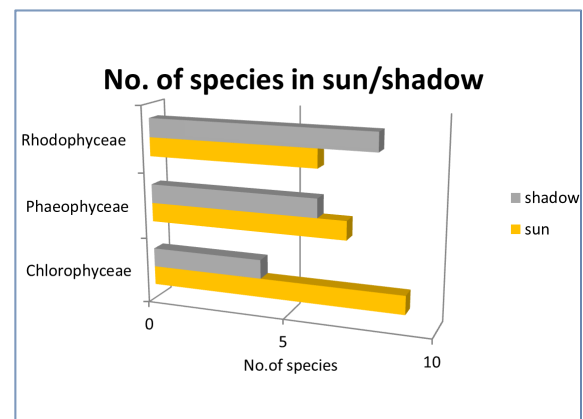


Fig. 5: Number of species on the sun and shadow sites.

abundance on the shadow sites is quite low in the shallow water. Finally, about the brown algae, the higher abundance was under the sun influence (3 to 5 species) but lower in shadow with 1 to 4 species.

## Discussion

A comparable amount of algae were collected in this year (34 species) in contrast to the previous year (37 species). Therefore we would not say, that there is a meaningful difference in the species composition of the different years. The difference could be due to the variable efficiency of determination.

In all depths all algae groups were found, what is not surprising, because of the small differences between the three depth groups. The distribution, especially for red algae, is much deeper than 3 m (until 300m), so a real separation of the different algae groups cannot be found.

The highest number of Rhodophyceae (7 species) were collected in the shadow sites in depths of 3+ meters. This is due to the fact that the red algae have the ability to grow in relatively deeper waters than green and brown algae. (Tschudy H.R., 1932) The presence of the pigment phycoerythrin reflects red light and absorbs blue light. The blue light penetrates water to a greater depth than light of longer wavelengths and these pigments allow red algae to photosynthesize and live at somewhat greater depths than most other algae. (Source internet, University of California, Berkeley, museum of paleontology, 6/12/2012)

Because of the distribution pattern (fig.6) it could be said, that the niches are perfectly occupied, because the number of species of green algae decreases towards the shadow and the species number of red algae is decreasing towards the sun. The brown algae have an intermediate number of species in all depths. This is also represented in the maximum number of brown algae species.

Finally it can be said, that there is an adaptation of red algae to the different light spectrum in deeper water and to more shady habitats. Some of the red algae are also really well adapted to the wave activity in the shallower water, due to calcium carbonate enclosure (Corallinacea). That's the reason for the red algae are also represented in the upper layers of the water.

As a conclusion, we compare our data with the data of 2010 and we found the same pattern of the distribution of the different algae groups, like the lack of green algae in the surface water layer.

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## Species list

Phylum	Family	Species	
Chlorophyceae	Codiaceae	<i>Codium bursa</i>	
		<i>Codium vermilaria</i>	
		<i>Codium effusum</i>	
		Udoteaceae	<i>Udotea petiolata</i>
		Halimedaceae	<i>Halimenda tuna</i>
		Cladophorales	<i>Cladofora prolifera</i>
			<i>Cladophora socialis</i>
		Anadomenaceae	<i>Anadomene stellata</i>
		Polyphysaceae	<i>Acetabularia acetabulum</i>
		Caulerpaceae	<i>Caulerpa racemosa</i>
			<i>Caulerpa prolifera</i>
		Palmophyllaceae	<i>Palmophyllum crassum</i>
	Phaeophyceae	Dictyotaceae	<i>Dictyota dictotoma</i>
<i>Dictyota linearis</i>			
<i>Padina pavonica</i>			
		Stypocaulaceae	<i>Halopteris scoparia</i>
			<i>Halopteris filicina</i>
		Cystoseiraceae	<i>Cystoseira cf. amentasea</i>
			<i>Cystoseira compressa</i>
			<i>Cystoseira palearica</i>
Rhodophyceae		Squamariaceae	<i>Peyssonelia squamaria</i>
	Corallinaceae	<i>Corallina officinalis</i>	
		<i>Amphiroa rigida</i>	
		<i>Corallina mediterranea</i>	
		<i>Titanoderma sp.</i>	
		<i>Lithophyllum lichenoides</i>	
		Rhodomelaceae	<i>Laurencia obtusa</i>
			<i>Brongniartella byssoides</i>
		Bonnemaisoniaceae	<i>Asparagopsis armata</i>
			<i>Falkenberg rufolanosa</i>
		Liagoraceae	<i>Liagora viscida</i>
		Delesseriaceae	<i>Nitophyllum punctatum</i>
		Ceramiales	<i>Calithamnion corymbosum</i>
	Halymeniaceae	<i>Grateloupia filicina</i>	



# Boulder Field

Julia Offer, Sebastian Peer and Julia Wunderer

## Introduction

Boulder field describes an ocean floor plain with rocks and blocks of different sizes, offering a variety of habitats in between-, under- and on the rocks themselves. The coast near the bay of STARESO (Station de Recherches Sous-Marine et Océanographique) is characterized by this type of substrate and shows a high diversity of all kind of organisms. The substrate benefits from the high diversity (f.e. sandy substrate<sup>1</sup> shows lower diversity) and leads to a good water quality.

The harbor basin itself, where we spent most of our time sampling, is characterized by relatively shallow water leading to massive algae-growth on the granite-blocks (fig.1), as well as the protection from tempests given by the mole. Moreover old bread is regularly thrown into the water offering an additional source of food, mostly for fish.

The granite blocks differ in size (fig.2), and depending on the size they are turned around at different rates by the waves. This is an important factor as some organisms cannot sustain a high turn-rate, for instance algae, which are reliant on sunlight.

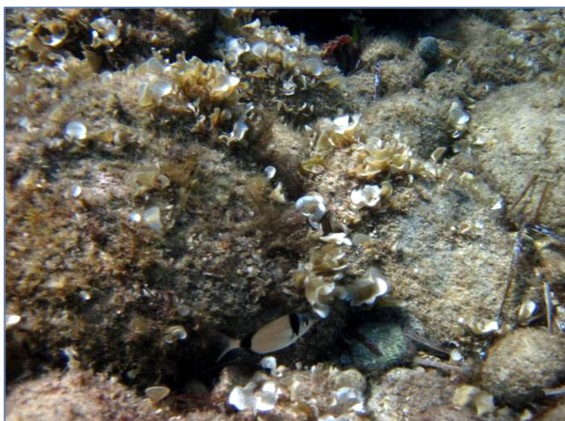


Fig.1: algae growth on granite blocks in the harbor



Fig.2: different block sizes

## Materials and Methods

The boulder field in the bay of STARESO was investigated by snorkeling. The students collected the organisms from different depths in several snorkeling sessions on one day. The organisms were collected by hand or with nets and were kept in small water basins. The organisms of the macro fauna were categorized, mainly using the classification guide "Fauna und Flora des Mittelmeeres" by R. Riedl and were removed into the sea.

Fishes that were present in the bay of STARESO were categorized and a list of all identified fish species in the habitat boulder field was made.

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<sup>1</sup> Environmental and benthic habitat factors structuring the spatial distribution of a summer infralittoral fish assemblage in the north-western Mediterranean Sea, Y.Letourneur, S. Ruitton, S. Sartoretto 2003

## Results

Most of the sampled organisms in the boulder field could be defined to species level, only two decapods could be determined only to family level (Diogenidae, Paguridae) and the sea cucumber *Holothuria* sp. to the genus level. Altogether 51 different organisms of the invertebrate fauna were found and categorized. Not counting the fishes, the molluscs presented the biggest group with 24 species, followed by the arthropods and the crustaceans with seven species respectively. The smallest groups were the annelids, the echiurids and the tunicates with one identified species each. For the first time since the beginning of the Calvi- excursions sponges were classified to the species level (tab.1).

**Table 1:** List of all categorized macro fauna organisms found in the boulder field in the bay of STARESO.

Phylum	Class	Subclass	Order	Family	Species		
Porifera	Demospongiae		Dictyoceratida	Irciniidae	<i>Sarcotragus muscarum</i>		
					<i>Ircinia variabilis</i>		
			Haplosclerida	Chalinidae	<i>Reniera fulva</i>		
					Poecilosclerida	Hymedesmiidae	<i>Hemimycale columella</i>
					Hadromerida	Spirastrellidae	<i>Spirastrella cuncatrix</i>
Cnidaria	Anthozoa	Hexacorallia	Actiniaria	Actiniidae	<i>Actinia equina</i>		
					<i>Condylactis aurantiaca</i>		
					<i>Anemonia sulcata</i>		
					Hormatiidae	<i>Calliactis parasitica</i>	
					Mollusca	Gastropoda	
<i>Thais haemastoma</i>							
Fascioliariidae	<i>Fasciolaria lignaria</i>						
Trochidae	<i>Monodonta turbinata</i>						
	<i>Gibbula divaricata</i>						
	<i>Gibbula umbilicalis</i>						
Patellidae	<i>Patella caerulea</i>						
	<i>Patella candei</i>						
	<i>Patella rustica</i>						
	Haliotidae	<i>Haliotis lamellosa</i>					
<i>Haliotis tuberculata</i>							
Cerithiidae		<i>Gourmya vulgata</i>					
Pyrenidae	<i>Columbella rustica</i>						
Ranellidae	<i>Charonia tritonis</i>						
Elysiidae	<i>Thuridilla hopei</i>						
Polyplacophora				Chitonidae			
				Ischnochitonidae	<i>Ischnochiton rissoi</i>		
Bivalvia			Arcoida	Arcidae	<i>Arca noae</i>		
					<i>Barbatia barbata</i>		
				Limidea	<i>Lima lima</i>		
					<i>Limaria hians</i>		
				Pectinidae	<i>Chlamys varis</i>		
				Pinnidae	<i>Pinna nobilis</i>		
Cephalopoda				Octopodidae	<i>Octopus vulgaris</i>		
Echinodermata	Ophiuroidea		Ophiurae	Ophiodermatidae	<i>Ophioderma longicaudum</i>		
				Ophiomyxidae	<i>Ophiomyxa pentagona</i>		



	Holothuroidea			Holothuriidae	<i>Holothuria sp.</i>
	Echinoidea			Arbaciidae	<i>Arbacia lixula</i>
				Echinidae	<i>Sphaerechinus granularis</i>
				Echinidae	<i>Paracentrotus lividus</i>
	Asteroidea			Echinasteridae	<i>Echinaster sepositus</i>
Arthropoda	Crustacea	Malacostraca	Decapoda	Xanthidae	<i>Xantho poressa</i>
					<i>Xantho incisus</i>
				Porcellanidae	<i>Pisidia longicornis</i>
				Diogenidae	
				Paguridae	
		Malacostraca	Isopoda	Cymothoidea	<i>Nerocila bivittata</i>
				Maiidae	<i>Pisa nodipes</i>
Annelida	Polychaeta			Polycirrinae	<i>Polycirrus aurantiacus</i>
Echiurida				Echiurinae	<i>Bonellia viridis</i>
Nemertini	Anopla		Heteronemertini	Lineidae	<i>Notospermus geniculatus</i>
Tunicata	Ascidacea		Stolidobranchiata	Styelidae	<i>Polycarpa sp.</i>

In the bay of STARESO a total of 31 fish species could be found. The Labridae and the Sparidae with ten and seven species presented the largest groups, whereas other families were present with only one species (e.g. Mullidae, Blenniidae, Belonidae, Mugilidae etc.) (tab.2).

**Table 2:** List of all categorized fish species seen in the bay of STARESO.

Phylum	Class	Subclass	Order	Family	Species
Chordata	Actinopterygii	Neopterygii	Perciformes	Labridae	<i>Coris julis</i>
					<i>Thalassoma pavo</i>
					<i>Symphodus ocellatus</i>
					<i>Symphodus rostratus</i>
					<i>Symphodus tinca</i>
					<i>Symphodus roissali</i>
					<i>Symphodus melanocercus</i>
					<i>Symphodus mediterraneus</i>
					<i>Labrus viridis</i>
					<i>Labrus merula</i>
				Apogonidae	<i>Apogon imberbis</i>
				Sparidae	<i>Boops boops</i>
					<i>Sarpa salpa</i>
					<i>Diplodus annularis</i>
					<i>Diplodus vulgaris</i>
					<i>Diplodus puntazzo</i>
					<i>Diplodus sargus</i>
					<i>Oblada melanura</i>
				Pomacentridae	<i>Chromis chromis</i>
				Mullidae	<i>Mullus surmuletus</i>
				Carangidae	<i>Seriola rivoliana</i>
				Blenniidae	<i>Parablennius sanguinolentus</i>
				Tripterygiidae	<i>Tripterygion delaisi</i>
			Beloniformes	Belonidae	<i>Belone belone</i>
			Scorpaeniformes	Serranidae	<i>Serranus scriba</i>

Phylum	Class	Subclass	Order	Family	Species
					<i>Serranus cabrilla</i>
					<i>Mycteroperca rubra</i>
				Scorpaenidae	<i>Scorpaena porcus</i>
			Atheriniformes	Atherinidae	<i>Atherina boyeri</i>
					<i>Atherina hepsetus</i>
			Anguilliformes	Muraenidae	<i>Muraena helena</i>
			Mugiliformes	Mugilidae	<i>Oedalechilus labeo</i>

### Species composition boulder field 2012

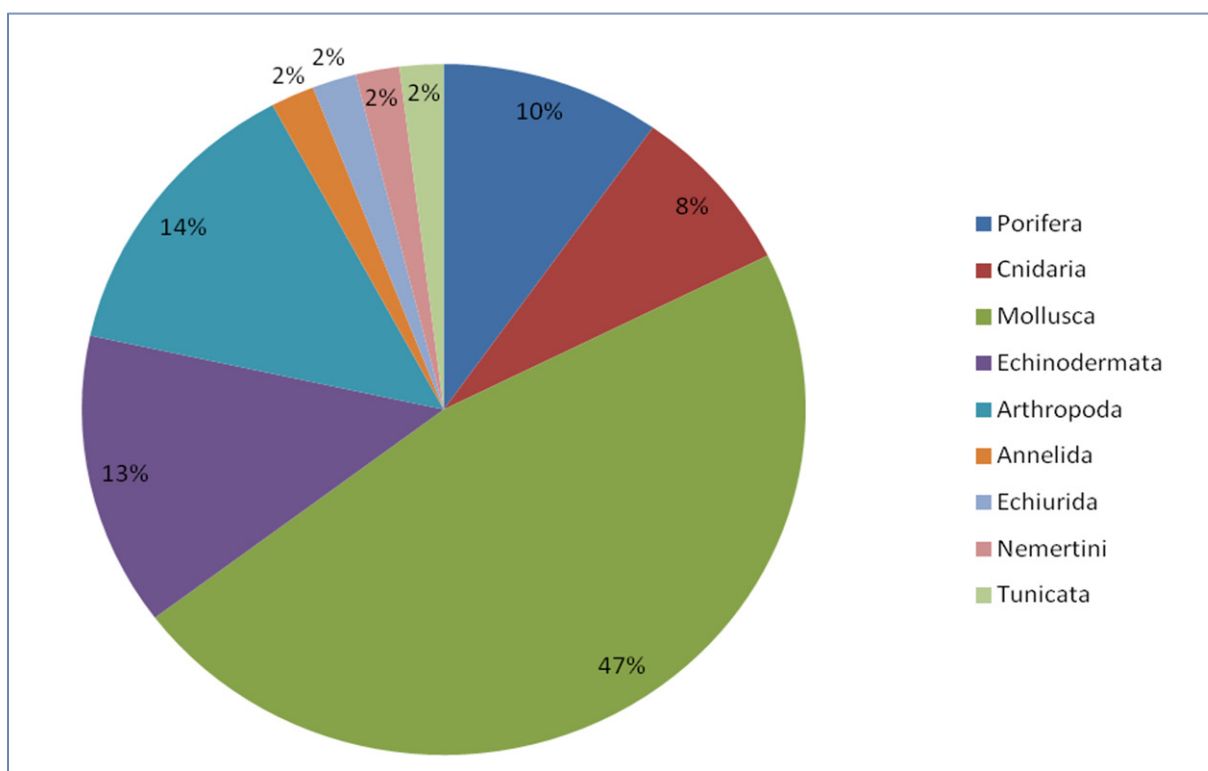


Fig.3: Species composition in the boulder field of STARESO bay.

### Discussion

Out of the 51 determined species 17 haven't been found near Stareso in 2010, namely *Condylactis aurantiaca* and *Calliactis parasitica* (Cnidaria), or *Fasciolaria lignaria*, *Haliotis tuberculata*, *Charonia tritonis*, *Thuridilla hopei*, *Ischnochiton rissoi*, *Lima lima*, *Limaria hians* and *Pinna nobilis* (Mollusca), *Xantho incisus*, *Nerocilla bivittata* and *Pisa nodipes* (Arthropoda) and *Notospermus geniculatus* (Nemertini).

In comparison to the years before *Sphaerechinus granularis* (Echinodermata) must be pointed out, as we sampled 7 individuals. This could be due to the increased water temperature in the summer of 2012. It has to be mentioned that in general the Echinoids were not as abundant as in past years.

In contrast to 2010, one individual of the Tunicate *Polycarpa* has been found. For the first time since the beginning of this course some Porifera were determined to the species level.

The huge number of Mollusca may be explained by the fact that they are easy to catch and a lot of empty shells can be found and determined. This is comparable to the years before.

In total this year more species were determined than in 2010, but the species composition differed. One reason is that in 2010 many organisms were collected by snorkeling and diving, whereas we collected them only by snorkeling (fig.4).

The catching success is dependent on the skills of the people involved, the weather conditions on the sampling days as well as luck. Therefore we cannot expect to find individuals of every abundant species in this area, it is more or less a snapshot of the species spectrum.

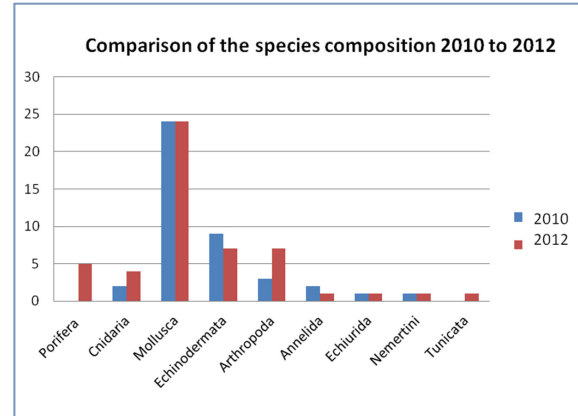
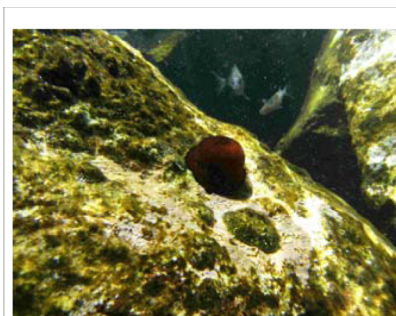


Fig.4: Comparison of the Phylum composition in 2010 and 2012 in the boulder field of STARESO bay. In 2012 51 species of invertebrates were collected in the boulder field of Stareso and we were able to determine them on species level (in 2010 there were found 42 species).

## Species



*Actinia equina*



*Anemonia sulcata*



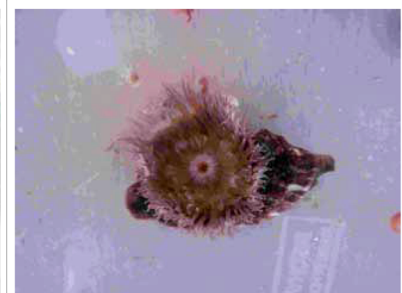
*Arbacia lixula*



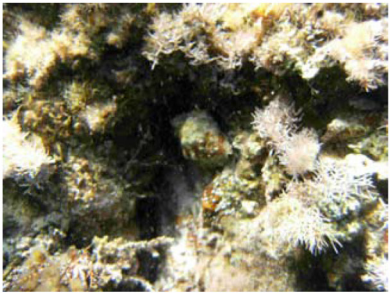

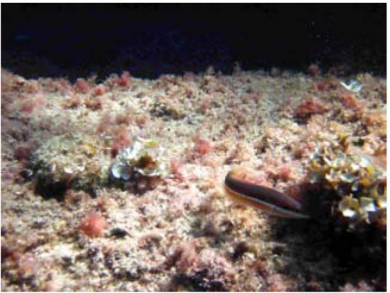








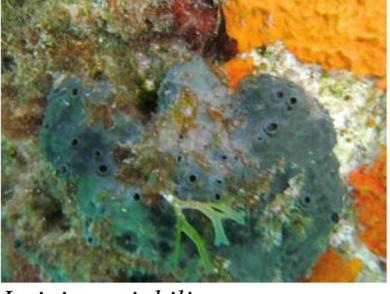
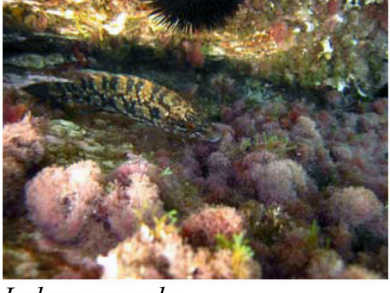
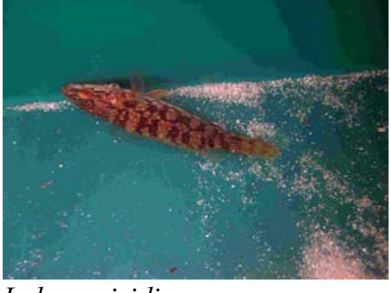

*Arca noae*

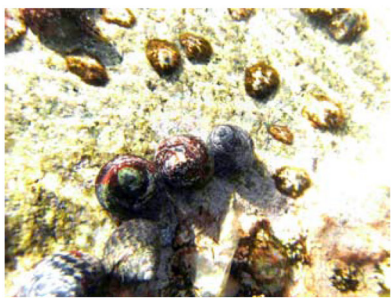
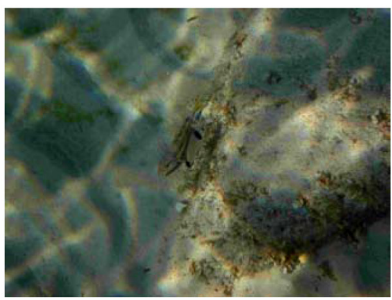
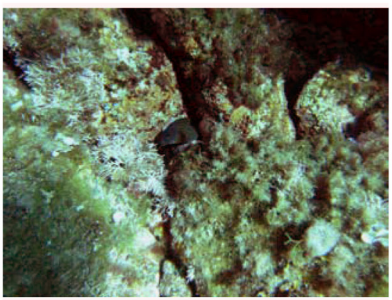










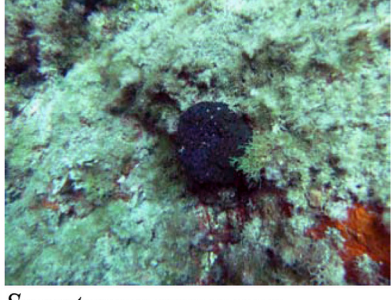




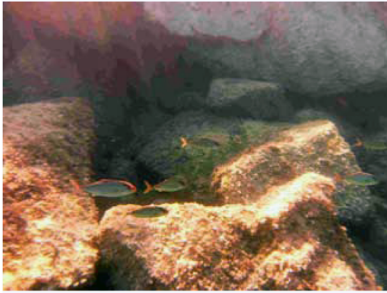


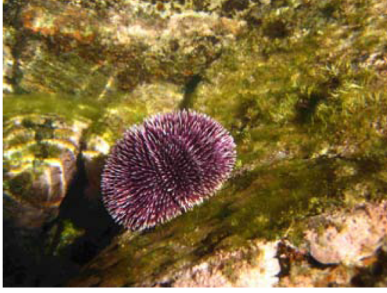
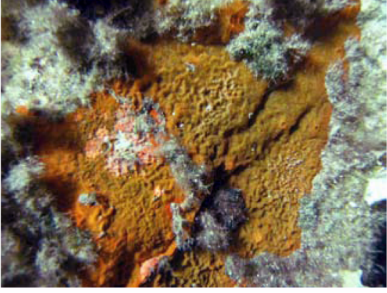



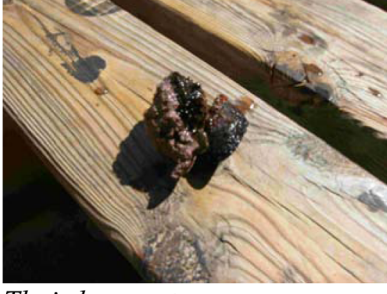


*Bonellia viridis*



*Calliactis parasitica*

		
<i>Charonia tritonis</i>	<i>Chromis chromis</i>	<i>Coris julis</i>
		
<i>Diplodus sargus</i>	<i>Diplodus vulgaris</i>	<i>Echinaster sepositus</i>
		
<i>Gibbula divaricata</i>	<i>Haliotis lamellosa</i>	<i>Haliotis tuberculata</i>
		
<i>Hemimyscale columella</i>	<i>Holothuria sp.</i>	<i>Ircinia variabilis</i>
		
<i>Labrus merula</i>	<i>Labrus viridis</i>	<i>Limaria hians</i>

		
<i>Monodonta turbinata</i>	<i>Mullus surmuletus</i>	<i>Muraena helena</i>
		
<i>Notospermus geniculatus</i>	<i>Oblada melanura</i>	<i>Octopus vulgaris</i>
		
<i>Oedalechilus labeo</i>	<i>Ophioderma longicaudum</i>	<i>Parablennius sanguinolentus</i>
		
<i>Paracentrotus lividus</i>	<i>Pinna nobilis</i>	<i>Polycarpa sp.</i>
		
<i>Reniera fulva</i>	<i>Sarcotragus muscarum</i>	<i>Sarpa salpa</i>

 <p>A photograph of a Scorpaena porcus fish, showing its characteristic spiny head and body, resting on a white surface.</p>	 <p>An underwater photograph of a Seriola rivoliana fish swimming near a rocky reef structure.</p>	 <p>An underwater photograph of a Serranus cabrilla fish swimming over a green, algae-covered seabed.</p>
 <p>An underwater photograph of a Serranus scriba fish, characterized by its dark, patterned body, swimming near a rocky reef.</p>	 <p>A close-up photograph of a Sphaerechinus granularis sea urchin, showing its purple, granular spines.</p>	 <p>A close-up photograph of a Spirastrella cunctatrix sponge, displaying its orange and brown porous structure.</p>
 <p>An underwater photograph of a Symphodus mediterraneus fish swimming near a large, yellowish, branching coral structure.</p>	 <p>An underwater photograph of a Symphodus roissali fish swimming over a dark, rocky seabed.</p>	 <p>An underwater photograph of a Symphodus tinca fish swimming over a green, grass-like seabed.</p>
 <p>A photograph of a Thais haemastoma crab resting on a piece of weathered wood.</p>	 <p>An underwater photograph of a Thalassoma pavo fish swimming over a rocky seabed.</p>	 <p>A photograph of a Thuridilla hopei worm, showing its long, segmented body, resting on a white surface.</p>

# Coralligène

Benedikt Zeindl, Thomas Pomberger and Nicolo Piacenza

## Introduction

The Coralligène is a secondary hard bottom which is mainly built by Corallinaceae. These algae have calcium deposits in their cell walls making them hard and fractural. A vast diversity of organisms live on the thalli of those Corallinaceae. If the thallus is covered too densely with organisms, the alga isn't able to make photosynthesis anymore and dies thusly. Although the calcified structures remain and build a new calcareous habitat.



Fig.1: Some sorted and determined species of the Coralligène

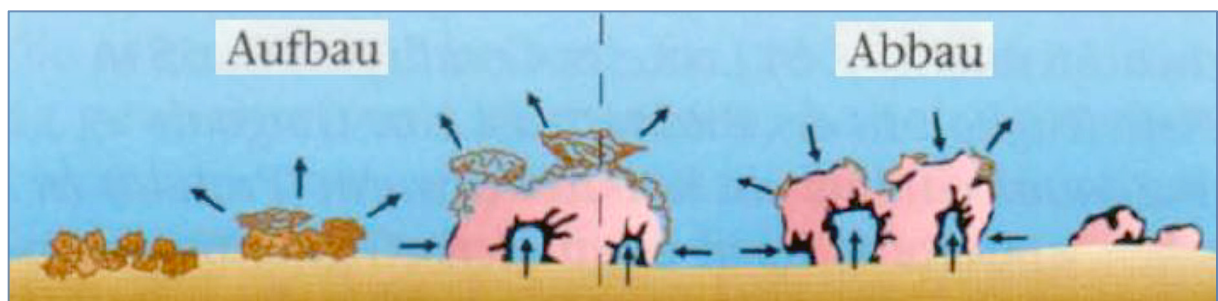


Fig.2: Assembling and Degradation of Corallinaceae (Schärer, 2006).

Beside Corallinaceae there are other organisms as well, like tube-building annelid worms, Bryozoans and Crustaceans which form this habitat by their skeletons and shells. Furthermore there are calcareous green algae and other red algae and moreover, drilling animals like piddocks (*Lithophaga lithophaga*) sponges use the Coralligène as substrate. This leads to a jagged biotope growing and declining at the same time and shelter a great biodiversity.



Fig.3: *Pseudophyllum expansum* ([http://fran.cornu.free.fr/affichage/affichage\\_nom.php?id\\_espece=531](http://fran.cornu.free.fr/affichage/affichage_nom.php?id_espece=531))

There are two different types of Coralligène:

One type is located on rocky surface (Coralligène de trottoir) and the other on a plane field on the sea ground (Coralligène de plateau).

The Coralligène on rocks is built out of 4 to 5 layers. One layer is made out of sea fans and sponges. The middle layers consist of Corallinaceae, Ascidians, Bryozoans, some Polychaets and Sponges. Beside Corallinaceae the third layer is built by some small animal species and the chalk layer which is formed by dead Corallinaceae.



Fig.4: Coralligène  
(<http://host.fatfish.fr/objectifapnee/photos.htm>)

The second Coralligène type, like the one in the bay of Calvi, is located deeper and on plane fields. It consists of flint, shell detritus, skeleton and shell fragments which are overgrown by Corallinaceae during succession.

## Materials and Methods

The sampling site was a plane Coralligène, located in the bay of Calvi in approximately 50 to 60 meters depth. For the sampling a “Dredge” was used (see fig. 5), which consisted out of a metal bucket with spikes in order to sample pieces out of the crusty layer covered with vegetation and a dragnet fixed on the bucket to collect the detached material.

To get quantitative results the Dredge was dragged by the STARESO boat for about 15 min over the plane Coralligène. The first sampling failed because of a twist in the net, so the sampling was repeated.

The collected material was packed in a plastic box and later on sorted and determined on species level, if possible.



Fig.5: Length relationship between the Dredge and BSc Benedikt Zeindl. The aperture was 40mm in the upper part and 5 mm in the lower part. The bucket opening was 60 cm.



## Results

Arthropoda, Cnidaria, Echinodermata, Mollusca and Sipunculida were found in the sampled Coralligène:

**Table 1:** Coralligène Species List

Class	Family	Species
Malacostraca	Diogenidae	<i>Paguristes eremita (Paguristes oculatus*)</i>
	Inachidae	<i>Macropodia</i> sp.
	Paguridae	<i>Anapagurus laevis</i>
Anthozoa	Sagartiidae	<i>Sagartiogeton undatus</i>
Holothuroidea	Holothuroiidae	<i>Holothuria (Holothuria) tubulosa</i>
Ophiuroidea	Amphiuridae	<i>Amphiura filiformis</i>
		<i>Amphiura chiajei</i>
	Ophiomyxidae	<i>Ophiomyxa pentagona</i>
	Ophiotrichidae	<i>Ophiotrix fragilis</i>
	Ophiuridae	<i>Ophiura albida</i>
		<i>Ophiura ophiura</i>
Bivalvia	Lucinidae	<i>Ctena decussata</i>
	Mytilidae	<i>Modiolula phaseolina</i>
	Pectinidae	<i>Aequipecten opercularis (Chlamys opercularis*)</i>
		<i>Pseudamussium clavatum</i>
	Veneridae	<i>Clausinella fasciata (Clausinella brongiartii*)</i>
<i>Venus verrucosa</i>		
Gastropoda	Cerithiidae	<i>Cerithium alucaster</i>
	Epitoniidae	<i>Epitonium turtonis</i>
	Turritellidae	<i>Turritella turbona</i>
Scaphopoda	Dentaliidae	<i>Dentalium</i> sp.
Polychaeta	Aphroditidae	<i>Aphrodita aculeata</i>
	Polynoidae	<i>Harmothoe extenuata (Lagisca extenuata*)</i>
Polyplacophora	Acanthochitonidae	<i>Acanthochitona fascicularis</i>
	Lepidochitonidae	<i>Lepidochitona (Lepidochitona) cinerea</i>
Sipunculidea	Sipunculidae	<i>Sipuncula</i> sp.

\* = Synonyms

## Discussion

In comparison to former years, some differences in species respectively class composition could be observed. In the year 2012, with four classes the greatest diversity of mollusca of all years could be determined. Further differences were that *Holothuria*, *Sipuncula* and *Anthozoa* could be found in the plane Coralligène for the first time. Beside these new classes some others like *Chordata* and *Nemertini* couldn't be observed during the observation period.

The abundance of classes differed as well, compared to preceding years. Despite the fact that this year most classes of *Mollusca* were sampled, the number of families and species were significantly less in comparison to the years before. On one hand the smaller quantity of collected material and on the other hand a shorter classification period could cause such an inconsistency.

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# Fish Fauna

Manuel Dureuil, Martina Stiasny and João Vicente de Camargo e Silva Gladek

## Introduction

The Mediterranean Sea is almost completely enclosed by Africa, Europe and Asia. It is the largest and deepest basin on Earth, and is connected via straits and channels to the Atlantic Ocean and Black Sea, and via the Red Sea to the Indian Ocean (Coll et al., 2010). In its geological history, the Mediterranean Sea was isolated, almost dried out and experienced severe climate changes, salinity and water level variations. As a consequence of this the semi-enclosed Mediterranean sea is today a hot spot of marine biodiversity, being one of the richest in marine flora species diversity (Boudouresque, 2004), with 28% of species being endemic. The origin of this biodiversity is the presence of species from the warm boreal, and tropical Atlantic and the Indo Pacific. The different gradient in temperature and salinity from west to east also further increases the diversity (Coll et al., 2010).

Despite only holding 1,7 % of the earth's surface, the Mediterranean Sea has approximately 7% of the world-wide marine fauna and 18 % of marine flora, of which 28 percent are endemic to the Mediterranean Sea (Abousamra, et al. 2005). The fish diversity found in fishbase.org at the present time is 720 species (Fishbase 2012).

## Material and Methods

The study was carried out in the harbor of the scientific station STARESO (Station de recherche océanographique et sous-marine), Calvi, Corsica, 42°35'N–8°43'E, and in adjacent beaches as far as Punta Oscellucia. In order to be able to identify the largest number of species possible, snorkeling observations were conducted during daytime and nighttime in the bay in front of the research station. There is a mix of rocky boulders and narrow valleys and a meadow of sea grass *P. oceanica* in depths up to 8 m (France & Pelaprat, 1999; Jadot et al., 2002).

The physical characteristics of Calvi bay and surrounding areas are influenced by a water circulation from south to north. It is furthermore highly influenced by the local wind, with currents of low speed (4-5 cm/s), semidiurnal tides of 50 cm, high salinity (38-41‰). The water is very clear and poor in nutrients with maximum temperatures in summer reaching 25,5°C in August (Bay, 1984).

The beaches in the vicinity of the station were studied during daytime. The sea floor in this area is characterized by *P. oceanica* meadows with large sandy patches and rocky boulders. Therefore, these different habitats were studied individually in order to draw a comparison. The observations while diving were made by either single researchers or larger groups. The species list consists of observations made by a group of 27 researchers between 26 August and 7 September 2012.

## Results

Overall 56 species were observed during the two weeks (Appendix I). On average 31 species have been seen by each single observer. The most remarkable members belonged to the families Labridae and Sparidae, which also formed the most numerous groups within the found fish genera. They can be differentiated by their shape and

their swimming behavior; wrasses use pectoral fins for swimming and caudal fins just to maneuver. However, members of other families, particularly *Artherina hepsetus* and *Chromis chromis*, were found in high densities with swarms of up to hundreds of individuals. Other regularly observed species included *Oedalechilus labeo*, *Serranus scriba*, *Belone belone* and *Mullus surmuletus*. One individual that was first identified as *Symphodus rosalli*, is likely to be *S. bailloni*, which has not been recorded for this area so far. *Symphodus bailloni* would be the only newly recorded species compared to the previous years. The results of a sequencing analysis will clarify this. Species of the family Gobiidae and Blenniidae were not directly targeted and are therefore just sparsely represented. Furthermore, *Epinephelus marginatus*, one of the top predators in this area, was found to be very rare and was just observed by a few people. Due to a lack of English identification literature, the systematic, biology and the most distinguishable features of some of the more common species are presented in the following (Information others than given by Reinhold Hanel pers. Comment (2012) were taken from [www.fishbase.org](http://www.fishbase.org) and [www.species-identificatoin.org](http://www.species-identificatoin.org) and Louisy (2002)). Images were taken from [www.fishbase.de](http://www.fishbase.de).

## Species

**Kingdom:** Animalia

**Phylum:** Chordata

**Class:** Actinopterygii (ray-finned fishes)

**Order:** Anguilliformes (Eels and morays)

**Family:** Muraenidae (Moray eels)

*Muraena helena*: The Mediterranean moray eel can reach a size of 1.5 m and feeds mainly on fish, crabs and squid. It can often be found in holes. Although the coloration is variable, often the species has a speckled pattern.

**Order:** Mugiliformes (Mulletts)

**Family:** Mugilidae (Mulletts)

*Oedalechilus labeo*: The boxlip mullet is a coastal species occurring throughout the Mediterranean and reaches a size of 25 cm. They can be found in small schools. The distinction from other mullets can be difficult and might not be possible without catching the animal. A characteristic feature is its anal fin with 11 soft finrays, whereas *Liza aurata* has 7-9 soft-rays in total.

**Order:** Atheriniformes (Silversides)

**Family:** Atherinidae (Silversides)

*Atherina hepsetus*: Like all silversides the Mediterranean sand smelt is swimming in jerky movements. It has a dark lateral line and is not as shiny in appearance as the smaller *A. boyeri*. It is feeding on pelagic copepods and benthic crustaceans and can be observed in schools near the surface.

**Order:** Beloniformes (Needle fishes)

**Family:** Belonidae (Needlefishes)

*Belone belone*: The garfish is hunting for smaller fishes in small loose groups and can be observed by following silversides in Corsica.



**Order:** Perciformes (Perch-likes)

**Family:** Serranidae (Sea basses: groupers and fairy basslets)

*Serranus scriba*: Painted combers are hermaphrodite fishes on rocky grounds and *Posidonia* beds. Another predator of its genus *S. cabrilla* (comber) has more variable patterns and colors, but it can easily be distinguished from *S. scriba* by having a light line on its side and a more round head.

**Family:** Moronidae (Temperate basses)

*Dicentrarchus labrax*: The European seabass can be identified by a dark patch on its rear edge of the operculum. This predator can reach a size of about 1 m. Its diet consists mainly of shrimps and molluscs, but also fishes especially with increasing age. It occurs also in brackish waters and occasionally rivers. Although listed here, it was just rarely observed.

**Family:** Apogonidae (Cardinalfishes)

*Apogon imberbis*: The cardinal fish is hiding at daytime but comes out at night where it's feeding mainly on crustaceans. The size is about 15 cm and it is red or pink colored. The big black eyes exhibit two white stripes.

**Family:** Sparidae (Porgies or sea breams)

*Boops boops*: The sides of the bogue are shining silver and yellow lines are present. Furthermore, it exhibits a black spot at the base of its pectoral fin. Bogue is a swarm fish, which can grow up to more than 30 cm but are commonly smaller. The young are mainly feeding on crustaceans. Adults can also be planktivorous.

*Dentex dentex*: The common *Dentex* was mainly observed in a juvenile stage. This predator has pronounced teeth and a straight forehead. Its maximum size is about 1 m. This sea bream is feeding on fishes and molluscs and can be found over rocky hard bottoms.

*Sarpa salpa*: The herbivorous salema is a silver-grey shining fish with yellow eyes and yellow stripes going from anterior to posterior. It can be found in areas with algal growth.

*Diplodus annularis*: A dark patch completely surrounding the caudal peduncle is characteristic for the annular seabream. These patterns are one of the main criteria to distinguish between *Diplodus* species. This predatory fish can be found mainly solitary over *Posidonia* beds, but also over sandy or rocky bottoms.

*Diplodus puntazzo*: As its name describes, the sharpnout seabream has a much more pointed snout and can thus be differentiated from *D. sargus* and other *Diplodus* species found in the area. This species often exhibits dark transversal stripes and a dark spot at the base of its pectoral fin. Its natural habitat is over sandy or rocky bottoms where it feeds on seaweeds, worms, molluscs and shrimps. Although it is considered to be very common in the Mediterranean, it was frequently less observed than *Diplodus sargus*.

*Diplodus sargus sargus*: The dark patch of the white seabream never completely surrounds the caudal peduncle. It feeds on invertebrates, which it picks off the sediment. As others, it inhabits rocky areas or can be found over *Posidonia* beds.

*Diplodus vulgaris*: As its name suggests, the common two-banded seabream exhibits two black bands. One band is behind the head, one in front of the caudal peduncle. This species occurs over rocky and sometimes sandy bottoms. Juveniles can also be found in seagrass beds.

*Oblada melanura*: The saddled seabream has a black patch at its upper caudal peduncle. It is formed like a saddle and surrounded by a white coloration and never encloses the whole caudal peduncle. *Oblada melanura* can be found above rocky bottoms or *Posidonia* beds.

*Sparus aurata*: The gilthead seabream is easily recognizable by its gold band going from one eye to the other and a dark spot on its operculum. It's a major commercial fish and very important in aquaculture.

*Lithognathus mormyrus*: The sand steenbras or striped sea bream mainly inhabits sandy grounds where it feeds on small crustaceans, molluscs and worms. Its forehead is gently curved. Some darker transverse stripes are on the silver grey body.

**Family:** Mullidae (Goatfishes)

*Mullus surmuletus*: The surmullet exhibits a pair of white barbels, which are used to detect benthic organisms. This digging attracts other species waiting to catch escaping prey.

**Family:** Pomacentridae (Damsel-fishes)

*Chromis chromis*: As very common throughout the whole family, the male damselfish guards the eggs. This family contains many reef fishes, but *Chromis chromis* inhabits also the whole Mediterranean. They can form large swarms and are easily identifiable by its forked caudal fin.

**Family:** Labridae (Wrasses)

*Coris julis*: The male Mediterranean rainbow wrasse is characterized by a black spot on its side. Females change into males and individuals with a total length above 18 cm are all considered as males. This species is identifiable in the Corsica region by its colorful body, which looks different from other colorful fish like *Thalassoma pavo*. However, the color is variable and changes from juvenile to adults. *C. julis* occurs near rocks and plants and buries itself into the sand if there is danger or by night.

*Labrus viridis*: *L. viridis*, also called green wrasse by the FAO, is classified as vulnerable on the IUCN Red List. This species is green colored if living in seagrass, brown colored if living on rocks. It has an elongated body and an almost straight forehead. The snout looks more bend in contrast to *Labrus merula*. Often this species has a white stripe along the body from the snout to the tail. It occurs around seagrass beds and rocks.

*Labrus merula*: The brown wrasse's fins are surrounded by a blue margin. The posterior soft part of the dorsal fin is higher than the spiny anterior part. This species feeds on sea urchins and other invertebrates. *L. viridis* and *L. merula* can be found within the same habitats.

*Symphodus mediterraneus*: The axillary wrasse has a patch on the basis of the pectoral fins and another dark patch on its upper caudal peduncle. It can be found over rocky grounds and *Posidonia* beds.



*Symphodus melanocercus*: *S. melanocercus* is a cleaner fish, which feeds on ectoparasites. Its caudal fin is dark, outlined with a vertical stripe on its base. This species does not exhibit any patches but it can easily be differentiated from *S. rostratus* by do not having an elongated snout.

*Symphodus ocellatus*: *S. ocellatus* has an oscillating patch on the upper posterior part of its operculum. Adults are mainly found over rocky bottoms, juveniles also inhabit *Posidonia* beds.



Fig.1: *Symphodus mediterraneus*

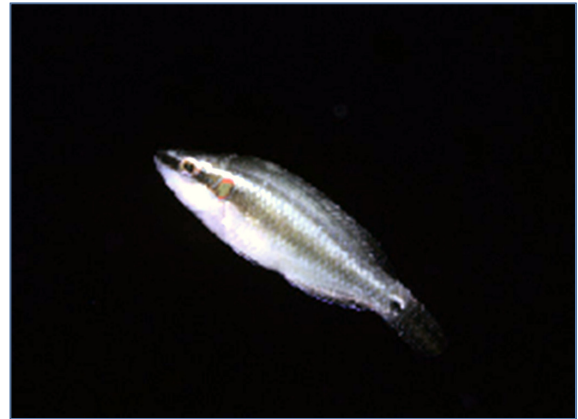


Fig.2: *Symphodus ocellatus*

*Symphodus roissali*: The five-spotted wrasse has five dark spots below its dorsal fin and a dark pattern under its eye. Often this species exhibits a dark spot in the middle of its caudal peduncle. *S. roissali* lives mainly solitary near rocks or plant covered habitats.

*Symphodus rostratus*: *S. rostratus* has an elongated pointed snout, which is directed upward. Inhabiting rocky areas, its color is browner, whereas in the vicinity of seagrass beds it's greener. Often *S. rostratus* can be found in groups with *S. ocellatus* and *S. tinca*.



Fig.3: *Symphodus rostratus*



Fig.4: *Symphodus tinca*

*Symphodus tinca*: The East Atlantic peacock wrasse can be identified by its duck-like mouth. Furthermore, this species has a little black dot in the middle of its caudal peduncle, which is more or less pronounced but sometimes absent. The peacock wrasse inhabits rocky areas or eelgrass beds, often found to be gregarious.

*Thalassoma pavo*: The ornate wrasse is one of the most colorful fishes that was observed. One of its characteristic features is a colorful to blue net like pattern on its head. Even though it can be found in small groups, it is usually solitary. The ornate wrasse lives in shallow coastal waters near rocks or eelgrass beds.

**Family:** Blenniidae (Combtooth blennies)

*Aidablennius sphynx*: *A. sphynx* is a representative of the Blenniidae, which was found more commonly than others. It can easily be differentiated from others by having vertical bars on its olive to brown body with blue margins. It can be found directly on very shallow rocks, exposed to sunlight and covered by algae.

## Discussion

Most of the fish species of the genus *Symphodus* that were found during the excursion in previous years were also found this year. Only one species, namely *Symphodus dodderleini*, was not observed in 2012. Furthermore *S. cinereus* was rarely seen. Both of these species are mainly found over sea grass beds. A more thorough study of these habitats would have likely shown that they were present as well. The same is true for species of the families Gobiidae and Blenniidae, which were not often observed this year. However, they were not specifically searched for and a higher effort may reveal higher diversity in these taxa.

Generally, less species were found than in previous years. This can be due to a variety of reasons. One variable that is not compared across years is the sampling effort in terms of time spent in the water.

It is possible that considerably less time was spent searching during this year's excursion than in previous years due to adverse weather conditions and other factors. Climate conditions may have also affected the species composition directly. This cannot be tested since we do not have a temperature record for this year, but it appeared to be warmer than in previous years. This can considerably change the species composition. It would be very interesting to compare the species number and composition across years and correlate them to the temperature record.

Possibly one new species was found. It was first identified as *Symphodus roisalli*, but a closer examination revealed that it might in fact be *S. bailloni*. This will be further tested using a DNA sequencing analysis. If the results show that it is *S. bailloni*, it would be the only species that was not found in previous years. However, since a closer examination outside the water and DNA sequencing is necessary to be sure, it is possible that the fish was misidentified in previous years.

A previous study (La Mesa et al., 2011) compared the pattern in fish assemblages in three different habitats; meadows of *Posidonia oceanica*, rocky reef and sandy bottom around Elba Island. Even though there are different water currents influencing this region, this study could give a clue in what diversity could be expect from our study site near Calvi. All the three habitats also occur in our study site. They found 59 fish species, distributed in 24 families. The Labridae (13 species) and Sparidae (11 species) were the most commonly found families, and the remaining had no more than 4 species. We found 56 species in 24 families including the Sparidae with 12 species and the Labridae with 11 species.

In general it can be said that the most common species for this region were found and no species was missing that is characteristic for this ecosystem or this region. Any differences in the species list between 2012 and the last couple of years are likely to be due to different sampling efforts and with more time in the water and more effort spent on less common species, it is likely that more species would have been found.





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Appendix I (species list)

Species	1996	1998	2000	2002	2004	2006	2008	2010	2012
<i>Torpedo marmorata</i>								X	
<i>Dasyatis pastinaca</i>					X	X	X	X	X
<i>Dasyatis violacea</i>						X			
<i>Myliobatis aquila</i>				X	X	X			
<i>Conger conger</i>		X	X		X		X		
<i>Anguilla anguilla</i>		X	X		X	X			
<i>Muraena helena</i>		X	X	X	X	X	X	X	X
<i>Engraulis encrasicolus</i>						X			
<i>Synodus saurus</i>								X	X
<i>Phycis phycis</i>				X		X			X
<i>Mugil cephalus</i>					X	X			
<i>Oedalechilus labeo</i>				X	X	X	X	X	X
<i>Liza aurata</i>		X	X	X	X	X	X	X	X
<i>Atherina boyeri</i>		X	X	X	X	X	X	X	X
<i>Atherina hepsetus</i>			X		X	X	X	X	X
<i>Belone belone</i>				X		X	X	X	X
<i>Hippocampus guttulatus</i>		X							
<i>Syngnathus typhle</i>									
<i>Scorpaena porcus</i>			X	X	X	X	X	X	X
<i>Scorpaena notata</i>		X	X	X	X	X	X	X	
<i>Scorpaena scrofa</i>		X		X		X	X	X	
<i>Chelidonichthys sp.</i>					X				
<i>Dactylopterus volitans</i>								X	
<i>Epinephelus marginatus</i>		X	X	X	X	X	X	X	X
<i>Serranus scriba</i>		X	X	X	X	X	X	X	X
<i>Serranus cabrilla</i>		X	X	X	X	X	X	X	X
<i>Anthias anthias</i>				X					X
<i>Dicentrarchus labrax</i>					X			X	X
<i>Apogon imberbis</i>		X	X	X	X	X	X	X	X
<i>Seriola carpenteri</i>						X	X	X	X
<i>Seriola dumerili</i>					X	X			
<i>Trachinotus ovatus</i>						X			
<i>Trachurus mediterraneus</i>					X				
<i>Spicara maena</i>			X	X		X	X		X
<i>Spicara smaris</i>								X	
<i>Boops boops</i>				X	X	X	X	X	X
<i>Dentex dentex</i>		X		X	X	X	X	X	X
<i>Diplodus annularis</i>	X	X	X	X	X	X	X	X	X
<i>Diplodus puntazzo</i>	X	X	X	X	X	X	X	X	X
<i>Diplodus sargus sargus</i>	X	X	X	X	X	X	X	X	X
<i>Diplodus vulgaris</i>	X	X	X	X	X	X	X	X	X
<i>Oblada melanura</i>	X	X	X	X	X	X	X	X	X



<i>Sarpa salpa</i>	X	X	X	X	X	X	X	X	X
<i>Sparus aurata</i>	X	X	X	X	X	X	X	X	X
<i>Lithognathus mormyrus</i>		X		X	X	X	X	X	X
<i>Pagellus acarne</i>				X			X		X
<i>Pagellus erythrinus</i>		X		X			X		
<i>Pagrus pagrus</i>				X					
<i>Spondyliosoma cantharus</i>	X		X	X	X	X	X	X	X
<i>Pomadasys incisus</i>								X	
<i>Sciaena umbra</i>				X	X	X		X	X
<i>Mullus barbatus</i>					X	X			
<i>Mullus surmuletus</i>		X	X	X	X	X	X	X	X
<i>Chromis chromis</i>		X	X	X	X	X	X	X	X
<i>Coris julis</i>	X	X	X	X	X	X	X	X	X
<i>Labrus viridis</i>	X	X		X	X	X	X	X	X
<i>Labrus merula</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus cinereus</i>	X	X		X	X	X	X	X	X
<i>Symphodus mediterraneus</i>			X	X	X	X	X	X	X
<i>Symphodus melanocercus</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus ocellatus</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus roissali</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus rostratus</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus tinca</i>	X	X	X	X	X	X	X	X	X
<i>Symphodus doderleini</i>					X				
<i>Thalassoma pavo</i>		X	X	X	X	X	X	X	X
<i>Trachinus draco</i>	X						X	X	X
<i>Trachinus araneus</i>					X				
<i>Uranoscopus scaber</i>	X						X		
<i>Tripterygion tripteronotus</i>	X	X	X		X		X	X	X
<i>Parablennius gattorugine</i>	X	X	X		X		X	X	X
<i>Parablennius sanguinolentus</i>	X	X	X		X		X	X	X
<i>Parablennius rouxi</i>		X	X		X		X	X	X
<i>Parablennius zvonimiri</i>		X	X		X		X	X	X
<i>Parablennius pilicornis</i>							X	X	
<i>Salaria pavo</i>		X	X		X				
<i>Lipophyrus nigriceps</i>					X	X	X	X	
<i>Lipophyrus trigloides</i>					X	X			
<i>Lipophyrus fluviatilis</i>					X	X	X	X	X
<i>Coryphoblennius galerita</i>							X	X	
<i>Aidablennius sphyinx</i>		X		X		X	X	X	X
<i>Clinitrachus argentatus</i>					X				
<i>Gobius bucchichi</i>		X		X	X	X			
<i>Gobius cobitis</i>				X	X	X	X		
<i>Gobius paganellus</i>						X			
<i>Gobius geniporus</i>					X				

Species	1996	1998	2000	2002	2004	2006	2008	2010	2012
<i>Pomatoschistus minutus</i>						X	X	X	
<i>Lepadogaster candollei</i>		X		X	X	X	X		
<i>Lepadogaster lepadogaster</i>								X	
<i>Diplecogaster bimaculata</i>								X	
<i>Gouania wildenowi</i>								X	
<i>Opeatogenys gracilis</i>					X	X			X
<i>Callionymus pusillus</i>							X	X	X
<i>Sphyræna viridensis</i>					X	X	X	X	X
<i>Bothus podas</i>						X	X		X
<i>Phrymorhombus regius</i>				X	X		X	X	
<i>Arnoglossus kessleri</i>		X							
<i>Solea lascaris</i>						X	X		
<i>Solea</i> sp.								X	
<i>Balistes carolinensis</i>		X					X		



# Plankton

Annika Fritschi and Marina Wanner

## Introduction

The term „plankton“ (greek: „to wander“) was introduced of Viktor Hensen, Professor at the University of Kiel, in 1887. He used it for the entire community of organisms and developmental stages, who live in the pelagic and whose motion depends on the motion of the water. So they cover distances by being drifted passively with the current. Their proper motion is just to change their vertical position within the water column.

The plankton community includes phytoplankton (photoautotrophic algae), zooplankton (heterotrophic protist and metazoans), bacterioplankton (prokaryotes) and virioplankton. It is a highly diverse community of various unicellular and multicellular organisms.

**Table 1:** Classification of plankton by size. Larink, Westheide (2011)

Type	Size range	Examples
Femtoplankton	0.02-0.2 $\mu\text{m}$	Viruses
Picoplankton	0.2-2.0 $\mu\text{m}$	bacteria, cyanophytes
Nanoplankton	2.0-20 $\mu\text{m}$	small phototrophic flagellates
Microplankton	20-200 $\mu\text{m}$	tintinnids, diatoms, dinoflagellates
Mesoplankton	0.2-20 mm	copepods, many larvae, hydromedusae
Macroplankton	2.0-20 mm	krill, arrow-worms
Megaplankton	0.2-2.0 m	large jellyfish and tunicate

Size classification for the enormous size range of planktic organisms (tab.1) has been used for a long time. The smallest planktic organisms are viruses, whereas the biggest ones are the large medusas of scyphozoa (Cnidaria).

You can also distinguish the species due to their life cycle. Pelagic species spending their whole life cycle as passively drifting plankton in the open water column are termed holoplankton. If they are spending just a particular developmental stage, usually the larvae stage, as plankton, they are called meroplankton. As an adult animal they belong to the neuston that are large actively moving organisms that can change their swim direction independent of the water motion. To this group belongs the sea urchin, the starfish, crustacean and marine worms.

Plankton plays an essential role in the aquatic ecosystem. As primary producer, photoautotrophic phytoplankton built the basis of the food chain and provides energy for heterotrophic zooplankton. Small zooplankton in turn serves as major food source for bigger zooplankton and many other sea dwellers like sponges, fish or baleen whale, which filter them with special organs out of the water.

Gelatinous planktonic organisms, consisting of aggregates of various live and dead organisms together with secreted sticky substances, build the so-called “marine snow”. Other zooplankton includes the planktonic turbellarians, rotifers, molluscs and chaetognaths.

The majority of the marine invertebrates produce planktonic larvae. This meroplankton dominates coastal waters in some seasons. Predation is the most significant reason for the high mortality of the planktonic larvae. They are eaten by planktonic predators like hydromedusae, siphonophores, shrimps and invertebrate larvae, and benthic predators like mussels, polychaets, ascidians. Also fish and larval cannibalism decrease the number of larvae but still it's sufficient to maintain stable populations.



Fig.2: Plankton net

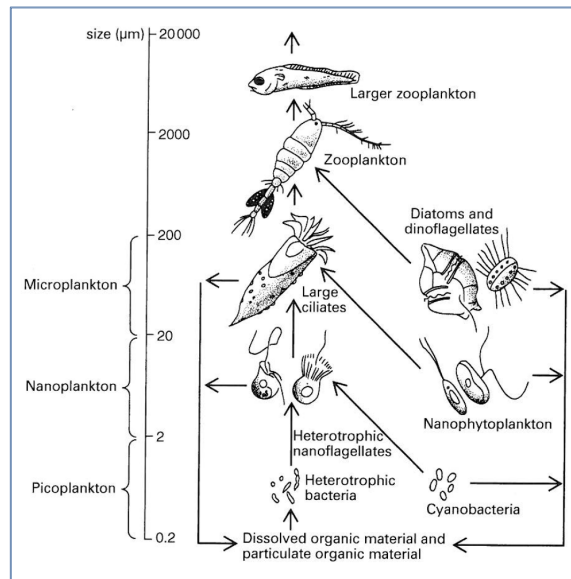


Fig.1: Diagram of the microbial loop (Levinton, 2001; from Larin and Westheide, 2011).

Within the water column, there is a vertical layering with various trophic levels. Phytoplankton can only live in the surface layer (photic zone), because it needs light for photosynthesis. Feeding on phytoplankton forces most of the zooplankton to live also in the photic zone. But beside the upper water layer, they can also populate the middle layer. Therefore most of the plankton is found in the upper 200 m of the sea (fig.1). In the aphotic zone, the bacterioplankton as destruent remineralizes organic material (fig.1).

The most important exogenous factor controlling dispersion and phenology of plankton is the regional temperature. Global warming may influence the composition of the organism. The abundance of plankton depends on seasonal variability, exposure to light (seasons), nutrient supply, water circulation and also on interspecific competition. Therefore the big fish stocks can be found in dull, cold and rich in plankton (eutrophic) sea, whereas clear and trophic sea is nutrient-poor. Too high nutrient contamination results in algal bloom, what may have a positive effect in the increase of the zooplankton. Dead organisms drop at the end of the bloom as marine snow into the depth and are recycled by Archaea and Bacteria.



## Material and Methods

We collected plankton in a depth of about 5m from the motorboat of the STARESO station with a plankton net that was pulled horizontally for 10 min on a low speed. The plankton net was pointed and had a diameter of 56 cm at the opening and 9,3 cm at the bottom. Its length was 3,0 m. At the end of the net is a collection container. The mesh size at the area in front is 500  $\mu\text{m}$ , at the area back it's 200 to 250  $\mu\text{m}$  (fig.2). We collected the material in a plastic container. In the lap of the station the material was pipetted into a petri dish. Afterwards the samples were observed with dissecting and regular light microscopes and identified as far as possible with suitable literature (Larink 2011).

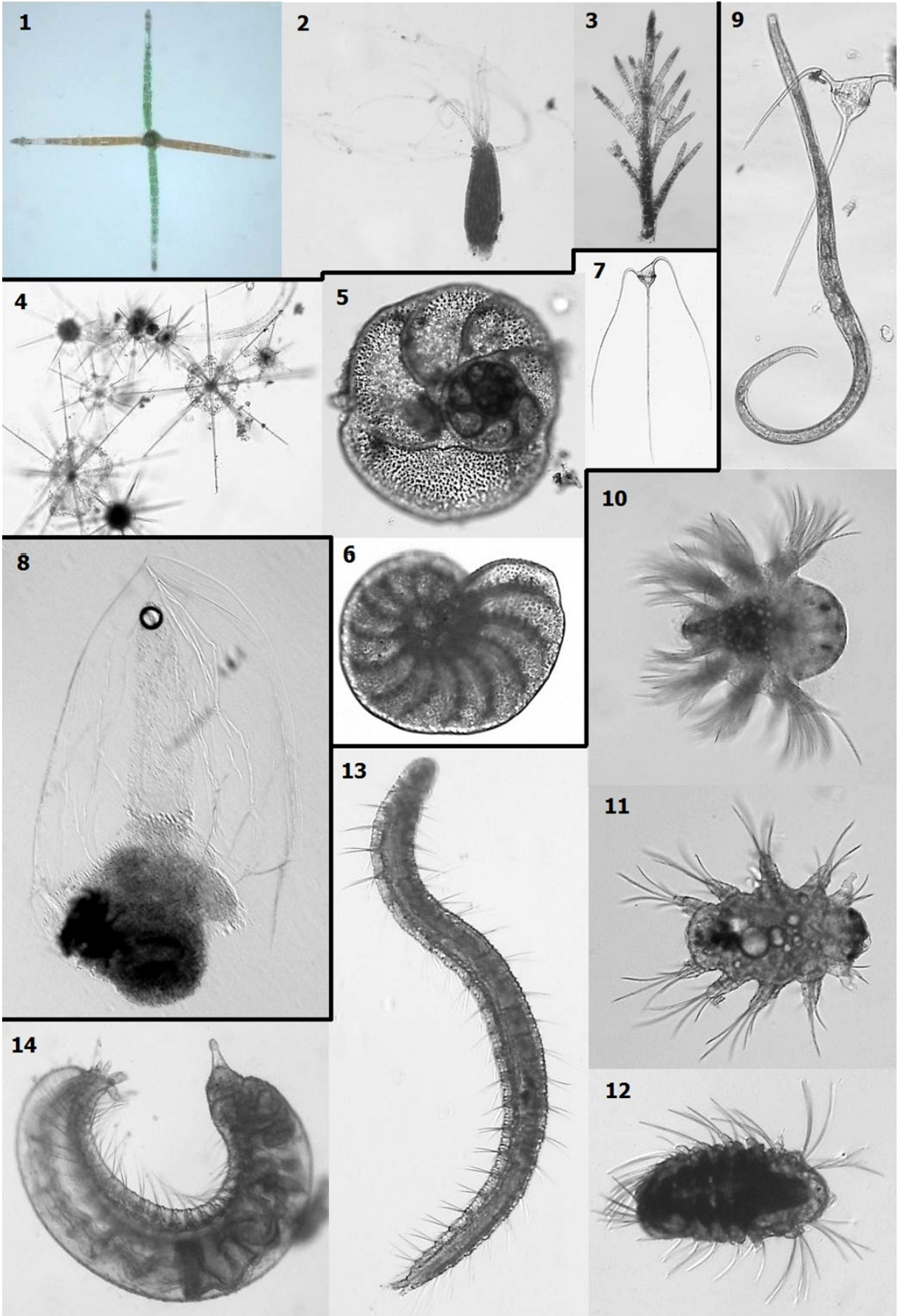
On the species list below, data from all groups are summarized. One group collected in the open water near the station, another group 200 m off the sandy beach of Revellata.

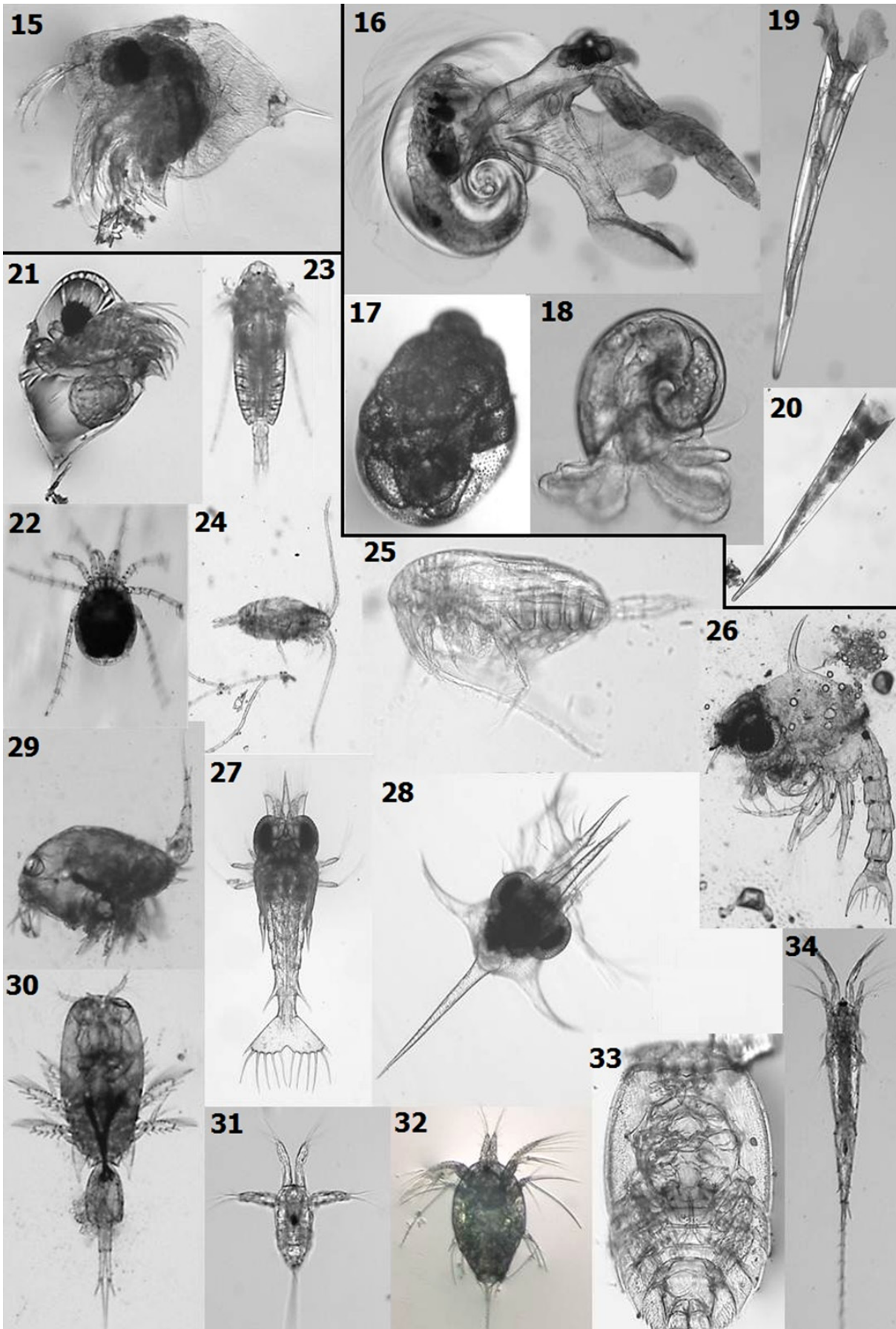
## Results

Species list								
Phylum	class	subclass	order	family	species	remark/ description		
Rhizopoda	Foraminifera							
	Radiolaria							
Actinopoda	Acantharia		Arthracanthida	Phyllostauridae	<i>Phyllostaurus siculus</i>			
Alveolata	Dinoflagellata		Gonyaulacales	Ceratiaceae	<i>Ceratium pavillandi</i>			
Phycophyta	Diatomeae							
Cnidaria	Hydrozoa		Siphonophora					
Mollusca	Gastropoda			Limacinidae	<i>Limacina</i> sp.			
			Opisthobranchia		<i>Creseis acicula</i>			
Annelida	Polychaeta					Trochophora		
Arthropoda	Crustacea	Copepoda						
		Copepoda	Harpacticoida	Harpacticidae	<i>Harpacticoida copepodid</i>			
			Calanoida	Calanidae	<i>Calanus</i> sp.			
			Calanoida	Acartiidae	<i>Acartia</i> sp.			
					<i>Calocalamus</i> sp.			
					Porcellidiidae	<i>Porcellidium</i> sp.	Exuvia	
			Ostracoda					
			Branchiopoda					
			Malacostraca	Cumacea			<i>Diastylis</i> sp.	
				Decapoda	Callianassidae		<i>Callianassa</i> sp.	
		Decapoda				Zoea-Larva		
Chaetognatha						Adult		
Echinodermata	Echinoidea					Pluteus larva (4 extremities)		

Phylum	class	subclass	order	family	species	remark/ description
	Asteroidea					
Chordata	Fish					eggs, larvae
	Ascidiae					
Tunicata	Asciacea					
	Appendicularia					







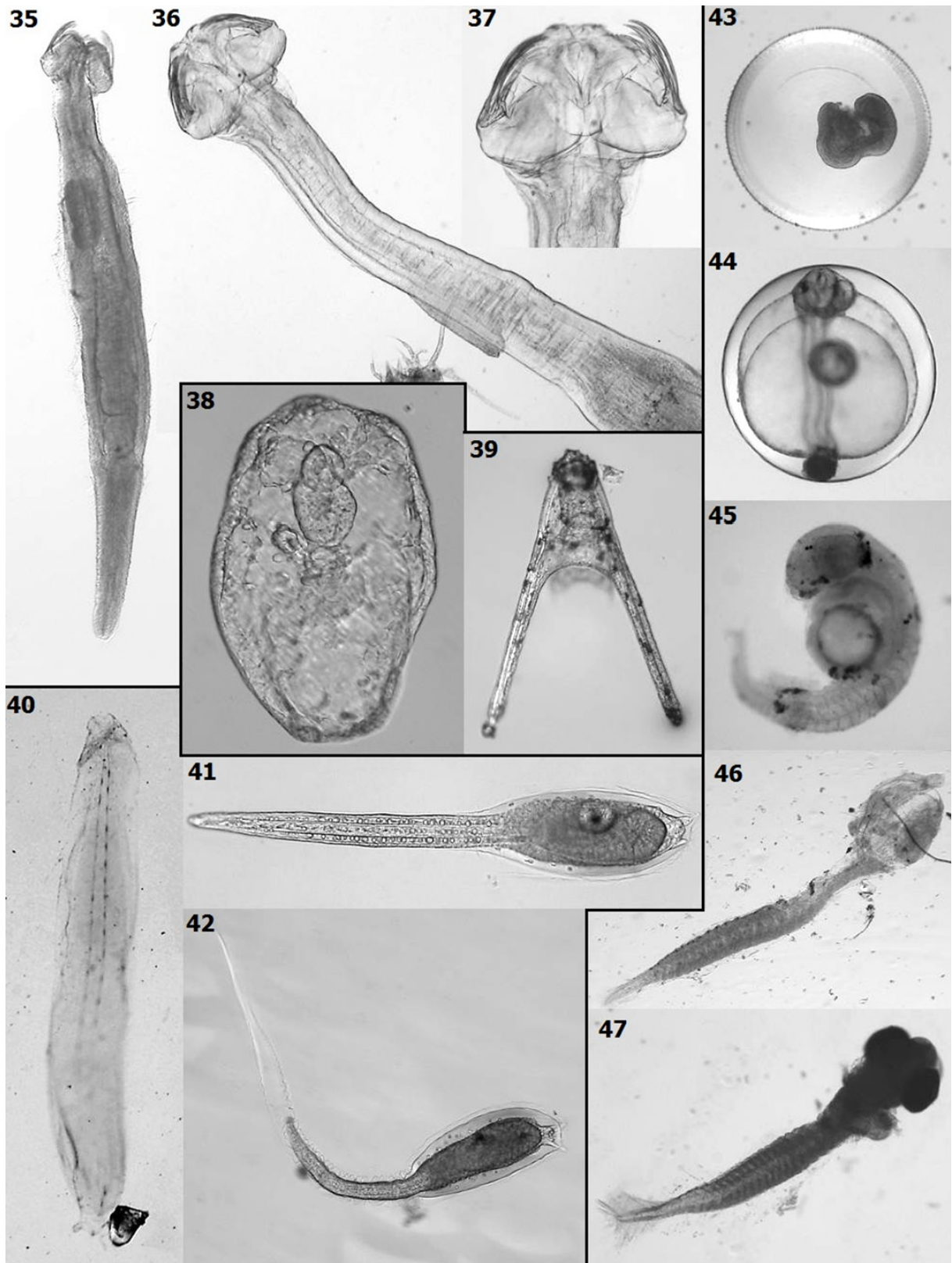


Fig.3: 1 - 47 diverse plankton organisms

Denomination of Organisms in figure 3:		Fig. 3/ 22	Acari
Fig. 3/ 1-3	Phytoplankton	Fig. 3/ 23-34	Crustacea
Fig. 3/ 4-7	Rhizopoda		23-25: Copopod
	4: Radiolaria		26-28: Zoea
	5-6: Foraminifera		29,30: Crustacean larvae
	7: Ceratium		31: Crustacea
Fig. 3/ 8	Cnidaria: Siphonophora		32: Ostracoda
			33: Porcellidium
			34: Calanoida
Fig. 3/ 9	Invertebrate worms: Nematoda	Fig. 3/ 35-37	Chaetognatha
Fig. 3/ 10-14	Polychaeta		35: whole Chaetognatha
	10-12 Larvae in differentiation		36: anterior part
	13,14: Adult		37: head
Fig. 3/ 15	Brachiopoda	Fig. 3/ 38+39	Deuterostomia: Echinodermata
Fig. 3/ 16	Mollusca		38: Bipinnaria larvae
	17: Trochophora	Fig. 3/ 40-42	Tunicata:
	18: Mollusc		40: Appendicularia
	19, 20: Strombidae		41, 42: Ascidia
	19: extracted	Fig. 3/43-47	Chordata
	20: retracted		43-45: Fish embryos
Fig. 3/ 21	Arthropoda: Cladocera		46, 47: Fish larvae

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# Macrofauna of the Sandy Beach

Friederike Engel and Leona Schulze

## Introduction

Sandy beaches dominate most temperate coastlines and are the most extensive intertidal system worldwide. In this harsh and highly dynamic environment the interaction of breaking waves, tides and sediment type determines the morphology of the beach (Short, 1999/Wright and Short, 1984). Often they represent important recreational assets as well as buffer zones against the sea (Davis, 1972).

In 1990, Brown and McLachlan defined sandy beaches as 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.

Sediment composition and physical characteristics of sandy beaches form a very specific environment, where only a few, but highly adapted, organisms live in. Sand as a bottom substrate does not provide good hiding places for motile organisms, algae cannot attach to the ground, and constant wave action leads to the overturning of sand grains and small stones making it nearly impossible for organisms to settle on them (Nybakken and Bertness, 2005). On the other hand, sand is an excellent buffer against large changes in temperature and salinity. Oxygen is usually abundant in sandy beach systems. These are just some of the favorable characteristics present in a sandy beach habitat (Nybakken and Bertness, 2005).

The beach of Revellata belongs to the dissipative beaches, as it is defined by strong wave energy which is dissipated over a broad, flat surf zone located with some distance from the face of the beach. The most prominent characteristic for this type of beach is a gently sloped beach face with maximal erosion (Wright and Short, 1984).

Contrary to the long believed assumption that sandy beach systems are organism poor habitats, it is now confirmed that these environments host a high diversity of organisms, which are not only interactive among themselves, but also influence processes in the water and the sediment they live in (Trush et al., 2004).

The abundance and diversity of macrofaunal organisms correlates most strongly with the particle size and slope of the beach and less with the wave action (McLachlan, 1983). Macrofauna is defined as the organisms living on or in the ground that are bigger than 0.5 mm (Nybakken and Bertness, 2005).

Organisms living in this special habitat are normally highly adapted to the unstable, constantly moving substrate. Some burrow deeply into the sand (e.g. clams) so they are below the depth affected by passing waves, and some other species burrow very quickly into the sand (e.g. annelid worms and crustaceans). Often, their bodies are short with modified limbs to dig quickly into the sediment (Nybakken and Bertness, 2005).

Another often observed adaptation is protective mimicry to compensate for the missing hiding places. Some organisms display sand like body colors (e.g. fish species), others even show morphological changes like for example flatfishes. They have turned their body axes, so that both eyes are on top of the head and they can observe the water column above them while moving across the seafloor (Levinton, 2001).

The beach of Revellata 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, because it is not a closed system.



Fig.1: Beach of Revellata, surrounded by boulder field (right and left side) and sea grass meadows (right and left side, but also off shore of the beach)

This protocol reports all organisms seen on 4 days of observing and collecting at the beach of Revellata, including also the organisms seen and found by the fish group and the mollusk group. It will highlight the species directly living in or on the sandy beach and describe their adaptations, but it also depicts the species depending on this habitat as feeding and hunting grounds.

## Materials and Methods

Snorkeling equipment was used to sample organisms in the deeper water of Revellata beach. Larger species, including many pelagic fish, were identified by observing the living organisms and noting their appearance. Others, especially benthic organisms, were caught with hand nets and transported back to shore where they were placed into plastic tubs for later identification.

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

Sieves were utilized to find macrofaunal organisms living within the substrate. The mesh sizes of the sieves were 1 and 2 mm, respectively. Sand at the shore and at different water depths from about 1 to 3 meters was sieved and the trapped organisms were placed into small plastic containers.

Larger organisms were released back into the water after identification, but smaller organisms were transported back to STARESO station and were later identified in the laboratory. In the lab, binoculars and identification guides were used to classify the species.

## Results

**Table 1:** Mollusks found at the beach of Revellata.

Phylum	Class	Family	Species	
Mollusca	Bivalvia	Carditidae	<i>Cardita calyculata</i>	
		Lucinidae	<i>Ctena decussata</i>	
		Lucinidae	<i>Loripes lucinalis</i>	
		Cardiidae	<i>Papillicardium papillosum</i>	
		Chamidae	<i>Chama gryphoides</i>	
		Chamidae	<i>Pseudochama gryphina</i>	
		Tellinidae	<i>Angulus planatus</i>	
		Veneridae	<i>Chamelea striatula</i>	
		Veneridae	<i>Polititapes aureus</i>	
		Arcidae	<i>Arca noae</i>	
		Arcidae	<i>Barbatia barbata</i>	
		Limidae	<i>Lima sp.</i>	
		Mytilidae	<i>Modiolus barbatus</i>	
		Ostreidae	<i>Ostrea edulis</i>	
		Pinnidae	<i>Pinna nobilis</i>	
		Pinnidae	<i>Pinna rudis</i>	
		Cephalopoda	Octopodidae	<i>Octopus vulgaris</i>
			Sepiidae	<i>Sepia officinalis</i>
		Gastropoda	Cerithiidae	<i>Bittium reticulatum</i>
	Cerithiidae		<i>Bittium scabrum</i>	
	Epitoniidae		<i>Epitonium turtonis</i>	
	Littorinidae		<i>Melarhaphe neritoides</i>	
	Rissoidae		<i>Alvania cimex</i>	
	Rissoidae		<i>Rissoa auriscalpium</i>	
	Collumbellidae		<i>Columbella rustica</i>	
	Conidae		<i>Conus ventricosus</i>	
	Muricidae		<i>Coralliophila squamosa</i>	
	Muricidae		<i>Hexaplex trunculus</i>	
	Nassariidae		<i>Cyclope neritea</i>	
	Nassariidae		<i>Cyclope pellucida</i>	
	Patellidae		<i>Patella caerulea</i>	
	Patellidae		<i>Patella ferruginea</i>	
	Patellidae		<i>Patella intermedia</i>	
Patellidae	<i>Patella nigra</i>			
Phasianellidae	<i>Tricolia speciosa</i>			
Trochidae	<i>Clanculus cruciatus</i>			
Trochidae	<i>Gibbula ardens</i>			
Trochidae	<i>Gibbula divaricata</i>			
Trochidae	<i>Gibbula racketsi</i>			
Trochidae	<i>Gibbula turbinoides</i>			
Trochidae	<i>Gibbula umbilicalis</i>			
Polyplacophora	Acanthochitonidae	<i>Acanthochitona fasciularis</i>		
	Chitonidae	<i>Chiton (Rhyssoplax) olivaceus</i>		
Scaphopoda	Dentaliidae	<i>Antalis vulgaris</i>		

\*Gray=Organisms adapted to and living on sandy beach habitats and found alive.

**Table 2:** Fishes found at the beach of Revellata.

Phylum	Class	Family	Species
Chordata	Actinopterygii	Atherinidae	<i>Atherina hepsetus</i>
		Atherinidae	<i>Atherina boyeri</i>
		Synodontidae	<i>Synodus saurus</i>
		Mugilidae	<i>Oedalechilus labeo</i>
		Callionomyidae	<i>Callionymus pusillus</i>
		Gobiidae	<i>Gobius sp.</i>
		Labridae	<i>Coris julis</i>
		Labridae	<i>Labrus merula</i>
		Labridae	<i>Labrus vividis</i>
		Labridae	<i>Symphodus (Crenilabrus) roissali</i>
		Labridae	<i>Symphodus (Crenilabrus) tinca</i>
		Labridae	<i>Symphodus (Symphodus) rostratus</i>
		Mullidae	<i>Mullus surmuletus</i>
		Pomacentridae	<i>Chromis chromis</i>
		Serranidae	<i>Serranus scriba</i>
		Sparidae	<i>Diplodus annularis</i>
		Sparidae	<i>Diplodus sargus</i>
		Sparidae	<i>Diplodus vulgaris</i>
		Sparidae	<i>Lithognathus mormyrus</i>
		Sparidae	<i>Oblada melanura</i>
Sparidae	<i>Sarpa salpa</i>		
	Sphyraenidae	<i>Sphyraena viridensis</i>	
	Trachinidae	<i>Trachinus draco</i>	
	Bothidae	<i>Bothus podas</i>	
	Elasmobranchii	Dasyatidae	<i>Dasyatis pastinaca</i>

\*Gray=Organisms adapted to and living on sandy beach habitats

### Description of the species adapted to and living on sandy beaches

**Loripes lucinalis** (Lamarck, 1818): This typical mollusk species buries into reducing sediments and hosts endocellular sulphur-oxidizing bacteria in its gills. The host is supplied with nutrients like organic carbon from the bacteria which in turn has a relatively safe living space (Johnson & Fernandez, 2001).

**Pinna rudis** (Linnaeus, 1758): This saltwater clam lives in soft sandy mud ground. It is buried into the sand, with its thick shell. Normally it occurs within seagrass meadows, but occasionally it can be found on sandy grounds outside of seagrass meadows ([www.marinespecies.org](http://www.marinespecies.org)).

**Sepia officinalis** (Linnaeus, 1758): The European cuttlefish, *Sepia officinalis*, is mostly found in coastal waters between 2-3 m depth on sandy or muddy substrate. One of the most obvious and beneficial adaptations of this species is its ability to change skin color and texture as camouflage. Depending on the substrate they are living on, they adjust their skin to blend in with the surroundings. There are three different cell structures in their skin that allow rapid color and texture changes: Chromatophores, leucophores, and iridophores. Even though this species can be present in many different habitat types, *Sepia officinalis* prefers sandy and muddy substrate, because it enables them to bury into the sediment quickly to hide. Most individuals of this species display a seasonal migration pattern. In spring and summer, they live close to the shore in shallower waters; in the winter, they migrate into deeper waters (up to 200 m depth). Their diet is mainly composed of crustaceans, small bony



fishes, mollusks, polychaetes and nemertean worms. They are quite flexible in their diet, however, and adjust to seasonal and daily variations in prey availability (Guerra, 2006).

***Octopus vulgaris*** (Cuvier, 1797): These animals are adapted to live in various different habitats from rocky shores to sandy beaches. Just like *Sepia officinalis*, they can change the color of their skin in response to the background they are on. Often this camouflage technique allows them to blend in almost perfectly with the background. Studies show that *Octopus vulgaris* usually prefers habitats that contain material to build dens as hiding places over open sandy beach areas (Katsanevakis & Verriopoulos, 2004).

***Epitornium turtonis*** (Turton, 1819): This gastropod specimen is a predatory species that lives within the substrate of sandy habitats. It feeds by inserting its proboscis into the prey and biting out small pieces of tissue ([www.marinespecies.org](http://www.marinespecies.org)).

***Cyclope neritea*** (Linnaeus, 1758): It is a small burrowing gastropod species that is adapted to live in the sand. The shell is small (10-15 mm in diameter) and flattened. This allows rapid burrowing into soft sediment when danger is in sight. *Cyclope neritea* feeds mainly on dead and decaying animal or plant matter on the sediment surface (Morton, 1960).

***Synodus saurus*** (Linnaeus, 1758): The Atlantic lizardfish belongs to the family Synodontidae and can reach a total length of 43 cm. It is commonly found on sandy bottoms, sometimes up to 400 m water depth (Esposito et al., 2009). It is characterized by camouflage and an immobile behavior (Galoni, 1993). The individuals spend most of the time buried in the sand and feed on mainly small pelagic gregarious fishes, crustaceans and cephalopods. Their predatory behavior is documented as highly motile, and nocturnal feeding was assumed by Esposito et al. in 2009. This species is listed on the red list of endangered species ([www.iucnredlist.org](http://www.iucnredlist.org)).

***Gobius sp.*** (Linnaeus, 1758): Many goby species live in shallow marine habitats, also on sandy bottoms. Their pelvic fin can be located anteriorly under their pectorals and by this it can be used as a help to stay in place on sandy grounds. Some species move forward by jumping-like movements. Also camouflage behavior is documented (Frank, 1998).

***Lithognathus mormyrus*** (Linnaeus, 1758): The sand steenbras can reach a total length of 55 cm and lives on the shelf over sandy and muddy bottoms. It feeds on worms, mollusks and small crustaceans ([www.fishbase.org](http://www.fishbase.org)). These animals use camouflage to mimic the ripple patterns in the sand caused by currents and tides by silver-grey-white colored bodies ([www.aquarium.co.za](http://www.aquarium.co.za)).

***Trachinus draco*** (Linnaeus, 1758): Due to the fact that individuals of this species are more or less always buried in the sand and live directly above it, they do not need a swim bladder. They normally live near the shore, up to 15 m water depth. Camouflaged with sandy-like color and buried in the sand they wait for their prey, attack it and bury themselves immediately after the attack again in the sand. Sometimes they hunt in swarms at night. This species is one of the most toxic species in European waters. It has spines on the first dorsal fin and on the gill covers, which are attached to venom glands releasing toxin if they are touched (Frank, 1998).

***Bothus podas*** (Delaroche, 1809): *B. podas* belongs to the family of the lefteye flounders (Bothidae). It lives in shallow waters on sandy or muddy sea bottom up to 400 m water depth. This species feeds on small benthic fishes and invertebrates ([www.fishbase.us](http://www.fishbase.us)). The most characteristic adaptation for this fish to its habitat is not the

camouflage color of the body but the whole body shape. While they are still young, they have a normal fish-like body shape, with eyes on both sides. But then their body starts to flatten and loses its bilateral symmetry. The right eye moves over the middle of the head, until it is on the other side next to the left eye. Now the fish turns to the right side, where it already has lost its pigmentation, and this side is hereafter on the ground side. The left side can be camouflaged with sandy colors ([www.mir-co.net](http://www.mir-co.net)).

***Dasyatis pastinaca*** (Linnaeus, 1758): The stingray is a typical species found on sandy or muddy ground in the Mediterranean Sea. It lives in calm shallow coastal waters from 15–400 m depth ([www.fishbase.org](http://www.fishbase.org)). *D. pastinaca* individuals bury themselves in the sand and feed by bottom-dwelling on crustaceans, cephalopods, bivalves and polychaete worms. They can reach body sizes of 2,5 m with a weight of 10 kg. The color differs from brownish to more yellow-brownish (Frank, 1998). The barbed poison spine can be 8–35 cm long ([www.fishbase.org](http://www.fishbase.org)).

## Discussion

Only few of the species found are specially adapted to sandy habitats. That so many more organisms are listed in table 1 and table 2 is due to the fact that the beach of Revellata is only a small beach habitat surrounded by other habitats, like seagrass meadows and boulder field.

Seagrass like *Posidonia* sp. can grow on sandy bottoms, while algae cannot. Algae normally attach themselves on hard bottom substrates like stones or second hard bottom substrates like seagrass. Single plants of seagrass can only survive on sandy bottoms by attaching themselves to other seagrass plants with their roots. By doing so they give the very unstable sandy bottom some stability and by growing into a meadow they build a totally new environment for other species. Organisms living in seagrass meadows do not always stay in their favorite environment. They move around for hunting or mating.

The species *Pinna nobilis* and *Pinna rudis* belong to the organisms living normally in seagrass meadows, but can also be counted as typical sandy ground inhabitants. They dig far into the sand and attach themselves to the sandy bottom, to be protected against wave energy. Without sandy bottom they do not exist in seagrass meadows, but they also do not exist in sandy bottoms without seagrass ([www.marinespecies.org](http://www.marinespecies.org)).

Many organisms living in the boulder field were also found at the sandy beach environment. For example, we observed many fish species at the sandy beach. Fishes are highly mobile animals that can move back and forth between different habitats easily. Most of the species we saw at the beach usually prefer other habitats like the boulder field where they have hiding places. Still, they will be seen at the beach from time to time, because it acts as an additional food source for them.

In addition, high energy waves usually transport a lot of material to the beach. Therefore many dead organisms, especially hard structures like shells, will be washed to the beach. These species found at the beach are not necessarily typical beach species. They could have lived in a surrounding habitat and when they died, the waves carried them to the beach.

An example for this is the bivalve *Pseudochama gryphina*, which was one of the most abundant bivalves we found at Revellata beach. Since the species lives in colonies and attaches to hard surfaces, we only found dead shells that were washed ashore. The numerous individuals we found, however, indicate that *Pseudochama gryphina*

is abundant in the boulder rocks surrounding the sandy beach area. Other than in species of the genus Chama, individuals of this species attach to the substrate with their right valve. This valve therefore is concave and has a large flat attachment area. The left or upper valve is more rounded ([www.marinespecies.org](http://www.marinespecies.org)).

Organisms typical for sandy beaches show a variety of adaptations to it. Mostly all of the bigger benthic organisms show camouflage and burrowing behavior, like the fishes. Smaller organisms living in the sand often show fast burrow behavior, protecting them from wave energy and predators. Examples for these animals are mollusks and worms. It is also interesting to note that even though worm and worm-like organisms are typically the most common and diverse species at sandy habitats, we did not identify any of these species. They are not in our list due to a lack of identification knowledge and time. By including them we would have seen a much more diverse system and a higher organism abundance.

Compared to the other habitats (see protocols of seagrass meadow and boulder field), the sandy beach is a less species rich community.

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# Meiofauna of the Mesopsammon

René Mähr and Sandro Reheis

## Introduction

It was long believed, that the litoral of sandy bays consists only out of anorganic mass and contains no live at all. At the early 19th century Adolf Remane Professor at the University of Kiel developed a new technique to extract organisms out of sand (grain size 0,06 – 2 mm) and was so able to proof that in sand a vast diversity of organsim find their habitat. This habitat is called Mesopsammon. In the 70's and 80's a numerousness of papers contemplate the subject benthos. It was in those days the terms Episammon and Meiobenthos were formed and lead to the present-day term of the Meiofauna. The Meiofauna is defined as the class of the Zoobenthos, which fits thru a mesh with an aperture of 1 mm.[1]

We examined sand specimens out of different depth (0,5 m; 1,0 m; 1,5 m; 2,0 m) from the proximate bay „Plage de la Revellata“. Organisms living in this habitat evolved specific adaptations, such as a slender build, reduced body height and suctional organs with which the animals adhere themselfe to the grains.

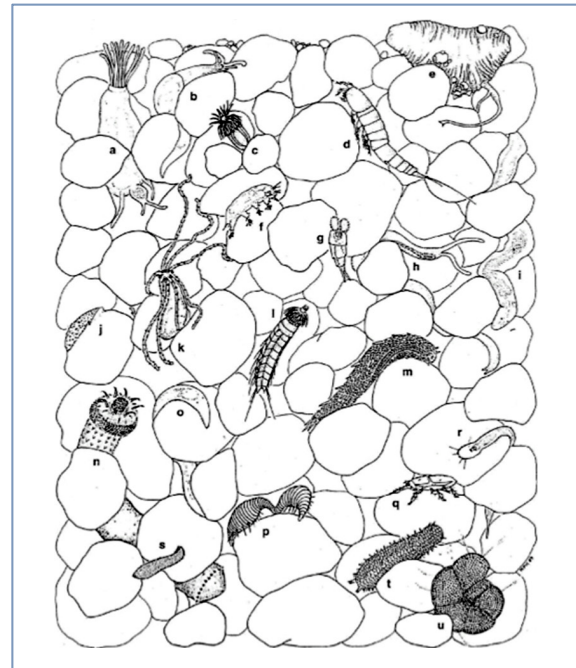


Fig.1: Meiofauna and Mesopsammom [2]

## Material & Methods

The degree of salinity was determined with a Rayleigh refractometer, which showed a degree of salinity of 36 promille. The upper sand layer in all the different depth was excavated with small cups and afterwards in the lab, rinsed with a  $MgCl_2$  dilution (36 g/ml). Due to the treatment with  $MgCl_2$  the musculature relaxes, the organsim fall of the grains and it becomes possible to extract all the organisms adhering to the grains.

## Results

### Chaetognatha

Commonly known as arrow worms they belong to their own phylum, the Chaetognatha. This indicates that they are a relatively unique form of life. Like many animal phyla, the Chaetognatha go back to at least the Cambrian era. [3]

Noticeable is the long tail, which adds up to almost half of the body. At the pairing Chaetognatha swim antipodal to one another and so over give their sperm.[5]

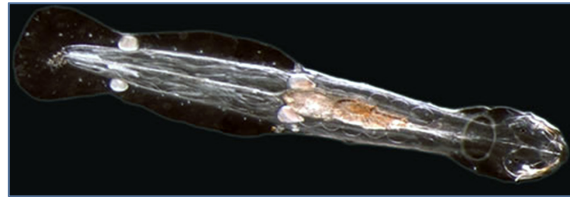


Fig.2: *Spadella cephaloptera* [4]

### Annelida

In the Mesopsammon Annelida are mainly represented by the class of the Polychaete, in particular Archaiannelida – small forms with in part larve character – most of them Protodrilus species.

Polychaetes are segmented worms, generally less than 10 centimetres in length, although ranging at the extremes from 1 millimetre (0.039 in) to 3 metres. They are often brightly coloured, and may be iridescent or even luminescent. Each segment bears a pair of paddle-like and highly vascularized parapodia, which are used for movement and, in many species, act as the worm's primary respiratory surfaces [7].



Fig.3: *Protodrilus* sp. [6]

### Acoelomorpha

*Symsagittifera corsicae* belongs to the Acoela and lives in a close symbiotic relationship with photosynthetic active alga, although the mouth is still present posteriorly to the statocyst. Due to that fact it is not surprising that it exclusively appears in the, with sunlight flooded, upper layers of the Mesopsammon. It doesn't possess any optical organ of perception, although it is able to find its way to the surface with the aid of its with receptor-cilia armed Statocyst [8].

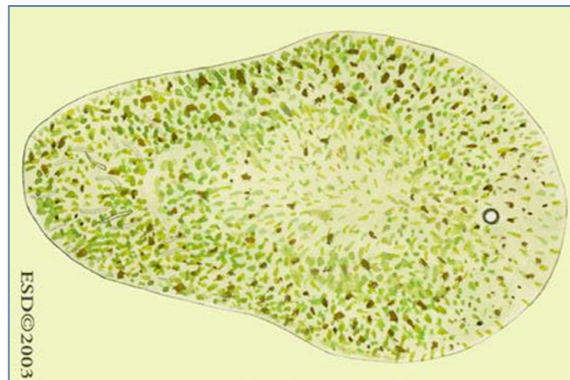


Fig.4: *Symsagittifera corsicae* [9]

### Rizaria

*Elphidium crispum* is a genus of foraminifera, and quiet abundant at the coast of Corsica.

*Elphidium* shows dimorphism with alternating generations. The complete cycle for *Elphidium crispum* takes two years in the shallower marine regions, although it may be delayed at deeper stations. Asexual reproduction reaches a peak in spring of first year. Sexual reproduction begins early in the second spring as temperatures begin to rise 10 °C [10].

**Ciliata**

*Tracheloraphis* is a very long, marine ciliate. Its body is very contractile and wider at the anterior end (mouth). [12] Some of the ciliate look pretty similar to Plathelminthes, an easy way to distinguish them is the fact that Ciliate can move forth and back, whereas Plathelminthes only can move in one direction [13].

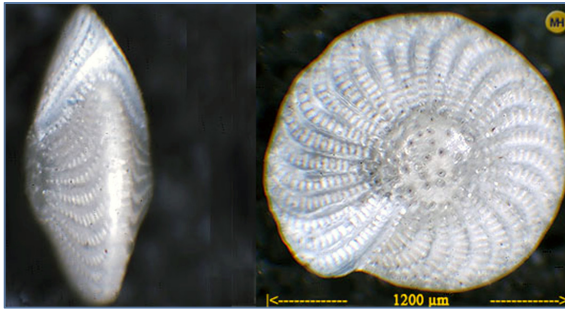


Fig. 5: *Elphidium crispum* [11]



Fig. 6: *Tracheloraphis* sp.[14]

**Table 1:** Species

Phylum	Class	Ordo	Family	Species
Rhizopoda	Radiolaria			
	Foraminifera	Rotaliida Lenkester		
		Bliminida Fursenko		
			Nonionidae	<i>Elphidium crispum</i>
				<i>Cassidulina carinata</i>
			Elphidiidae	<i>Criboelphidium vadescens</i>
Ciliata				<i>Tracheloraphis</i> sp.
Acoelomorpha		Acoela	Sagittiferidae	<i>Symsagittifera corsicae</i>
Plathelminthes		Turbellaria	Polycladida	
Annelida	Polychaeta			<i>Sabellidae</i> sp.
Arthropoda				
Mollusca	Scaphopoda			
Chaetognata	Sagittoidea			<i>Spadella</i> sp.

**Discussion**

We found a plurality of typical inhabitants of the Mesopsammon (see tab.1), the most abundant ones are Rhizopoda with its classes Foraminifera and Radiolaria. The higher organised Ciliata are also found, with it ciliates they are perfectly adapted to manoeuvre through the interspace of the sand grains. Other Metazoa found are Acoelomorpha, Plathelminthes, Annelida, Arthropoda and Mollusca.

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# Seagrass *Posidonia Oceanica*

Nadja Parth, Robert Pieta and Sophie Riccabona

## Introduction

Together with *Zostera marina* (Linnaeus, 1753), *Zostera noltii* (Hornemann, 1832), *Cymodacea nodosa* (Ascherson, 1870) and *Ruppia maritima* (Linnaeus, 1753), *Posidonia oceanica* ((Linnaeus) Delile, 1813) is one of five species of seagrass naturally occurring in the Mediterranean Sea, there *P. oceanica* is endemic. In the temperate region seagrass meadows are monotypic, meaning that the meadows are composed by one species in contrast to seagrass meadows in the tropics which could be formed by two or three species (Hofrichter 2002). Seagrasses are perennial (Sommer 2005).



Fig.1: *Posidonia oceanica* seagrass in the Bay of STARESO

A: *Posidonia oceanica* meadow; B: Seagrass cut beneath the rhizome; C: Fauna and flora upon the surface of *Posidonia* (from the top to the bottom of the picture: organism density increases with the distance from the rhizome); D: *Electra posidoniae*, Bryozoa, growing on the seagrass surface

*Posidonia oceanica* is not a grass in a strict sense, taxonomically it belongs to the angiosperms within the Monocotyledoneae. They have developed from plants which lived terrestrially and have come back into the water secondarily. Together with green algae they are the only ones with chlorophyll a and b (Hofrichter 2003). They have roots, blossoms and fruits, and pollination occurs under water. Normally the single grasses are 60 – 80 cm in length, whereas the maximal leaf length can be up to 140 cm (Hofrichter 2002, Hofrichter 2003).

*P. oceanica* is the most important species which forms extended seagrass meadows upon sediment bottoms in the Mediterranean Sea. Depending on light availability and intensity it occurs from surface waters as deep as 40 meters (Hofrichter 2002). A study in the Bay of Calvi shows that light intensity is one of the most important factors for the growth of seagrass (Elkalay et al., 2003). In ideal conditions, up to 1000 *Posidonia* leaves can grow per squaremeter. The climax of the growth of *P. oceanica* is in autumn and winter, it grows from the meristem and the leaf tip dies and shears off (Hofrichter 2002).

#### **Ecological importance of *P. oceanica***

Seagrass meadows as *Posidonia* - meadows fulfill a variety of important functions. They stabilize sediments, and nutrients in fine sediments are held back in between the leaves. That is essential for the plant itself as for filter feeders like Porifera, Hydrozoa, Bryozoa and Polychaeta which live upon the grass. Additionally seagrass meadows moderate water movements and can thus create slack water regions. Because of so many single leaves standing closely together there is an enormous enlargement of the surface that forms a completely different habitat as the one outside the seagrass meadow at the same time. This habitat is used by small organisms and as nursery for invertebrates and fish as it provides hatcheries, protection from predators and food (Hofrichter 2002).

#### **Posidonia – meadows are threatened habitats**

Well established meadows are very stable, they grow slowly and the regression occurs also in a moderate speed. Thereby they are very sensitive against environmental changes, for example pollution. In the last decades in many regions regression of *Posidonia* - meadows was noticed. The most important problems are mechanical harms by anchors or dredges, suffocation of the meadows under algae because of eutrophication, and change of the flow conditions by shipping or new buildings (Hofrichter 2002).

There are many studies on this topic. Cancemi et al. (2002) detected that a fish farm in a bay of Corsica harms the *Posidonia* – population because of the nutrient enrichment.

## **Materials and Methods**

In the beginning of September 2012 three groups retrieved samples from the seagrass *Posidonia oceanica* from different depths (3 m, 5 m, 7 m, 9 m, 11 m) at the research station STARESO (Station de Recherches sous-marine et oceanographique), near Calvi, Corsica. Depths have been determined by using a depth sounder and a dive computer. The average between these two methods was used.

To reach the greatest diversity possible of different epiphytic organisms, a plastic bag was put over the plant, which was subsequently cut off carefully to prevent the organisms to get lost on the way to the water surface. To determine the variety of the species living on the surface of the rhizome we had to be aware that together with the leaves also the upper parts of the rhizome were cut off (see fig 1B). Additionally scoop nets were used to strip off organisms from the plant surface.

Then the surface of the seagrass was investigated in the lab by using stereo microscopy and the biodiversity was determined using Riedl (1983).

The seagrass was cut into three sections by one group to determine the density of the organisms on the surface of each piece. The organism density increases with the distance from the rhizome and decreases with the depth.

## Results and Discussion

All sampled specimens are shown in table 1.

The most abundant unicellular Eucaryota we found were Foraminifera, which we could identify in all depths. All other unicellular Eucaryota were ciliates which were exclusively found in the upper sampling sites. This might be because they are able to use photosynthesis and so are dependent on upper layers with more light available. In this course we have identified a lot more cnidarian species than during earlier courses. A highlight was a juvenile anthozoan from 11 meters. Most cnidarians we found were hydrozoans. Cnidarians were totally missing in samples from five and seven meters. It is possible that, due to the short sampling time of approximately one hour we just didn't catch them. In 2010 only one hydrozoan from five meters depth could be identified.

Annelids, most of them belong to the "Polychaeta", were found in all sampled depths except in five meters. During the 2010 course two species of Polychaeta were found in five meter depth, so we concluded that annelids can be found in all depths of the upper Posidonia meadow. With *Chrysopetalum debile* and *Perinereis cultifera* we could identify two additional annelid species which weren't found in 2010.

Arthropods of the Posidonia meadow mainly belong to the Crustaceae. Our sampling experiments showed that crustaceans can be found very often in sampling depths up to nine meter. Interestingly at 11 meters, only two larvae were found but adults were missing. This gives the impression that adult crustacean inhabitants of Posidonia meadows can mostly be found in the upper layers, whereas larval stages are more abundant in greater depths. This data is also consistent with the sampling of 2010 where crustaceans were found up to a depth of five meters. Unfortunately the students didn't sample at 11 meters in the year 2010. The fact that they also didn't find any larvae in the upper layers supports this theory, but to be sure, it needs a lot more sampling.

Bryozoans have the highest abundance at three meters. Three of four bryozoan species were found in this depth. Only *Myriapora truncata*, the "false coral", was sampled at 11 meters. The depth range for this species is from two or three meters down to approximately 60 meters, so this depth is not unusual for this taxon (Ballesteros, 2006).

From all five echinoderm divisions, we were able to identify two, namely Asteroidea and Ophiura, in *Posidonia* seagrass. They were abundant in all samples except in samples from three meters.

The mollusc species we found in *Posidonia* seagrass belong to the Polyplacophora, Bivalvia and Gastropoda and are abundant in all sampled depths. In all samples also Nematodes were found in great numbers.

We found three animals belonging to the Platyhelminthes in our investigation, one was determined as the polyclad species *Prosthiostomum siphunculus*. This worm, together with a not determined flatworm, was found in a depth of three meters.

Additionally we found one Nemertini at three meters and two sponge species in seven and nine meters.

Algae were found epiphytic on the *Posidonia* leaves mostly at lower depths. Chlorophyta and Ochrophyta were mainly at three to seven meters sites whereas Rhodophyta could be identified also in deeper zones.

**Table 1:** Taxa found in *Posidonia oceanica* seaweed at STARESO – Calvi in 2012

Animals										
Phylum	Division	Class	Family	Species	additional Information	Depth				
						3	5	7	9	11
Alveolata	Ciliophora					x	x	x		
Alveolata	Ciliophora		"Holotricha"				x			
Rhizaria	Foraminifera					x	x	x	x	x
Porifera								x	x	
Cnidaria	Anthozoa				juvenil					x
Cnidaria	Anthozoa					x				
Cnidaria	Hydrozoa		Aglaopheniidae	<i>Aglaophenia sp.</i>		x				
Cnidaria	Hydrozoa		Cladonematidae	<i>Cladonema radiatum</i> (Dujardin, 1843)		x				
Cnidaria	Hydrozoa								x	
Cnidaria	Hydrozoa		Sertulariidae						x	
Plathelminthes						x				x
Plathelminthes		Polycladida		<i>Prosthostomum siphunculus</i> (Delle Chiaje, 1822)		x				
Nemertini			Tetrastemmatidae			x				
Mollusca	Polyplacophora									x
Mollusca	Gastropoda				dead animal (7m)	x	x			
Mollusca	Gastropoda		Rissoidae	<i>Rissoa violacea</i> (Desmarest, 1814)						x
Mollusca	Bivalvia									x
Mollusca	Bivalvia		Arcidae	<i>Arca noae</i> (Linnaeus, 1758)						x
Mollusca	Bivalvia					x				
Annelida									x	
Annelida		"Polychaeta"				x	x	x	x	
Annelida		"Polychaeta"	Chrysopetalidae	<i>Chrysopetalum debile</i> (Grube, 1855)						x
Annelida		"Polychaeta"	Nereidae	<i>Perinereis cultifera</i> (Grube, 1840)		x				
Annelida		"Polychaeta"	Serpulidae							x
Annelida		"Polychaeta"	Serpulidae	<i>Spirorbis sp.</i>		x				
Annelida		"Polychaeta"	Syllidae							x
Arthropoda	Arachnida	Acari								x
Arthropoda	Crustaceae	Copepoda				x	x	x		
Arthropoda	Crustaceae	Copepoda			Larva					x
Arthropoda	Crustaceae	Copepoda				x				
Arthropoda	Crustaceae	Malacostraca	Caprellidae	<i>Pseudoprotella phasma</i> (Montagu, 1804)			x	x	x	
Arthropoda	Crustaceae	Malacostraca	Gammaridae			x	x			
Arthropoda	Crustaceae	Malacostraca	Isopoda							x
Arthropoda	Crustaceae	Malacostraca		"Amphipoda sp."		x				
Arthropoda	Crustaceae	Malacostraca				x	x	x		
Arthropoda	Crustaceae	Malacostraca	Pandalidae							x
Arthropoda	Crustaceae				Zoea larva (11m)	x				x
Nematoda						x	x	x	x	x
Bryozoa		Gymnolaemata	Electridae	<i>Electra posidoniae</i> (Gautier, 1954)		x				
Bryozoa		Gymnolaemata	Microporellidae	<i>Microporella sp.</i>		x				
Bryozoa		Gymnolaemata	Myriaporidae	<i>Myriapora truncata</i> (Pallas, 1766)						x
Bryozoa						x				
Echinodermata		Asteroidea								x
Echinodermata		Asteroidea								x
Echinodermata		Ophiura								x
Echinodermata		Ophiura	Amphiuridae							x
Echinodermata		Ophiura	Ophiocomidae							x
Echinodermata										x

Algae										
Phylum	Division	Class	Family	Species	additional Information	Depth				
						3	5	7	9	11
Chlorophyta		Ulvophyceae	Cladophoraceae	<i>Chaetomorpha sp.</i>		x				x
Chlorophyta							x			
Ochrophyta		Phaeophyceae	Desmarestiaceae	<i>Desmarestia viridis</i> (J.V. Lamouroux, 1813)		x				
Ochrophyta		Phaeophyceae					x			
Ochrophyta		Bacillariophyceae	Rhizosoleniales	<i>Rhizosolenia calcar-arvis</i> (Schultze, 1858)						x
Rhodophyta		Floridaeophyceae	Corallinaceae	<i>Amphiroa fragillissima</i> (J.V. Lamouroux, 1816)		x				
Rhodophyta		Floridaeophyceae	Corallinaceae	<i>Fosliella sp.</i>		x				
Rhodophyta		Floridaeophyceae	Acrochaetiaceae	<i>Colaconema daviesii</i> (Stegenga, 1985)						x
Rhodophyta						x				x

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# Excursion to Fango

Martin Kinzner and Magdalena Tratter

## Introduction

The Fango is a river in the north-west of Corsica, rising from two springs at the Capo Tafunatu and at the Haut Asco, fusing near Barghiana, then running about 13.5 km westwards and entering in the Gulf of Galéria into the Mediterranean Sea (fig.1).

At the estuary of the river there is a high energy beach (fig.2) influenced by the precipitation in the catchment area: after a dry period, the water level of the river is low and there is only a subsurface connection to the sea.

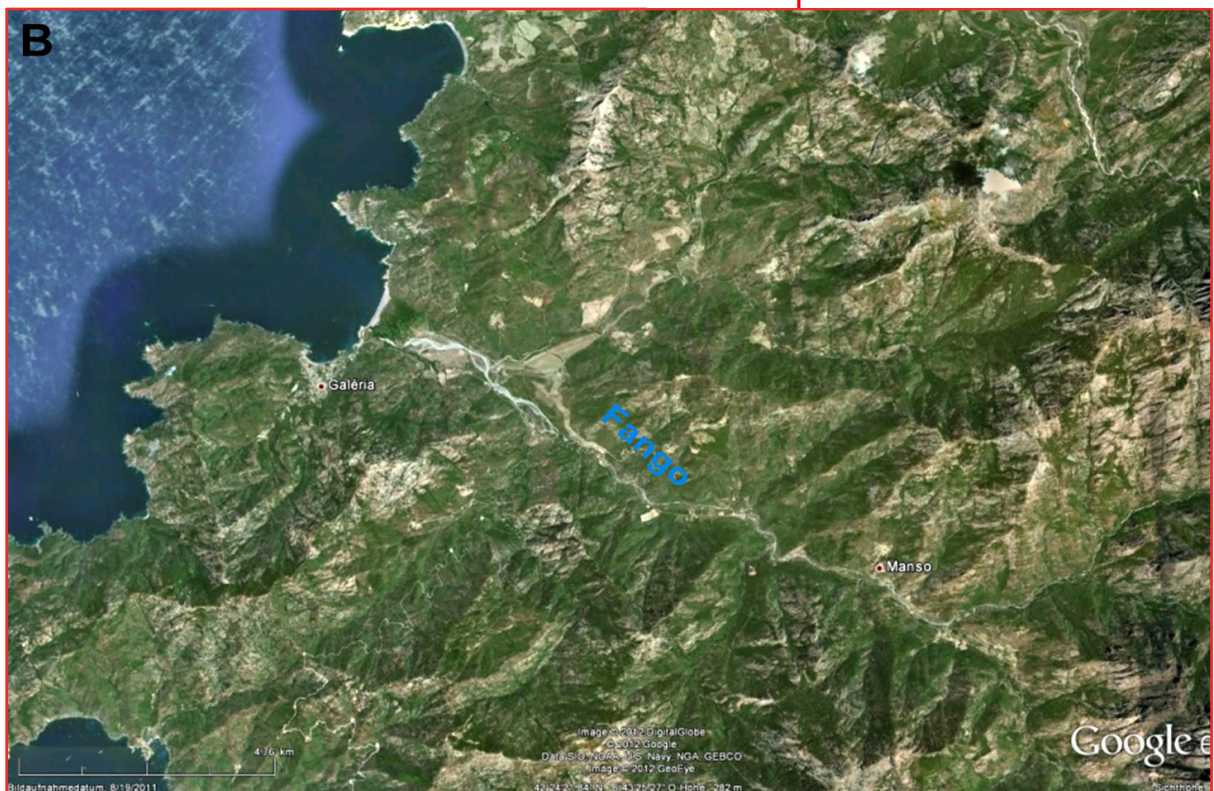


Fig.1: Geographical position of the Fango river: (A) map of northern Corsica, (B) Fango river. Scale of map at the bottom left corner of each picture (Google earth).

In periods of a high water level, parts of the beach are flooded. This fact gives the possibility for marine species to migrate into the freshwater and vice versa.

The high energy beach is characterized by an exposed shore, where the waves can reach the coast without or with little slowdown, and so the sand consists of mainly coarse particles. This can vary from coast to coast and there is also variation during the year (Hofrichter 2002).

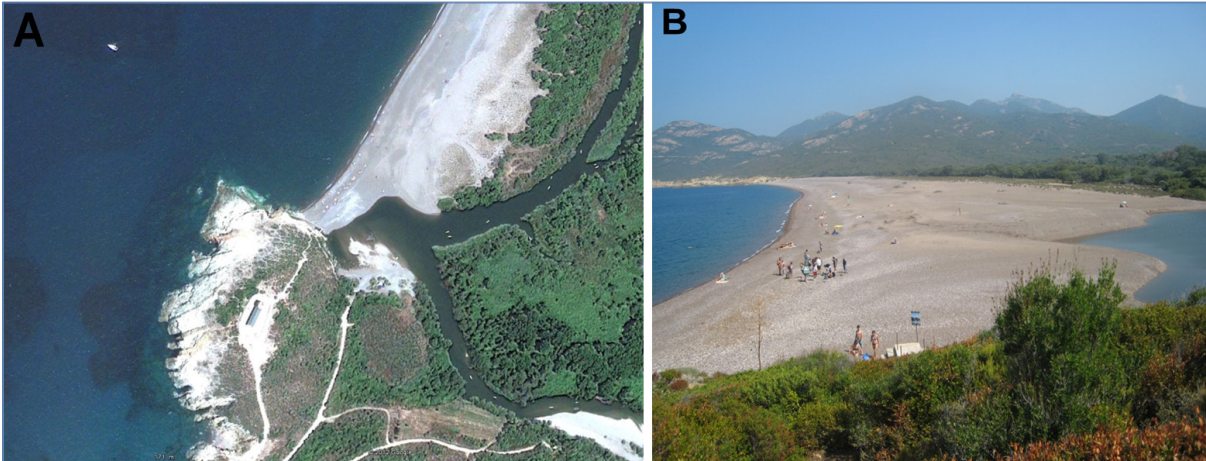


Fig.2: Geographical position of the Fango delta (42°25'12"N, 8°39'30"E): (A) map of the Fango delta. Scale of map at the bottom left corner (Google earth), (B) photography of the beach. At the left side there is the sea and at the right side the Fango.

The road from the delta upstream goes more or less always directly along the river, the water is clear and there are several small pools, so that the Fango is a popular vacation destination for tourists and locals. We stopped at such a pool about 10 km upstream of the estuary (42°22'42"N, 8°45'46"E) at 120 m above sea level, where we observed the limnic and terrestrial fauna.



Fig.3: Typical pools along the Fango river (A, B).



## Material and Methods

The marine, terrestrial and limnic fauna of the sample site was recorded by snorkeling and observation. Shells and insects were collected by hand. Some insects were put into 75% EtOH and taken to Innsbruck for identification. For species determination we used: Riedl (1983), Debelius (1998) and Bergbauer & Humberg (2009) for fish, Riedl (1983) and Poppe & Yoshihiro (1991, 1993) for molluscs and Bährmann (2005), Schaefer (2006) and Seifert (2007) for terrestrial fauna. One beetle was sent to Manfred Kahlen of the Ferdinandeum, Naturwissenschaftliche Sammlungen for determination.

## Results and Discussion

In total we found 50 different species, of which 25 were fish, 15 insects, seven molluscs, one sea urchin, one lizard and one snake (tab.1); nematodes and triclads are not included in the calculation because they couldn't be determined at a lower level. Of the 25 fish only two are not marine species. The Mediterranean trout (*Salmo cetti*) and the Freshwater blenny (*Salaria fluviatilis*) are two of the three fresh water species in the Fango; the third, the Common Eel (*Anguilla anguilla*), could not be seen. The list of the marine fish include more or less all common representatives of the Corsican coastal fish fauna e.g. *Coris julis* or *Oblada melanura*. We could find also some typical fish for this gravel habitat as the Common Stingray (*Dasyatis pastinaca*) or some flatfish. The molluscs were under-represented, maybe because of few motivated mollusc collectors.

The ants that were found near the Fango were mainly harvester ants (*Messor* sp.), the species could not be determined because of taxonomic uncertainties (Schlick-Steiner et al. 2006; Steiner et al. 2011). The beetle has been identified as *Vesperus* sp. (fig.4), which is the only genus of the Subfamily Vesperinae in the family of longhorn beetles (Cerambycidae). For a species determination, it would be necessary to contact a specialist for Vesperinae (Kahlen, pers. comm.).

We also saw some popular Lepidoptera e.g. the Old World Swallowtail (*Papilio machaon*) or the Foxy Emperor (*Charaxes jasius*), the biggest European diurnal butterfly.

The comparison of our results with the results of former excursions to the Fango shows that we observed much more marine species, what can be explained by the good weather this year. In other years before, more terrestrial fauna e.g. *Saltatoria* species were found; we did not see even one.

All in all, we have found as many species in and near the Fango as never before and especially the molluscan and the terrestrial fauna are not yet totally recorded in this course.



Fig.4: The female of the beetle *Vesperus* sp.

**Table 1:** Species list from the excursion to Fango, recorded for the different habitats. On the left the taxonomic group and the scientific name, in the middle the popular name in English and German, if both were available, and on the right the family.

phylum	class	order	family	species
1.) Fango Delta				
1.1) Marine fauna				
Chordata	Chondrichthyes	Rajiformes	Dasyatidae	<i>Dasyatis pastinaca</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Apogonidae	<i>Apogon imberbis</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Blenniidae	<i>Aidablennius sphyinx</i> (Valenciennes, 1836)
	Actinopterygii	Perciformes	Blenniidae	<i>Parablennius sanguinolentus</i> (Pallas, 1814)
	Actinopterygii	Perciformes	Labridae	<i>Coris julis</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Labridae	<i>Symphodus rostratus</i> (Bloch, 1791)
	Actinopterygii	Perciformes	Labridae	<i>Symphodus tinca</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Labridae	<i>Thalassoma pavo</i> (Linnaeus, 1758)
	Actinopterygii	Mugiliformes	Mugilidae	<i>Oedalechilus labeo</i> (Cuvier, 1829)
	Actinopterygii	Perciformes	Mullidae	<i>Mullus surmuletus</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Pomacentridae	<i>Chromis chromis</i> (Linnaeus, 1758)
	Actinopterygii	Scorpaeniformes	Scorpaenidae	<i>Scorpaena porcus</i> Linnaeus, 1758
	Actinopterygii	Perciformes	Serranidae	<i>Serranus cabrilla</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Serranidae	<i>Serranus scriba</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Serranidae	<i>Epinephelus marginatus</i> (Lowe, 1834)
	Actinopterygii	Perciformes	Sparidae	<i>Diplodus annularis</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Sparidae	<i>Diplodus puntazzo</i> (Cetti, 1777)
	Actinopterygii	Perciformes	Sparidae	<i>Diplodus sargus</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Sparidae	<i>Diplodus vulgaris</i> (G. Saint-Hilaire, 1817)
	Actinopterygii	Perciformes	Sparidae	<i>Lithognathus mormyrus</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Sparidae	<i>Oblada melanura</i> (Linnaeus, 1758)
	Actinopterygii	Perciformes	Sparidae	<i>Sarpa salpa</i> (Linnaeus, 1758)
	Actinopterygii	Pleuronectiformes		Gen. sp.
Mollusca	Cephalopoda	Octopoda	Octopodidae	<i>Octopus</i> sp.
	Gastropoda	Archaeogastropoda	Haliotidae	<i>Haliotis turbeculata</i> Lamarck, 1822
	Gastropoda	Archaeogastropoda	Patellidae	<i>Patella caerulea</i> Linnaeus, 1758
	Gastropoda	Archaeogastropoda	Patellidae	<i>Patella ferruginea</i> Gmelin, 1791
	Gastropoda	Archaeogastropoda	Patellidae	<i>Patella</i> sp.
	Gastropoda	Neogastropoda	Muricidae	<i>Thais haemastoma</i> (Linnaeus, 1767)
	Bivalvia	Limoida	Limidae	<i>Lima lima</i> (Linnaeus, 1758)
Echinodermata	Echinoidea	Arbacioida	Arbaciidae	<i>Arbacia lixula</i> (Linnaeus, 1758)
1.2) Terrestrial fauna beach				
Arthropoda	Insecta	Hymenoptera	Apidae	<i>Bombus</i> sp.
	Insecta	Hymenoptera	Vespidae	<i>Vespula</i> sp.
	Insecta	Lepidoptera		<i>Papilionoidea</i> Gen. sp.
2) Fango river				
2.1) Limnic fauna				
Chordata	Actinopterygii	Perciformes	Blenniidae	<i>Salaria fluviatilis</i> (Asso, 1801)
	Actinopterygii	Salmoniformes	Salmonidae	<i>Salmo cettii</i> (Rafinesque, 1810)

Platyhelminthes	Turbellaria	Tricladida		Gen. sp.
2.2) Terrestrial fauna				
Arthropoda	Insecta	Hemiptera	Gerridae	<i>Gerris lacustris</i> (Linnaeus, 1758)
	Insecta	Lepidoptera	Nymphalidae	<i>Charaxes jasius</i> (Linnaeus, 1766)
	Insecta	Lepidoptera	Papilionidae	<i>Papilio hospiton</i> (Guenée, 1839)
	Insecta	Lepidoptera	Papilionidae	<i>Papilio machaon</i> Linnaeus, 1758
	Insecta	Lepidoptera	Nymphalidae	<i>Vanessa atalanta</i> (Linnaeus, 1758)
	Insecta	Lepidoptera	Nymphalidae	<i>Limenitis reducta</i> Staudinger, 1901
	Insecta	Hymenoptera	Formicidae	<i>Messor</i> sp.
	Insecta	Hymenoptera	Formicidae	<i>Solenopsis</i> sp.
	Insecta	Hymenoptera	Formicidae	<i>Formicinae</i> Gen. sp.
	Insecta	Hymenoptera	Vespidae	<i>Vespula</i> sp.
	Insecta	Odonata		Gen. sp.
	Insecta	Coleoptera	Cerambycidae	<i>Vesperus</i> sp.
Nematoda				Gen. sp.
Chordata	"Reptilia"	Squamata		<i>Ophidia</i> Gen. sp.
Chordata	"Reptilia"	Squamata	Lacertidae	Gen. sp.

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# Projects



## Molluscs in the Bay of Revellata

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## Introduction

The predominantly marine Mollusca (mollis = soft), firstly described by Georges Cuvier in 1795, are a very species-rich taxon whose representatives can display many different body types. The difference in size between distinct species is the highest known in the animal kingdom as it can vary from 0.5 mm to 18 m. The phylum Mollusca comprises about 100,000 extant species (Tardent 2005, Westheide and Rieger 2007).

Molluscs live nearly everywhere on the planet except in regions with permafrost, like Polar Regions and glaciers. Most species, however, live in marine environments, especially in the benthic zone. Others live in freshwater and some gastropods can even live terrestrially (Westheide and Rieger 2007).

Even though there is such a high richness in shape and body-type within the molluscs, some common features unite all species in this phylum. The body has two main components: cephalopodium and visceropallium. The very highly developed cephalopodium contains the nervous system, the sensory organs and the mouth. All of the inner organs can be found in the visceropallium. This structure also has glands that secrete the calcareous shell of the organisms (Westheide and Rieger 2007).

The Molluscs are primarily bilateral symmetric organisms but many of them are secondary asymmetric. The development occurs by spiral cleavage resulting in a veliger larva, or more uncommon, in a trochophora larva (Westheide and Rieger 2007).

The phylum Mollusca can be divided into two subclasses, the “Aculifera” and the Conchifera.

### “Aculifera”

“Aculifera” are marine molluscs with a distinct anterior-posterior axis. A calcareous shell is either missing or consists of eight plates which are located on the dorsal side of the animal.

The “Aplacophora” (~320 species) have no shell, but often they are equipped with calcareous spiculae localized within the cuticula. Their habitat extends from the sublittoral zone up to a depth of 4,000 meters. Caudofoveata (~250 species) and Solenogastres (~70 species) are the extant representatives of this group.

Polyplacophora (~1,000 species) have an oval-shaped body with eight shell plates on the dorsal part. They are surrounded by a scaled perinotum. A ventral located muscular foot is used mainly for attachment and locomotion. They can be found on rocky beaches, even in the surf zone.

### Conchifera

The Conchifera comprise all higher molluscs. They have a more or less uniform shell which provides shelter for the inner organs.

The Tryblidia (~ 25 extant species) were discovered in Costa Rica in 1952. They live in depths from about 170 m up to 6,500 m. They are also known as living fossils.

Scaphopoda (~600 extant species) have a cone-shaped single shell which is open on both ends. They are typical inhabitants of the deeper zones and the habitat of some species can reach up to 6,000 m. Scaphopods live buried in the sand with only the smaller opening reaching out into the water. Through this opening, they can let fresh seawater flow into the mantle cavity for oxygen supply. Foraminifers are the main food source amongst detritus and other sediment fauna. Food gets crushed by a relatively large radular organ.

With about 38,000 extant species, the Gastropoda have the highest diversity in the phylum Mollusca. This relatively young class (fossil records since the early Cambrian) can be found in marine and fresh water as well as on land. This makes gastropods the most successful molluscs on the planet. Their body has a well-developed head and the visceropallium shows a typical counter clockwise torsion of 180°. Some taxa show a secondary retorsion. Cephalopoda are a quite small, but the most highly developed group within the molluscs. They are exclusively marine organisms and contain about 750 extant species. In addition to the species living in the benthos (e.g.

*Octopus vulgaris*) there are also some cephalopod species that live in the pelagic zone. Most species, with some exceptions like nautilus, do not have an outer shell. Sepia have an inner cuttlebone made from aragonite, others lack the calcareous tissue completely. Cephalopods are famous for their highly developed nervous- and visual systems.

Bivalvia live in marine environments as well as in brackish water and some species can even live in freshwater. Their size is quite diverse, ranging from 2 mm to 1 m with a maximum weight of more than 400 kg (*Tridacna gigas*). About 15,000 bivalve species have been described. Although most Bivalvia live in shallow water, they can also be found on deep sea floors up to about 10,000 meters. All head structures, except for the ingestion opening, have been lost during evolution - they also don't possess a radula. They can attach tightly to the surface of hard substrates by secreting "glue" out of their byssus glands. The two-piece shell is built up by glands which are localized in the outer layer of the pallium.

## Materials and Methods

### Sampling Sites

During evolution, species had to undergo several adaptations to be able to populate new habitats. Over time, speciation events created a unique fauna for each habitat. Our main goal for this course was to determine as many Mediterranean mollusc species as possible. To find the most diverse range of species possible, we sampled five different habitats (see also fig.1). Sampling took place on each day from September 2nd to September 6th, 2012.

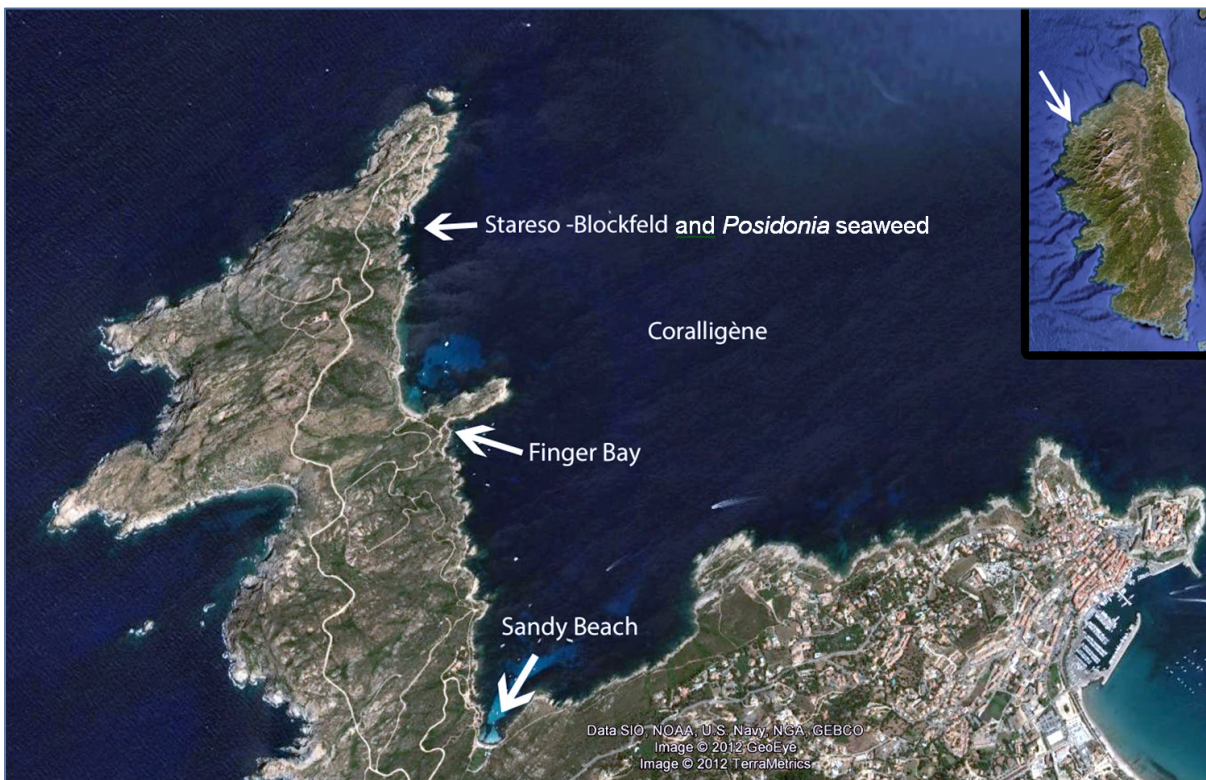


Fig.1. Map of the gulf of Revellata near Calvi, Corsica showing the sampling sites: STARESO Boulder Field ("Blockfeld") and *Posidonia* seaweed meadow, Finger Bay, Sandy Beach, and Coralligène.



The sampled habitats were:

- Boulder Field, Bay of STARESO
- Posidonia Seaweed Meadows, Bay of STARESO
- Coralligène
- Sandy Beach, Bay of Revellata
- Finger Bay

The bay in front of STARESO is bounded by the pier of the little harbor in the north and by cliffs in the south. The sea floor is composed of two main structures. On the one hand, closer to the edges of the bay, there is the Boulder Field with rocks of different sizes. More in the middle of the bay, a *Posidonia oceanica* meadow is growing. It acts as a nursery for small animals, including some molluscs.



Fig.3 The „Finger Bay“ was sampled on one day for the project. It is surrounded by boulder field but also contains partly sandy substrate.

between STARESO and the sandy beach “Plage de l’Alga”.

The sandy beach we investigated was “Plage de l’Alga”. Sandy beaches are special habitats since the beach area is wider and the slope is less steep than in the other types of coast we sampled. At the sandy beach, there was again boulder field and cliffs on both sides of the sandy beach. At sandy beaches it is not always clear if the animals which are found, particularly the shells of molluscs, really are inhabitants of this area or if they were only washed ashore. The sampling site at the tip of the cape beneath the lighthouse could not be visited because of the weather.



Fig.2: The coralligène sample is being pulled out of the water and lifted onto the boat. The dredge content was poured into the green box and transported back to STARESO for identification.

The Coralligène was sampled a little further away from the land near STARESO Bay (figure 2). Coralligène is an accumulation that is formed by various red algae belonging to the Corallinaceae which produce calcium carbonate. This is the main component of coralligène bottoms. Coralligenous structures serve as a shelter for many benthic animals like some mollusc species (Tardent 2005; <http://www.archipelago.gr/>).

Another sampling place was a small bay that we called “Finger Bay” (fig.3). It is a combination of boulders near the coast and small stones and sand further out. It is located on Cape Revellata halfway



Fig.4. The sandy beach that we sampled (“Plage de l’Alga”). It is surrounded by boulders. Due to heavy winds and waves, large amounts of dead seaweed had been washed onto the beach area and accumulated in the shallow water. This made sampling more difficult.

### Sampling Methods

Snorkeling gear was used for sampling in all of the habitats and specimens were placed into zip-lock plastic bags or small plastic containers with screw-on lids. They were transported back to the station where living organisms were placed into saltwater-filled plastic tubs, and shells were stored in Petri dishes.

The Coralligène was sampled with a dredge. The dredge was 150 cm long and the front opening was 59 x 20 cm wide (see fig.5). The mesh size of the front net was 5 cm and the back net had a mesh size of 1 cm. The dredge was towed behind a slowly moving motor boat about 500 m off STARESO. The sampling water depth was around 50 m. The material found in the Coralligène sample was sorted by hand into broad categories. The different groups were placed into different plastic tubs and then they were identified in the laboratory.



Fig.5: The dredge that was used to sample the Corraligène.

Organisms living within the sandy sediment at Plage de l'Alga were collected by sieving the sand. Sieves with a diameter of 20 cm, a depth of 6.5 cm, and a mesh size of 1 and 2 mm, respectively, were used (figure 6a). The sediment was sampled in different water depths (about 0-3 m depth). In addition to the sieving, dead shells and larger organisms washed up to the beach were collected by hand.

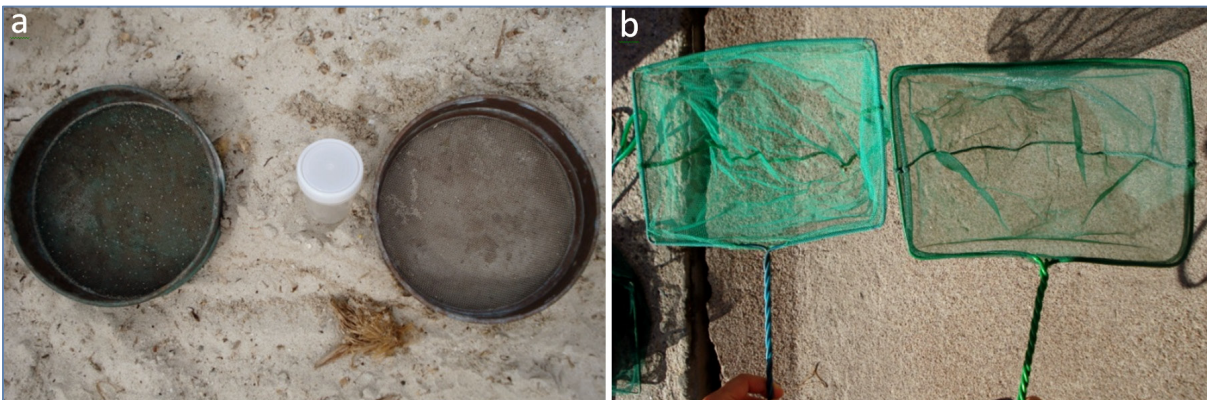


Fig.6: The sieves that were used at the sandy beach (left) and the nets for sea grass sampling (right).

The boulder field was sampled on multiple days throughout the project week. The organisms were collected by hand. The depth for sampling ranged from 0 up to about 5 m depth. Stones were picked up or turned slowly to find specimens that were hiding beneath the stones. The same methods were also used for the Finger Bay.

Mollusks in the *Posidonia* meadow in the Bay of STARESO were sampled with the help of hand-held nets (fig.6b). The nets had a mesh size of 2 mm and the front opening was 19 x 15 cm wide. The nets were placed against the bottom of the plant right above the seafloor and then moved upward while shaking the plant softly so that the animals would fall into the net.

### Identification and Archiving

The identification took place in the dry lab at STARESO. Binoculars were used to identify smaller species.

Our main identification guides were European Seashells Vol. 1 und 2 by Poppe & Goto and Fauna und Flora des Mittelmeeres by Riedl. In addition we used pictures from the internet and the picture guides compiled by previous courses.

After identification, the specimens were photographed and archived. Information recorded included species and family names, original descriptor, descriptor, location of the finding place, and date of identification.

After documentation was done, living organisms were released back into the water whereas empty shells were kept and transported back to the University of Innsbruck.

### Analysis of Data

We used the software program Primer 6 (vers. 6.1.15, Plymouth Marine Laboratory, Plymouth, United Kingdom) to compile Bray-Curtis similarity matrices. This was done for the species list as a whole comparing the different years, as well as for the different habitats for the data of the current year (2012). The calculated matrices then were used to create nonmetric multidimensional scaling diagrams (MDS, 25 restarts, minimum stress: 0.01).

The MDS diagrams were used to estimate the similarity of the years/habitats. The MDS output shows this similarity graphically as two-or three-dimensional distances (see fig.8 & 9). The "2-D stress" indicates how accurate the two-dimensional figure actually depicts the multi-dimensional structure of the data set. The closer the value to zero, the better is the fit. Therefore a stress value of "0" would mean a perfect depiction of the structure in a two-dimensional diagram.

## Results and Discussion

In 2006, about 2,100 mollusc species were recorded for the Mediterranean Sea (Ramazzotti et al. 2006). Four years earlier Hofrichter (2002) only noted 1,376 species. This big difference of more than 700 species in such a short time shows that the Mollusca are a "mega diversity group" of which still many species have to be discovered in the Mediterranean Sea and in other oceans.

The total number of mollusc species in the Gulf of Revellata found this year in the course (2012) was 131 (see tab.1). From 2004-2010, in the years previous courses did mollusc identification at STARESO, a combined list of 282 different species had been compiled. This year we found 15 new species that had never been identified in this area before. Among these were two scaphopods, seven snails and six mussels. In total, there now are 297 species known to occur in the Gulf of Revellata, which is more than 14% of the discovered Mediterranean molluscs.

**Table 1:** List of all mollusks found in the Gulf of Revellata during the 2012 course, including the habitat and sample state.

Co = Coralligène, Bo = Boulder Field, Fi = Finger Bay, Po = Posidonia Field, Sa = Sandy Beach, PI = Plankton; L = Living specimen, S = Shell only, L/S = Living specimens and shells found, n.a. = state not available.

class/family	species	habitat						state
		Co	Bo	Fi	Po	Sa	PI	
<b>Cephalopoda</b>								
Octopodidae	<i>Octopus vulgaris</i> Lamarck, 1798		x					L
Sepiidae	<i>Sepia officinalis</i> Linnaeus, 1758		x	x		x		L
<b>Scaphopoda</b>								
Dentaliidae	<i>Dentalium agile</i> Sars, 1872	x						S
	<i>Dentalium dentalis</i> Linnaeus, 1758	x						S
	<i>Dentalium vulgare</i> da Costa, 1778	x				x		S
	<i>Dentalium mutabile inaequicostatum</i> Dautzenberg, 1891	x						S
Fustiariidae	<i>Fustiaria rubescens</i> (Deshayes, 1825)	x						S
<b>Polyplacophora</b>								
Acanthochitonidae	<i>Acanthochitona communis</i> (Risso, 1826)			x		x		L
	<i>Acanthochitona fascicularis</i> (Linnaeus, 1767)	x						L
Chitonidae	<i>Chiton olivaceus</i> Spengler, 1797		x	x		x		L
Ischnochitonidae	<i>Callochiton laevis</i> (Montagu, 1803)			x				L
	<i>Ischnochiton rissoi</i> (Payraudeau, 1826)		x	x				L
	<i>Lepidochitona cinerea</i> (Linnaeus, 1767)	x						L
Lepidopleuridae	<i>Lepidopleurus cajetanus</i> (Poli, 1791)			x				L
<b>Gastropoda</b>								
Aporrhaidae	<i>Aporrhais pespelecani</i> (Linnaeus, 1758)	x						S
Atyidae	<i>Haminoea hydatis</i> (Linnaeus, 1758)	x						L
Buccinidae	<i>Cantharus dorbigny</i> (Payraudeau, 1826)			x				L
	<i>Cantharus scacchianus</i> (Philippi, 1844)		x					L
Calyptraeidae	<i>Calyptrea chinensis</i> (Linnaeus, 1758)	x						L
Cavoliniidae	<i>Creseis acicula</i> Rang, 1828						x	L
Cerithiidae	<i>Bittium reticulatum</i> (da Costa, 1778)		x	x		x		S
	<i>Bittium scabrum</i> (Olivi, 1792)					x		S
	<i>Cerithium alucaster</i> (Brocchi, 1814)	x						S
	<i>Cerithium vulgatum</i> (Bruguère, 1792)	x		x				S
	<i>Rhinoclavis kochi</i> (Philippi, 1848)				x			L
Columbellidae	<i>Mitrella minor</i> (Scacchi, 1836)		x					S
Conidae	<i>Conus ventricosus</i> Gmelin, 1791			x		x		L/S
Cypraeaecae	<i>Luria lurida</i> (Linnaeus, 1758)		x					S
Epitoniidae	<i>Epitonium clathrus</i> (Linnaeus, 1758)	x						S
	<i>Epitonium turtonis</i> (Turton, 1819)					x		L
Eratoidea	<i>Erato voluta</i> (Montagu, 1803)	x						S
Fascioliariidae	<i>Fasciolaria lignaria</i> (Linnaeus, 1758)		x	x				L
	<i>Fusinus labronicus</i> (Monterosato, 1884)	x						S
	<i>Fusinus rostratus</i> (Olivi, 1792)	x						S
Fissurelidae	<i>Diodora gibberula</i> (Lamarck, 1822)	x						S
Haliotidae	<i>Haliotis turbeculata lamellosa</i> Lamarck, 1822		x	x				S
Littorinidae	<i>Littorina neritoides</i> (Linnaeus, 1758)					x		S
Melongenidae	<i>Columbella rustica</i> (Linnaeus, 1758)		x	x		x		S
Muricidae	<i>Hexaplex trunculus</i> (Linnaeus, 1758)		x			x		L
Nacticidae	<i>Tectonatica filosa</i> (Philippi, 1844)	x						S
Nassariidae	<i>Cyclope donovania</i> Risso 1826					x		L
	<i>Cyclope neritea</i> (Linnaeus, 1758)					x		L
	<i>Nassarius corniculus</i> (Olivi, 1792)		x					S

	<i>Nassarius cuvieri</i> (Payraudeau, 1826)			x				L
	<i>Nassarius incrassatus</i> (Ström, 1768)			x				L
Patellidae	<i>Patella caerulea</i> Linnaeus, 1758		x	x	x	x		S
	<i>Patella ferruginea</i> Gmelin, 1791		x			x		L/S
	<i>Patella intermedia</i> Murray, 1857					x		S
	<i>Patella nigra</i> (da Costa, 1771)					x		S
	<i>Patella rustica</i> Linnaeus, 1758			x				S
Phasianellidae	<i>Tricolia speciosa</i> (von Mühlfeldt, 1824)					x		S
	<i>Odostomia conoidea</i> (Brocchi, 1814)	x						S
	<i>Turbonilla acutissima</i> Monterosato, 1884	x						S
Ranellidae	<i>Cymatium nicobaricum</i> (Röding, 1798)	x						n.a.
Rissoidae	<i>Alvania cimex form fusca</i> (Philippi, 1836)					x		n.a.
	<i>Rissoa auriscalpium</i> (Linnaeus, 1758)					x	x	L
	<i>Rissoa decorata</i> Philippi, 1846					x		L
	<i>Rissoa dolium</i> Nyst, 1845					x		L
	<i>Rissoa variabilis</i> (von Mühlfeldt, 1824)					x		L/S
	<i>Rissoa ventricosa</i> (Desmonest, 1814)					x		L
	<i>Rissoa violacea</i> Desmonest, 1814					x		L
Thaididae	<i>Thais haemastoma</i> (Linnaeus, 1767)		x	x				L
	<i>Coralliophila squamosa</i> (Bivona, 1838)					x		S
Trividae	<i>Trivia arctica</i> (Salander, 1797)	x						S
Trochidae	<i>Clanculus cruciatus</i> (Linnaeus, 1758)					x		S
	<i>Gibbula ardens</i> (von Salis, 1793)	x		x		x		L/S
	<i>Gibbula divaricata</i> (Linnaeus, 1767)					x		L/S
	<i>Gibbula racketti</i> (Payraudeau, 1826)					x		L
	<i>Gibbula rarilineata</i> (Michaud, 1829)					x		L/S
	<i>Gibbula turbinoides</i> (Deshayes, 1832)					x		S
	<i>Gibbula umbilicalis</i> (da Costa, 1778)					x		n.a.
	<i>Gibbula varia</i> (Linnaeus, 1767)		x	x	x			L/S
	<i>Jujubinus exasperatus</i> (Pennant, 1777)	x				x		L
	<i>Monodonta turbinata</i> (Born, 1780)		x	x				L
Turridae	<i>Raphitoma purpurea</i> (Montagu, 1803)			x				S
Turritellidae	<i>Turritella communis</i> Risso 1826	x	x					S
	<i>Turritella monterosatoi</i> Kobelt, 1887	x						S
	<i>Turritella turbona</i> Monterosato, 1877	x						S
<b>Bivalvia</b>								
Arcidae	<i>Arca noae</i> Linnaeus, 1758	x	x	x		x		S
	<i>Barbatia barbata</i> (Linnaeus, 1758)		x	x		x		L/S
Astartidae	<i>Astarte fusca</i> (Poli, 1795)	x						S
	<i>Astarte sulcata</i> (da Costa, 1778)	x						S
	<i>Plagiocardium papillosum</i> (Poli, 1795)	x				x		S
Cardiidae	<i>Acanthocardia echinata</i> (Linnaeus, 1758)	x						S
	<i>Acanthocardia spinosa</i> (Solander, 1786)	x						S
	<i>Laevicardium crassum</i> (Gmelin, 1791)	x						S
	<i>Parvicardium exiguum</i> (Gmelin, 1791)	x						S
	<i>Parvicardium minimum</i> (Philippi, 1836)	x						S
	<i>Parvicardium pinnulatum</i> (Conrad, 1831)	x						S
	<i>Parvicardium scrabum</i> (Philippi, 1844)	x						S
Carditidae	<i>Cardita calyculata</i> (Linnaeus, 1758)					x		S
Chamidae	<i>Chama gryphoides</i> Linnaeus, 1758					x		S
	<i>Pseudochama gryphina</i> (Lamarck, 1918)					x		S
Glycymerididae	<i>Glycymeris bimaculata</i> (Poli, 1795)	x						S
Limidae	<i>Lima lima</i> (Linnaeus, 1758)		x					S
	<i>Lima</i> sp.					x		S

class/family	species	habitat					state
	<i>Limaria hians</i> (Gmelin, 1791)		x				n.a.
	<i>Limatula gwyni</i> (Sykes, 1903)	x					S
Lucinidae	<i>Ctena decussata</i> (O. G. Costa, 1829)					x	S
	<i>Loripes lucinalis</i> (Lamarck, 1818)					x	S
	<i>Lucinella divaricata</i> (Linnaeus, 1758)	x					S
Mactridae	<i>Mactra stultorum</i> (Linnaeus, 1758)	x					S
Mytilidae	<i>Modiolula phaseolina</i> (Philippi, 1844)	x					n.a.
	<i>Modiolus barbatus</i> (Linnaeus, 1758)					x	S
	<i>Musculus costulatus</i> (Risso, 1826)		x				S
	<i>Mytilus edulis</i> Linnaeus, 1758		x				L
Ostreidae	<i>Ostrea edulis</i> Linnaeus, 1758		x			x	S
Pectinidae	<i>Chlamys bruei</i> (Payraudeau, 1826)		x				S
	<i>Chlamys commutata</i> (di Monterosato, 1875)	x					S
	<i>Chlamys flexuosa</i> (Poli, 1795)	x					S
	<i>Chlamys opercularis</i> (Linnaeus, 1758)	x					n.a.
	<i>Chlamys varia</i> (Linnaeus, 1758)	x	x				S
	<i>Pecten jacobaeus</i> (Linnaeus, 1758)	x					S
	<i>Pecten maximus</i> (Linnaeus, 1758)	x					S
	<i>Pseudamussium clavatum</i> (Poli, 1795)	x					n.a.
	<i>Pseudamussium peslutrae</i> (Linnaeus, 1771)	x					S
Pinnidae	<i>Pinna nobilis</i> Linnaeus, 1758		x		x	x	L
	<i>Pinna rudis</i> Linnaeus, 1758			x		x	L
Tellinidae	<i>Tellina compressa</i> Brocchi, 1814	x					S
	<i>Tellina donacina</i> Linnaeus, 1758	x					S
	<i>Tellina planata</i> Linnaeus, 1758					x	S
	<i>Tellina pulchella</i> Lamarck, 1818	x					S
Veneridae	<i>Callista chione</i> (Linnaeus, 1758)	x					S
	<i>Chamelea striatula</i> (da Costa, 1778)	x				x	S
	<i>Clausinella brongniartii</i> (Payraudeau, 1826)	x					n.a.
	<i>Dosinia exoleta</i> (Linnaeus, 1758)	x					S
	<i>Dosinia lupinus</i> (Linnaeus, 1758)	x					S
	<i>Paphia aurea</i> (Gmelin, 1791)					x	S
	<i>Pitar rudis</i> (Poli, 1795)	x					S
	<i>Tapes philippinarum</i> (Adams and Reeve, 1850)	x					S
	<i>Venus verrucosa</i> Linnaeus, 1758	x					n.a.

\*Orange marked rows show the new species 2012

As seen in figure 7a, we identified fewer species than the groups in the previous three courses. For example the 2010 course found an additional 37 species compared to the 131 of this year. The main reason for this decrease in mollusc species identified is that in 2010 there were more people responsible for the search and that divers (such as H. and I. Schatz) contributed to the collection of molluscs. Also, there were more people available for species determination. In addition we had several days with bad weather conditions this year that inhibited a more intensive search.

By comparing the single mollusc classes, it becomes clear that the decrease is almost exclusively found in the gastropods: there are 37 snails missing to reach the same snail species number as two years ago (figure 7b). In addition, we found a little less bivalves and cephalopods (fig.7b&c).

On the other hand, this year we identified more scaphopods and polyplacophores than in all previous courses. Still, there are only two scaphopod species (*Dentalium agile*, *D. mutabile inaequicostatum*), that are newly identified for the Gulf of Revellata (fig.7c). The fact that every year there is at least one new species of scaphopods identified, shows that we certainly have not reached the species identification limit yet. A focused search on these

cryptic invertebrates would most likely increase the scaphopod species list for the Bay of Revellata considerably. For the whole Mediterranean, there are only 16 species of scaphopods known (Ramazzotti et al. 2006). Over the years, the different courses already located 6 of those 16 species in the Gulf of Revellata. For the Polyplacophora the situation is similar: 35 species are known to occur in the Mediterranean (Ramazzotti et al. 2006) and nine species were found from 2004-2012 in the courses. Out of the nine species, seven were also identified this year (fig.7c). Cephalopods are represented with 25 species in the Mediterranean (Ramazzotti et al. 2006) and in total four species have been found in the Gulf of Revellata. This year we only found two species.

The largest part of the 2,100 marine molluscs in the Mediterranean Sea is comprised of mussels and snails. This is also reflected in the recent species list in which 117 out of 131 species are either in the class Bivalvia or Gastropoda. The same is true for the species lists of the previous courses (see fig.7a-c).

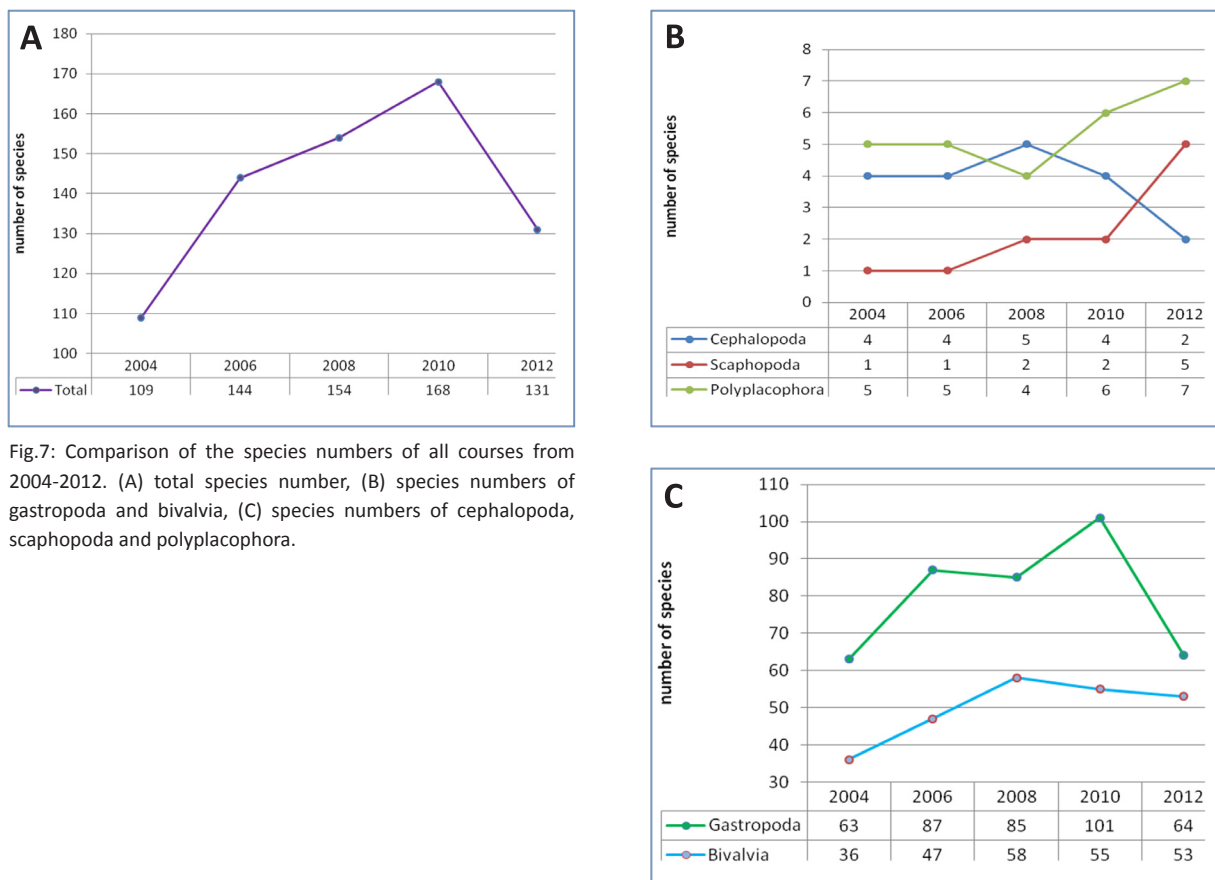


Fig.7: Comparison of the species numbers of all courses from 2004-2012. (A) total species number, (B) species numbers of gastropoda and bivalvia, (C) species numbers of cephalopoda, scaphopoda and polyplacophora.

Figure 8 is a graph comparing species lists of the different years. It shows that the species composition found in 2010 and 2008 are the most similar (about 64% similarity), whereas the species from 2004 are the most dissimilar to all other species lists (41-50%). The species list from 2012 is only a little closer to that from 2010 and 2008 (about 57%) than to the species list from 2006 (about 54.5%).

Only 27 species have been found in every course. Of those there are two Cephalopoda, three Polyplacophora, 15 Gastropoda and seven Bivalvia. The most prominent representatives found every year include *Octopus vulgaris*, *Thais haemastoma* or *Venus verrucosa*. This year we found 50 species that are the same as in 2004, 66 species that were also found in 2006, 80 species overlap with the list from 2008 and 84 species are the same as in 2010. For comparison: 2008 and 2010 share 101 same species.

In Table 2, the number of species for each sampled habitat for the year 2012 can be seen. The highest number of species was found in the Coralligène (62 species), followed by the sandy beach (43 species). The samples from

the sandy beach are difficult to evaluate, however, because most of the specimens we found were only dead shells. These could have also been washed ashore from elsewhere and may not belong to the sandy beach habitat.

Only one mollusc species (*Creseis acicula*) could be identified in the plankton. There were also several undeterminable Veliger-larvae present. This is not surprising, because only few adult molluscs are pelagic swimmers and therefore part of the plankton. The majority of adult species have benthic life styles. In the *Posidonia* seaweed meadow, we also only found relatively few species. The reasons for this could be one or more of the following: (i) *Posidonia* meadows in the Gulf of Revellata are really unfavourable habitats for molluscs, (ii) the distribution of molluscs in a *Posidonia* meadow is not homogeneous and we had bad luck by sampling or (iii) our sample method by using nets is not efficient enough, so we always only see a small part of the diversity of *Posidonia* meadows. All of these possible problems could be controlled by sampling calmly via scuba diving.

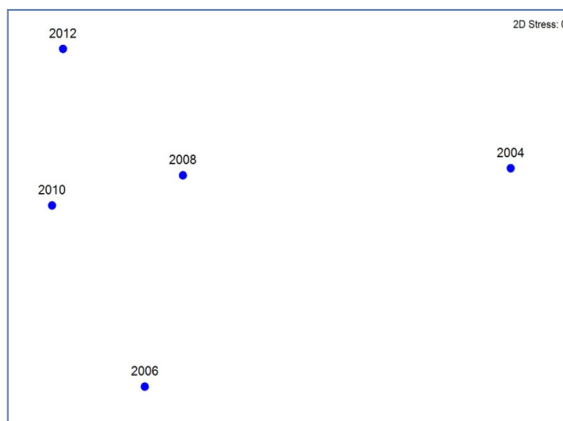


Fig.8: Similarity of the mollusc species lists of the different course years 2004-2012. The distance of the dots reflects the relative similarity of species. In how far the two-dimensional graphic is in accordance with the real multidimensional structure in the data set is shown as the “2D-stress”. A stress of “0” shows a perfect fit.

**Table 2:** Species numbers for the different habitats and molluscan classes for this year 2012.

	Coralligène	Boulder Field	Finger Bay	Posidonia meadow	Sandy Beach	Plankton
Cephalopoda	-	2	1	-	1	-
Scaphopoda	5	-	-	-	1	-
Polyplocophora	2	2	5	-	2	-
Gastropoda	20	15	16	11	23	1
Bivalvia	35	10	3	1	16	-
Total	62	29	25	12	43	1

It is not surprising that we have not found any cephalopods in the Coralligène, the *Posidonia* meadow, or the plankton, because cephalopods are swimming too fast for the sampling methods we used for those habitats. In addition, they also generally avoid open water.

The result of the nonmetric multidimensional scaling (MDS) for the similarity of species composition in the different habitats is shown in figure 9. Finger Bay and Boulder Field are the most similar habitats (48% similarity). Also, the sandy beach is very similar to the Finger Bay habitat (32%). This is probably caused by the structural similarity of those habitats since the Finger Bay is a combination of sandy beach and Boulder Field. Therefore, typical species for both habitats, the Boulder Field and the sandy beach, can be found in the Finger Bay. The plankton sample did not share any species with the other habitats, so its similarity to all others is 0%. The species found in the Coralligène were also very different from all other habitats; the similarity ranges from 0% (plankton) to 9.5% (sandy beach).

Almost all the scaphopods we found were living in the Coralligène, and only one species (*Dentalium vulgare*) was also detected in the sandy beach habitat. Other typical representatives for the Coralligène were different species of the genus *Turritella*, *Chlamys*, as well as of the families Veneridae or the Cardiidae.



The Boulder Field was dominated in abundance by different *Patella* species. The Patellidae can store water in their shell and therefore they are able to tolerate periods of dryness without desiccation (e.g. at low tide). The species *Patella ferruginea*, the Ripped Mediterranean Limpet, is a protected species in Corsica. This mussel has a very high shell with longitudinal ribs. Other common species for this habitat are *Monodonta turbinata*, *Thais haemastoma* and *Octopus vulgaris*.

Sandy areas are typical habitats for the impressive bivalves *Pinna nobilis* and *P. rudis*, both endangered species. The collection of specimens is therefore prohibited. We also found many Patellidae shells, which are not typical for sandy habitats and probably have been washed ashore from the Boulder Field. We also found several species of the genus *Gibbula*, which normally also live in rocky habitats. The only species of cuttlefish (Sepiidae) we found in this course, *Sepia officinalis*, is a common representative of the sandy beach, where it is swimming on the ground and displays protective mimicry.

As mentioned before, the Finger Bay is a combination of Boulder Field and sandy beach, so a mixture of species typical for rocky and for sandy habitats can be observed. Typical for this combined habitat is *Conus ventricosus* (= Syn. *C. mediterraneus*) the only representative of the Conidae. The World Register of Marine Species (WoRMS) accepted *Conus ventricosus mediterraneus* as a subspecies in 2001 (Costello et al. 2001).

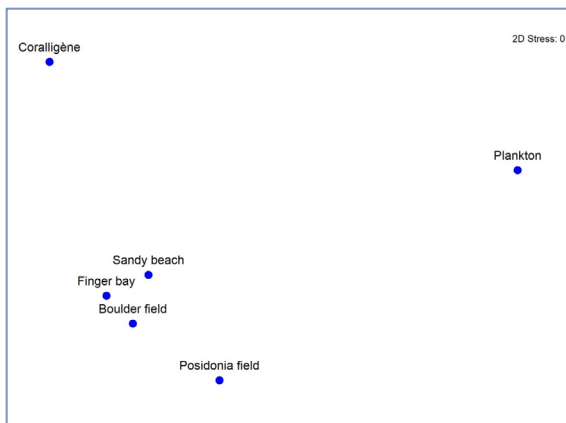


Fig.9: Similarity of the mollusc species in the different habitats. The distance of the dots reflects the relative similarity of species. How the two-dimensional graphic is in accordance with the real multidimensional structure in the data set is shown in the "2D-stress". A stress of "0" shows a perfect fit.

The molluscs from the *Posidonia* meadow were rather small species (a few mm in size). Among these were species found of the genus *Rissoa*, *Jujubinus exasperates* and *Rhinoclavis kochi*. These snails are small enough to live on the leaves of *Posidonia*. One beautiful representative of the *Posidonia* meadow is *Smaragdia viridis*, a bright green snail, but this year it has not been found.

As mentioned before, in the plankton there were very few molluscs; one exception is the group of the Thecosomata, also called sea butterflies, including the family Cavoliniidae of which we found *Creseis acicula* (Riedl 1983; Poppe & Yoshihiro 1991, 1993).

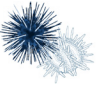
## Conclusion

All in all, we found fewer species than in the previous three courses. One of our main problems this year was the difficult weather conditions, which did not allow sampling every day and in every location we wanted. Also, a small group size led to the problem that not all species found could be identified at STARESO. We also did not have samples from scuba diving tours, which had led to a large increase in the numbers of Gastropoda identified in the course of 2010.

On the other hand, we found more species in the groups Polyplacophora and Scaphopoda than in any other year. In total, we found 15 species that had never been recorded for the Gulf of Revellata before. We were also able to take many of the unidentified species back to Innsbruck so that more species can be identified over time.

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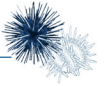
# Projects



## Sea Urchin Development

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# 1) Developmental Stages

Annika Fritschi and Leona Schulze

## General introduction

Echinoida (sea urchins) belong to the phylum Echinodermata. Around 950 sea urchin species are described worldwide. All Echinodermata belong to the Bilateria, which means that in some stage they still have bilateral symmetry. For sea urchins this is only true in the larval stage. Later in their development, after the metamorphosis phase, they show the typical pentamere radial symmetry. Regular sea urchins have a main axis from mouth to after (regularia) (fig.2), irregular sea urchins show a secondary bilateral symmetry (fig.1), also known as Irregularia (Westheide and Rieger, 2004).



Fig.1: Skeleton of an irregularia ([www.naturamediterraneo.com](http://www.naturamediterraneo.com))

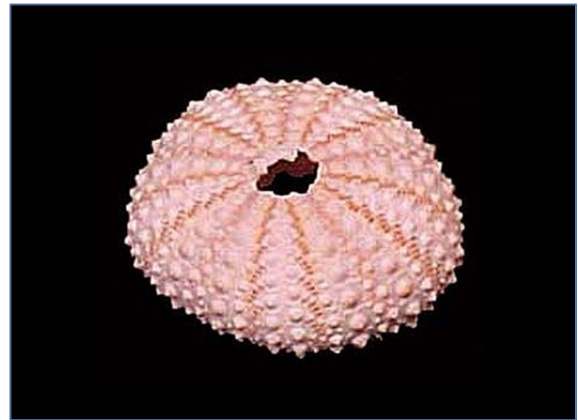


Fig.2: Skeleton of a regularia ([www.larcadinoe.com](http://www.larcadinoe.com))

Regularia can be found on hard bottom, like rocky shores, but also in coral reefs, sea grass meadows or within brown algae meadows. Their important ecological role within this habitats are that they serve as food for a lot organisms, for example some fishes, crustaceans, big starfishes, cephalopods, but they also provide hiding places between their spines for small fishes and other small organisms. Sea urchins normally feed on algae and by doing so they clean the substratum where the food algae are growing and hold their abundance in balance, which is very important for the surrounding ecosystem (Westheide and Rieger, 2004).

Sea urchins have been used for developmental studies for a long time. One reason for using them as preferred organism in this study field is that sea urchins are easy to obtain and they live on almost every sea coast. But also because they yield a large number of eggs and spawn rapidly (Hinegardner, 1969). An other specialty is that their eggs are transparent and allow scientists to follow all the development stages easily with a light microscope. Also the first test-tube fertilization was done with sea urchin eggs by Oscar Hertwig in 1887 (Westheide and Rieger, 2004).

Sea urchins have separate sexes and sexual dimorphism, if existing, is limited to different length of their genital papilla. The time of spawning is critical for free spawning organisms like sea urchins, because of the rapid delusion and longevity of gametes (Leviton and Petersen, 1995). Sperm can be vital for several hours, depending on the species, eggs can survive for several days in some species and under the right environmental conditions (Meidel and Yund, 2001/Yund and Meidel, 2003). Fertilization rates are strongly influenced by spawning behavior and

synchronal spawning might rise the success of fertilization (Lamare and Stewart, 1998). But also the environmental conditions play a major role for fertilization and larval survival rates. With the right conditions a higher probability for developing into the pelagic larvae is given (Gaudette et al, 2006/Yund and Meidel, 2003 ).

Some synchronal spawning were documented in association with environmental changes, like temperature changes (Lamare and Stewart, 1998) or lunar periodicity (Gaudette et al., 2006/Lamare and Stewart, 1998). Also the size and density of a population might play a role for mass spawning (Gaudette et al., 2006).

Fertilization is the fusion of a sperm and an egg (Munk, 2002). It is important that only one sperm fertilizes an egg. To avoid polyspermy several blockades come into effect. The moment the first sperm fuses with the egg, the egg depolarizes and its surface is rearranged by the cortical reaction. During this reaction the vitellin layer elevates and converts into the so called fertilization membrane (Guidice, 1973/Vacquier et al., 1972).

This fertilization membrane is later, in the blastula stage, dissolved again by a hatching enzyme, which is released from the embryo (Ishida, 1936; cited in Foerder and Shapiro, 1977). Another block for polyspermy is releasing of a trypsin like protease, which cleaves sperm receptors from the egg (Vacquier et al., 1972).

Sea urchins exhibit radial holoblastic cleavage (Gilbert, 2000), which means that the fertilized eggs cleave totally, and all new cells have the same size (Munk, 2002). The first two cleavages (2-cell stage and 4-cell stage) are meridional and perpendicular to each other (Gilbert, 2000). The third cleavage is equatorial and the blastomeres are in a radially symmetric order around a central axis going from the animal to the vegetal pole. This stage is called 8-cell stage (Munk, 2002).

With the next cleavage the cells at the animal pole divide differently from the cells at the vegetal pole. The cells at animal pole divide meridionally into eight equally sized blastomeres, so called mesomeres. The cells at the vegetative pole divide unequal into four larger cells, known as macromeres, and four smaller cells, known as micromeres. With this cleavage the 16-cell stage is reached (Gilbert, 2000).

By completing the next cleavage to the 32-cell stage two animal tiers are build by the mesomeres (an1 and an2), laying above each other. The macromere cell divide and form a eight cell tier below an2 before the micromere cells divide and produce a layer beneath the macromere tier. The cleavage to the 64-cell stage takes place by equatorial division followed by the meridional division of the seventh cleavage (Gilbert, 2000).

This seventh cleavage is also the point where the blastula starts to develop (blastula stage). The meanwhile equally sized cells form a hollow sphere surrounded by blastocoel. This blastocoel is in contact with every cell and completely encircled by a epithelial sheet built by united blastomeres (by tight junctions). The blastocoel expands by an influx of water while cell division continues (Gilbert, 2000).

After a few more cell divisions, the cells develop cilia on their outer surfaces. The blastula starts to rotate and begins to form a vegetal plate by thickening the cells at the vegetal pole. At the end of this stage (around 1000 cells), cells ingress into the blastocoel after dissociating from the epithelial monolayer. Due to the fact that they will later form the larval skeleton they are called skeletogenic mesenchyme. By fusing into syncytial cables later, they give the ground for axis forming of the larval skeletal spiculae (Gilberts, 2000).

The next developmental stage is called gastrula stage and the timing, when this stage is reached differs slightly in the references (Gilbert, 2000/Munk, 2002/Westheide and Rieger, 2004). According to Westheide and Rieger (2004) the gastrula stage starts with the differentiations of 60 macromere cells to the larval gut. The big macromere cells move as primary mesenchyme into the blastocoel. The smaller macromere cells divide slowly and eight of them become the appendix for the coelom system. The fate of each cell layer can be followed through its movement during gastrulation (Cameron et al., 1991/Gilberts, 2000). Building of the coelom starts at the end of gastrulation by chocking of the smaller micromere cells around the gut, while the mouth breaks through (Westheide and Rieger, 2004).

Due to the newly built mouth breaking through with the gut system, sea urchins belong to the group Deuterostomia (new mouth building organisms), which means that the old mouth will develop to an after (Munk, 2002). Shortly

before the new mouth breaks through, triaxial spikes are built and the early prism larvae stage is reached. Now the skeleton develops and the larvae develop into the pluteus stage (Munk, 2002). The moment the larvae reaches the pluteus stage, spicules are fully formed and the process of endoskeleton formation starts (Beniash et al, 1997).

After 4 to 6 weeks the larvae undergo metamorphosis and the planktonic larval stage ends. Within an hour, they become benthic juvenile sea urchins which grow to mature sea urchins (Westheide and Rieger, 2004).

The time through the different stages differs between species and can easily be followed in a light microscope (Beniash et al., 1997) (see results fig.8a-c).

## Introduction of the Organisms

### ***Arbacia lixula* - Black sea urchin -**

Classification:

Phylum: Echinodermata

Subphylum: Echinozoa

Class: Echinodidae

Subclass: Euechinoida

Infraclass: Carinacea

Superorder: Echinacea

Order: Arbacioida

Family: Arbaciidae ([www.marinespecies.org](http://www.marinespecies.org))



Fig.3: *Arbacia lixula* (Linnaeus 1758)

Description: Slightly depressed body with black spines and an orange, pinkish or brown skeleton (Fig. 3) and a diameter of about 5 cm. They feed on algal biofilm on rocks ([www.seadb.net](http://www.seadb.net)).

Habitat: This species lives on rocky shores and *Posidonia oceanica* meadows in shallow marine waters ([www.marinespecies.org](http://www.marinespecies.org)). In well enlightened areas they can live up to 50 m water depth ([www.seadb.net](http://www.seadb.net)).

Distribution: Mediterranean Sea, East Atlantic Ocean, Brazil Coast, North Africa, Morocco ([www.marinespecies.org](http://www.marinespecies.org)).

### ***Paracentrotus lividus* - Purple sea urchin -**

Classification:

Phylum: Echinodermata

Subphylum: Echinozoa

Class: Echinodidae

Subclass: Euechinoida

Infraclass: Carinacea

Superorder: Echinacea

Order: Arbacioida

Infraorder: Echinidea

Family: Parachenidae ([www.marinespecies.org](http://www.marinespecies.org))



Fig.4: *Paracentrotus lividus* (Lamarck 1816)

Description: The body reaches a size of around 7 cm diameter. The skeleton is normally greenish with purple spines (fig.4), sometimes they occur also with brownish or olive green spines ([www.habitas.org.uk](http://www.habitas.org.uk)). They feed on red, green or brown algae as well as on sea grass. This species is also known for their high sensitivity to salinity, organic pollution and heavy metals in the sea water (Lozano et al., 1995). In the Mediterranean Sea they are the most abundant echinoid species in sub littoral habitats down to 20 m depth (Verlaque, 1984 cited in Lozano et al., 1995). In some places like in the Mediterranean Sea and at the Irish Coast they are depleted by commercial harvesting. Their gonads are considered as a delicacy ([www.marinespecies.org](http://www.marinespecies.org)).

Habitat: This species occurs from sub-littoral up to 30 m depth, but only near shore and normally on stony ground ([www.marinespecies.org](http://www.marinespecies.org)), but they also occur in sea grass meadows and loose boulders. So their habitat changes from areas of hydrodynamic surrounding in the upper first meters down to more calmer waters in deeper zones or bays (Verlaque, 1984). Some of them dig holes with their mouth into soft rocks, till their body fit perfectly into their self made cavity (Lozano et al., 1995).

Distribution: Mediterranean Sea, European Waters, Canaries, South Coast of England and Ireland, West Coast of Scotland, Azores and Morocco ([www.marinespecies.org](http://www.marinespecies.org)).

### ***Sphaerechinus granularis* - Violet sea urchin -**

Classification:

Phylum: Echinodermata

Subphylum: Echinozoa

Class: Echinodidae

Subclass: Euechinoida

Infraclass: Carinacea

Superorder: Echinacea

Order: Arbacioida

Infraorder: Echinidea

Superfamily: Odontophora

Family: Toxopneustidae ([www.marinespecies.org](http://www.marinespecies.org))



Fig.5: *Sphaerechinus granularis* (Lamarck 1816)

Description: The color of their skeleton is purple and the spines are purple with sometimes white endings (fig.5). The body can reach a diameter of 15 cm and is sometimes covered by loose algae. This species also feeds on algae and is used for commercial harvesting for their gonads ([www.habitas.org.uk](http://www.habitas.org.uk)).

Habitat: They live in shallow waters up to 30 m water depth, mostly on rocky and gravel sand beds within algae ([www.habitas.org.uk](http://www.habitas.org.uk)).

Distribution: European Waters, North East Atlantik Mediterranean Sea, Cape Verde ([www.marinespecies.org](http://www.marinespecies.org)).

## Material and Methods

The three sea urchin species (*A. lixula*, *P. lividus*, *S. granularis*) were directly collected around the coast of the STARESO institute in Calvi. If it was possible, they were directly brought to the laboratory, sometimes, especially with the more rare or hard to find species *P. lividus* and *S. granularis*, they were shortly hold in sea water aquaria. To gain the gametes, a single organism was set on a glass filled with sea water and either stressed by injecting KCL (*A. lixula*) or in addition also stressed by centrifugation in a towel (*P. lividus*, *S. granularis*) (fig.6).

This processes were repeated, if necessary, until they released their eggs or sperm (fig.7).



The first found male of each species was directly cut open with a scissor, the gametes were removed with tweezers and put into an eppendorf tube and stored in the fridge. This way they could be used for more than one fertilization experiment if no further male was found.

Female sea urchins were left on the water glasses till they released their eggs. After a while, the eggs settled down to the bottom of the glass and the supernatant was carefully thrown away. Now the eggs were dispensed in petri dishes and sperms were add to them.



Fig.6: Stressing by injecting KCL (on the left) or by centrifugation in a towel (on the right)



Fig.7: Sperm releasing male of the species *S. granularis* (on the left) and egg releasing female of the species *A. lixula*(on the right)

Directly after addition of the sperm, the process of fertilization success was checked under a light microscope. If 100 % of the eggs were fertilized, the stages of 100 eggs were counted under the light microscope at different time points and noted in an excel-sheet. This was repeated until 100% of the embryos became pluteus larvae. Embryos between cleavages were not measured. The experiments were done separately few times with different eggs and sperms. Because the experiments took place over several days, the water in the petri dishes was renewed every 12 hours to provide a healthy environment for the embryos.

**Table 1:** Definition of the stages

Stage	Characteristica
Fertilized egg	Fertilization membrane
2-cell	2 same sized cells, completely divided
4-cell	4 same sized cells, completely divided
8-cell	8 cells
16-cell	16 cells
32-cell	> 16 cells, but without blastula
Blastula	Blastula could be seen, embryo starts moving
Gastrula	Gut starts to develop
Prism	3 angled pluteus larvae without feet
Pluteus	The feet start to develop

## Results

Part 1 of the report will concentrate on the time, which is needed by 50% of fertilized sea urchin eggs from different species (*A. lixula*, *P. lividus* and *S. granularis*) to accomplish each development stage till pluteus larva.

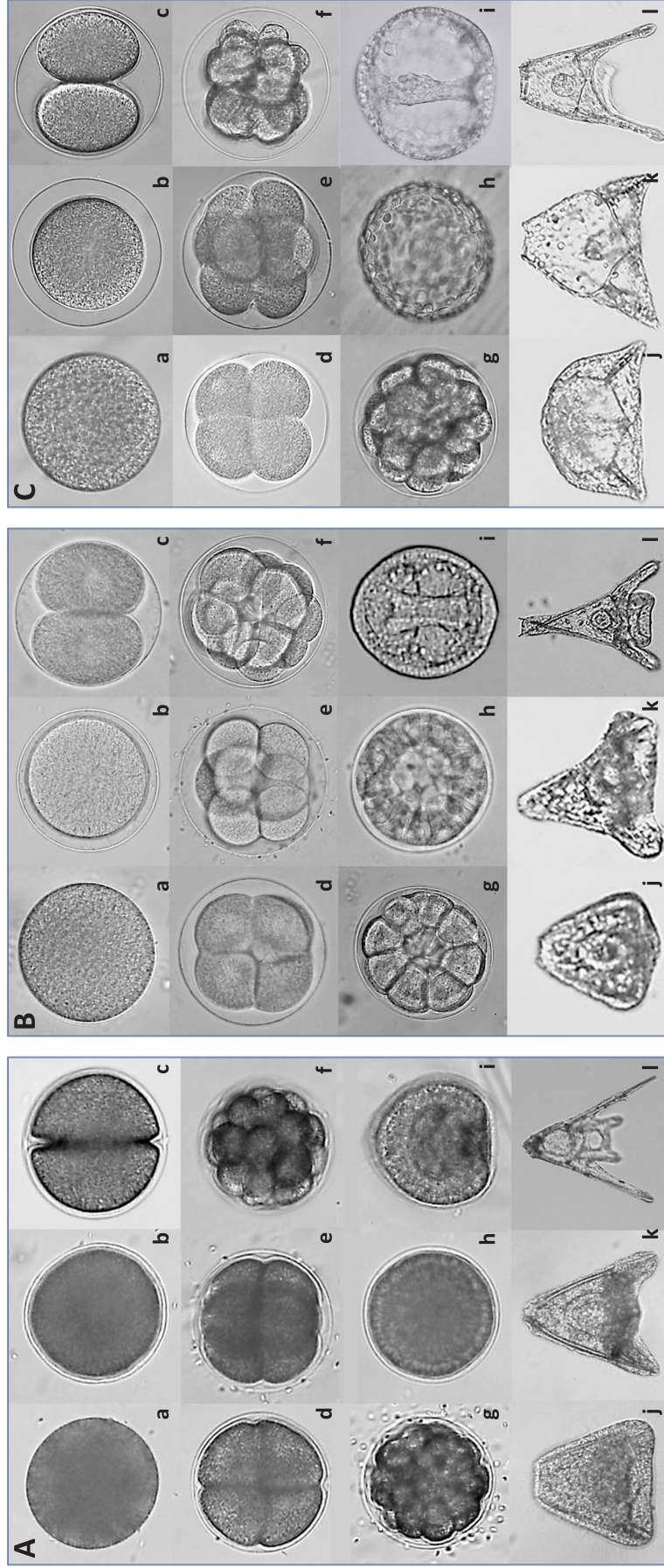


Fig.8: Developmental stages of *Arbacia lixula* (A), *Paracentrotus lividus* (B) and *Sphaerechinus granularis* (C).

- a) unfertilized egg;
- b) fertilized egg;
- c) 2-cell stage;
- d) 4-cell stage;
- e) 8-cell stage;
- f) 16-cell stage;
- g) 32-cell stage/early blastula stage;
- h) Blastula;
- i) Gastrula;
- j) Prism larvae;
- k) Early pluteus larvae;
- l) Late pluteus larvae

*Arbacia lixula*

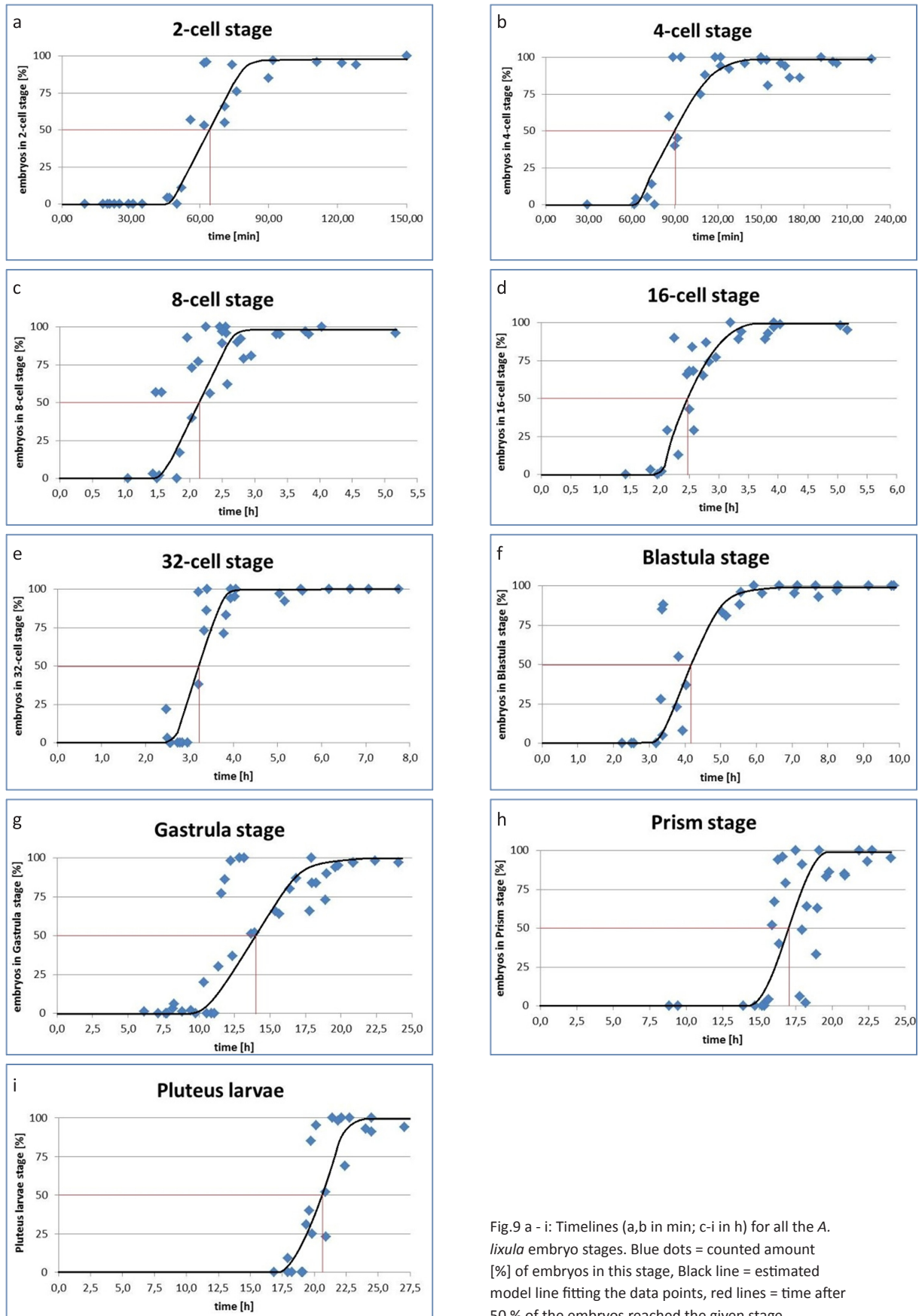


Fig.9 a - i: Timelines (a,b in min; c-i in h) for all the *A. lixula* embryo stages. Blue dots = counted amount [%] of embryos in this stage, Black line = estimated model line fitting the data points, red lines = time after 50 % of the embryos reached the given stage

*Paracentrotus lividus*

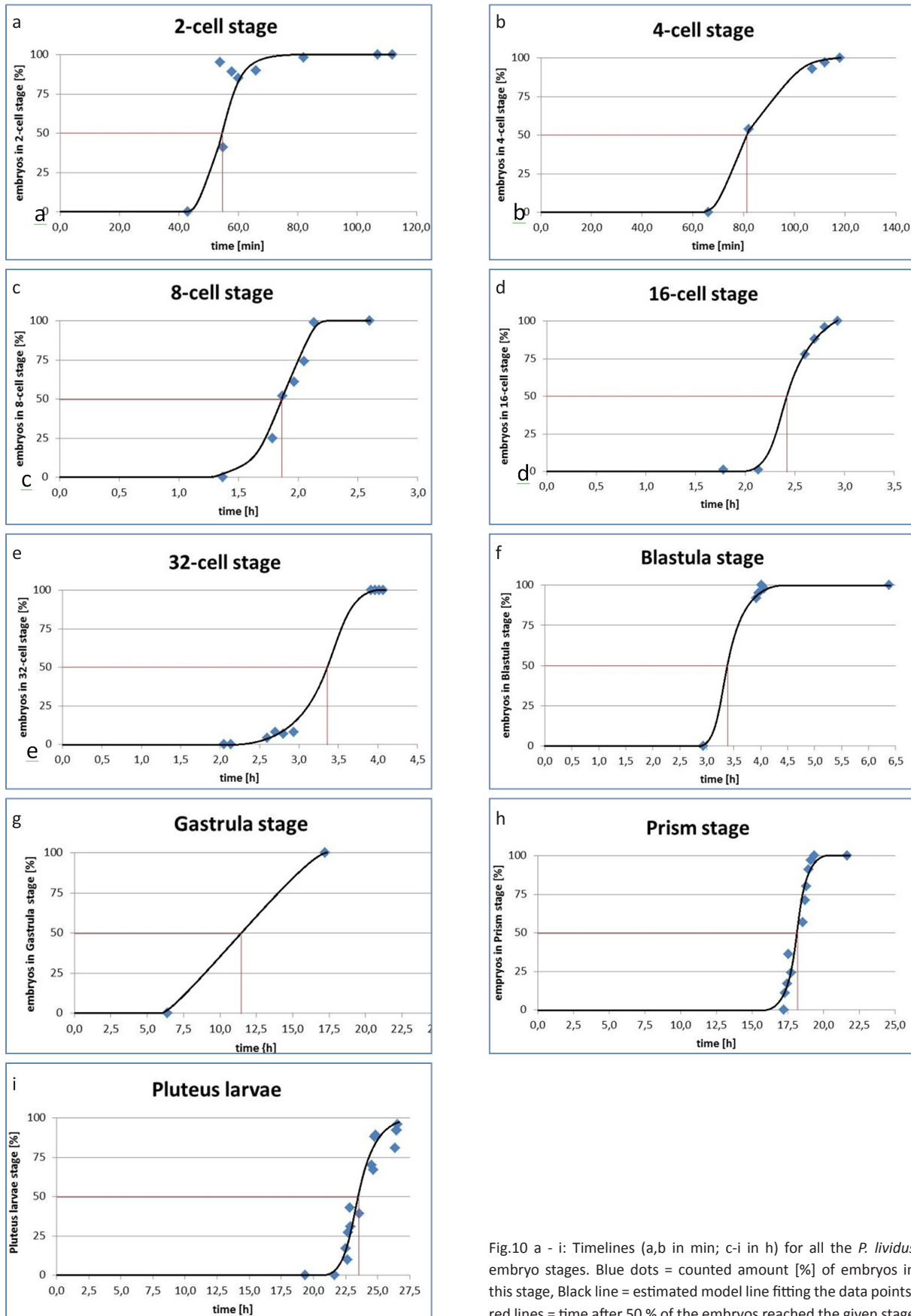


Fig.10 a - i: Timelines (a,b in min; c-i in h) for all the *P. lividus* embryo stages. Blue dots = counted amount [%] of embryos in this stage, Black line = estimated model line fitting the data points, red lines = time after 50 % of the embryos reached the given stage

*Sphaerechinus granularis*

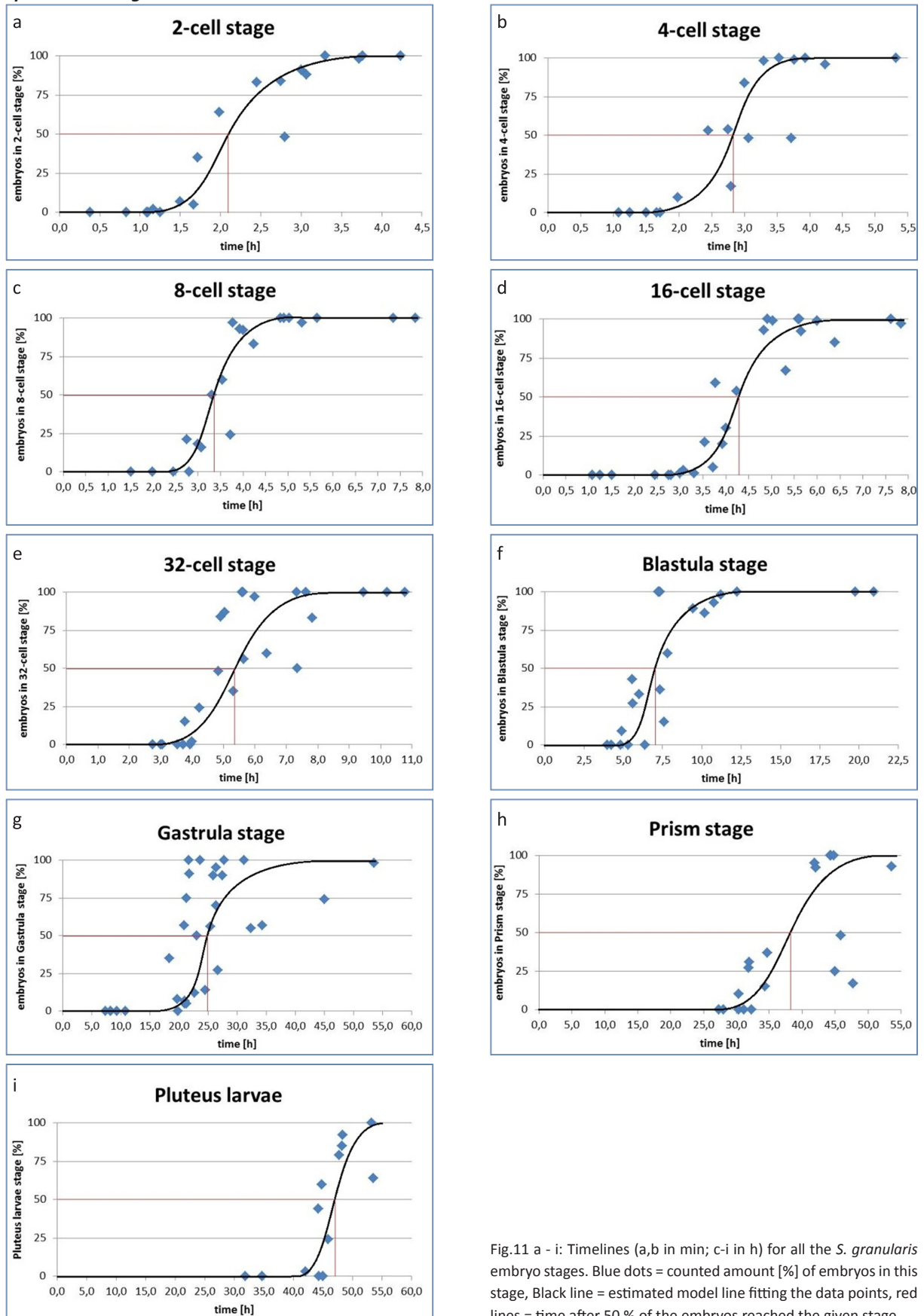


Fig.11 a - i: Timelines (a,b in min; c-i in h) for all the *S. granularis* embryo stages. Blue dots = counted amount [%] of embryos in this stage, Black line = estimated model line fitting the data points, red lines = time after 50 % of the embryos reached the given stage

## Discussion

The figures 9 to 11 show the different time tables for each embryonic stage of all three species of the experiment. To summarize the time after which 50 % of the counted embryos have reached each stage, figures 12 to 14 were produced.

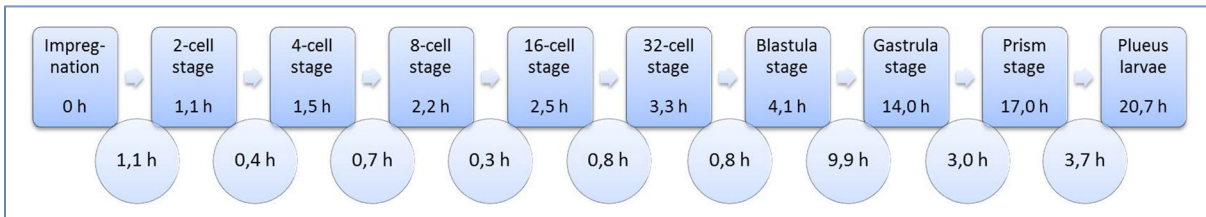


Fig.12: Time between the single stages for *A. lixula*

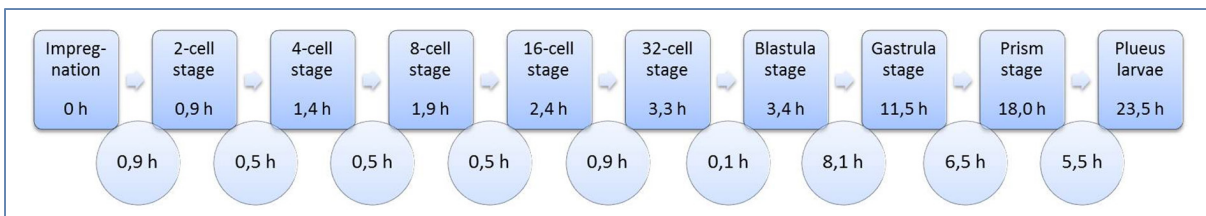


Fig.13: Time between the single stages for *P. lividus*

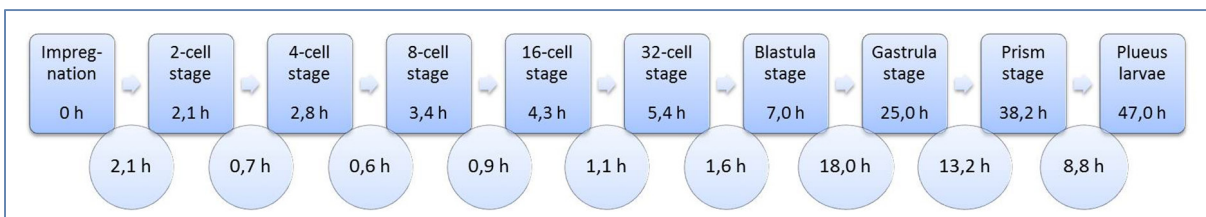
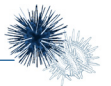


Fig.14: Time between the single stages for *S. granularis*

Comparing the times between the stages of the three species, the figures 12 to 14 show that *Arbacia lixula* and *Paracentrotus lividus* are more similar as compared with *Sphaerechinus granularis*. This might be due to the fact that we handled them all the same way, ignoring that *S. granularis* is coming from a slightly different habitat. This species is normally living in deeper water than the other two, so they are used to colder water temperature. For the experiment, the embryos were in petri dishes with room tempered water, which is more like the water temperature near to the surface of the Mediterranean.

Figures 12 to 14 also show that the time gets longer between the different stages the more complex the embryo gets, except for the first cleavage from the fertilized egg to the 2-cell stage embryo. After this cleavage the longest time step is between the blastula and gastrula stage, with 9,9 hours (*A. lixula*), 8,1 hours (*P. lividus*) and 18,0 hours (*S. granularis*). This long time step could be explained by all the cell differentiation and cleavage steps within this stage (Gilbert, 2000), but also by the fact that it was hard to distinguish the early gastrula stage. Also that they started to move around really fast under the microscope was a challenge to distinguish between late blastula and early gastrula stages.



In total *A. lixula* needed 20,7 hours, *P. lividus* 23,5 hours and *S. granularis* 47,0 hours till 50 % of the embryos reached the pluteus larvae stage. This shows again that *A. lixula* and *P. lividus* are closer to each other than both of them to *S. granularis*.

Comparing our results with the results from the group 2010 (shown in tab.2), it seems that our results for *A. lixula* and *P. lividus* are close to the results of the group before. This year was the first year *S. granularis* was fertilized and monitored throughout all the embryo stages with several parallels. In the year 2010, this experiment was also done, but in smaller scales and not till the pluteus larvae stage, so there are no results we can compare to.

The results in both years for *P. lividus* are similar. Interesting is, that for *A. lixula* there are two differences between last year and this year. One is that the time from 16-cell stage to blastula stage needs twice as long in our experiment, but only half of the time from gastrula stage to prism stage. But big differences exist also in the comparing the time of *S. granularis*. In 2010 *S. granularis* needed 9,2 h for the cleavage from 4-cell stage to 8-cell stage. Our experiment gave us a time step of 0,6 hours. This might be due to the fact that we had more time and parallels than the group in 2010. In total it is questionable in how far the results of 2010 for this species are reliable, because also the 0,5 hour time step from 16-cell stage to blastula stage is really short. In total we would suggest that the results for the species *S. granularis* can not be compared and should be handled separately.

**Table 2:** Time steps [h] for every cleavage, missing the 32-cell stage (for comparing with the data from 2010)

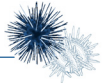
Cleavage step	<i>A. lixula</i>	<i>P. lividus</i>	<i>S. granularis</i>
fertilized egg to 2-cell	1,1	0,9	2,1
	0,8	0,9	1,2
2-cell to 4-cell	0,4	0,5	0,7
	0,5	0,5	0,4
4-cell to 8-cell	0,7	0,5	0,6
	0,8	1,4	9,2
8-cell to 16-cell	0,3	0,5	0,9
	0,5	0,6	1,0
16 cell to blastula stage	1,6	1,0	2,7
	0,7	0,9	0,5
blastula stage to gastrula stage	9,9	8,1	18,0
	9,4	7,6	15,8
Gastrula stage to prism larvae	3,0	6,5	13,2
	6,1	6,0	
Prism larvae to pluteus larvae	3,7	5,5	8,8
	3,7	5,5	

\*white = our results, light grey = results from 2010, dark grey = missing results, simplified to only 1 decimal place

It should be mentioned that counting larval stages was only done to the stages we could definitely identify, so embryos we were not sure to which stage they belong, we just ignored, which also might influence our results. Another problem were insufficient numbers of data points, as shown in fig.10g. Here only two countings took place. This lack of data points is probably due to no counting over night time.

To take this data seriously, it is important to keep in mind that not all the experiments within one species were done with the eggs and sperms from the same organisms. This can lead to a stronger variation between the single data points (see fig.9f-h), because the fitness of the “parents” play an important role for the fitness of their eggs and sperms. But also the lab conditions play an important role in such experiments. Small changes in water temperature can already stress or comfort some embryos. The petridishes were all hold in the same lab, but some were probably stored in a cooler place than others, so there is no guarantee that the terms were the same for all experiments, which also causes a higher variability in needed time for cleavage.





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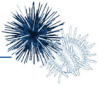
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### Figure References

- Figure 1: [http://www.naturamediterraneo.com/forum/topic.asp?TOPIC\\_ID=79753](http://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=79753)
- Figure 2: <http://www.larcadinoe.com/scheda/Regularia%20%28round%20sea-urchins%29/Echinometra+mathaei/11301>





## 2) Cross species fertilization

Nadia Parth and Marina Wanner

### Introduction

The aim of the experiment was to cross the three not very closely related sea urchin species that appear at Corsica (*Arbacia lixula*, *Sphaerechinus granularis* and *Paracentrotus lividus*).

#### The course of fertilization

Before the sperm can fertilize the egg, it has to pass several barriers to enter the egg. To be able to fuse with the plasma membrane the sperm must get successfully through the jelly layer and the vitelline membrane, which surrounds the egg (see fig.1) (Wolpert, 2011: 342-348).

The fusion between sperm and jelly layer releases the contents of acrosomal vesicles, which are located on the surface of the sperm head, whereupon the sperm penetrates the jelly layer. Afterwards the sperm adheres to the surface of the vitelline membrane (Gilbert, 2012: 128-130). Binding receptors on the surface of the vitelline envelope are typical for each sea urchin species and prevent that sperms of other species can fertilize the egg. Finally, a hole is lysed into the vitelline membrane by enzymes and the sperm advances to the plasma membrane (see fig.2).

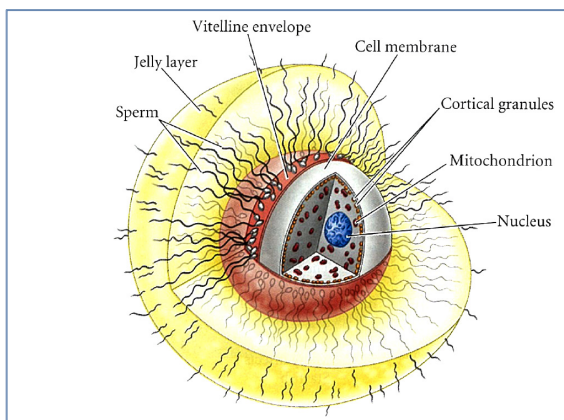


Fig.1: Structure of a sea urchin egg at fertilization (Gilbert, 2012: 125)

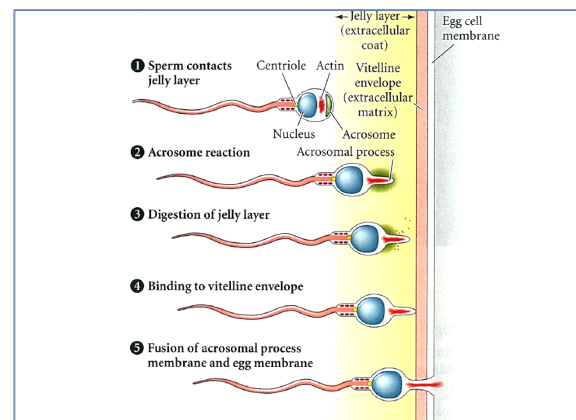


Fig.2: Summary of events leading to the fusion of egg and sperm cell membranes in the sea urchin (Gilbert, 2012: 128)

After the fusion of sperm and plasma membrane two mechanisms protect the egg from polyspermy (Gilbert, 2012: 132).

#### 1. The fast block to polyspermy

As soon as the sperm enters the egg the plasma membrane depolarizes for a minute and prohibits in his time that more than one sperm penetrates the egg. Until now it is not exactly known, how the change in the membrane potential prevents the entrance to a second sperm (Gilbert, 2012: 135-136).

#### 2. The slow block to polyspermy

After the sperm binds on the surface of the plasma membrane, a calcium wave disperses throughout the egg that causes the fusion of the cortical granules with the plasma membrane and the release of

their contents (several enzymes) between the plasma membrane and the vitelline membrane. One of the enzymes is a trypsin-like protease called cortical granule serine protease, which clips off the binding receptors on the surface of the vitelline membrane and releases the vitelline membrane from the cell membrane by cleaving of vitelline envelope proteins.

The vitelline membrane converts into the fertilization membrane and constitutes a sustained protection against polyspermy. This slow block to polyspermy uses to last for about 30 minutes to an hour after fertilization (Wolpert, 2011: 342-348; Gilbert, 2012: 136; Campbell, 2006: 1198-2202).

The protection against polyspermy is very important for the correct development of organisms. In most of the organisms the entrance of more sperms results in disastrous consequences. Theodor Boveri (1902) demonstrated that the fertilization by sea urchin with two sperms leads the cell to die or to develop abnormally (Gilbert, 2012: 135).

## Material and Methods

To avoid the embryonal blockade that prevents under normal circumstances the fertilization with sperm from other species, the eggs were treated with trypsin to destroy proteins on the vitelline membrane (see also above "The slow block to polyspermy").

The right trypsin concentration and the length of the treatment were very important for the experiments. A too high enzyme concentration or a too long impact time would damage the eggs, – and as a result a high number of them would die, – and a too low concentration or a too short impact time wouldn't clip off the binding receptors on the vitelline membrane. The fertilization with dissimilar sperm would be possible but the risk of polyspermy would be high. So the best concentration of enzyme was detected by conducting different trypsin concentration series. After successful fertilization with a high concentration of sperm, the egg should regularly enter the cleavage stages.

Trypsin concentration series:

- 0,2g/l seawater
- 0,4g/l seawater
- 0,8g/l seawater
- 1,2g/l seawater
- 2,0g/l seawater

High concentrations of *Arbacia lixula* eggs were treated for 15 min and for 30 min in petri dishes with different trypsin concentrations (see above). Afterwards they were washed three times with fresh sea water. For this, the petri dishes were rotated until all eggs were centered in the middle and could be collected easily with a plastic pipette. Then they were transferred into a new petri dish with fresh seawater. After the washing the eggs were fertilized with *Arbacia lixula* sperm. Fertilizations were followed under the microscope and were recorded. The destroyed, unfertilized and fertilized eggs were counted 1h and 18h after the fertilization.

The best results were obtained at a concentration of 1,2g/l trypsin in sea water and a treatment time of 15 minutes (97% of the eggs were fertilized, 3% unfertilized and 0% destroyed after 1h). This concentration was therefore used for the cross species experiment.

The fertilization series were conducted with fresh eggs and sperms of *Arbacia lixula*, *Sphaerechinus granularis* and *Paracentrotus lividus* and all following combinations were done:

- Arbacia lixula* (♀) x *Sphaerechinus granularis* (♂)
- Arbacia lixula* (♀) x *Paracentrotus lividus* (♂)
- Sphaerechinus granularis* (♀) x *Arbacia lixula* (♂)
- Sphaerechinus granularis* (♀) x *Paracentrotus lividus* (♂)
- Paracentrotus lividus* (♀) x *Arbacia lixula* (♂)
- Paracentrotus lividus* (♀) x *Sphaerechinus granularis* (♂)

Development of the cross species was followed and recorded after 3h, 5h and 20h.

## Results and Discussion

In figure 3 big differences between the various combinations of species are visible. We observed that the fertilization efficiency varied a lot and depended on the combination of the different species.

### *Arbacia lixula* (♀) x *Sphaerechinus granularis* (♂)

3 hours after fertilization (see fig. 3):

- Unfertilized: 16%
- Fertilized irregular: 54%
- Fertilized regular: 30%

After 20 hours, most of the embryos reached the gastrula stage.

### *Arbacia lixula* (♀) x *Paracentrotus lividus* (♂)

3 hours after fertilization (see fig. 3):

- Dead: 18%
- Unfertilized: 81%
- Fertilized irregular: 1%

After 20 hours of observation all of the cells were dead. Many eggs were not fertilized and an inefficient development was recognized.

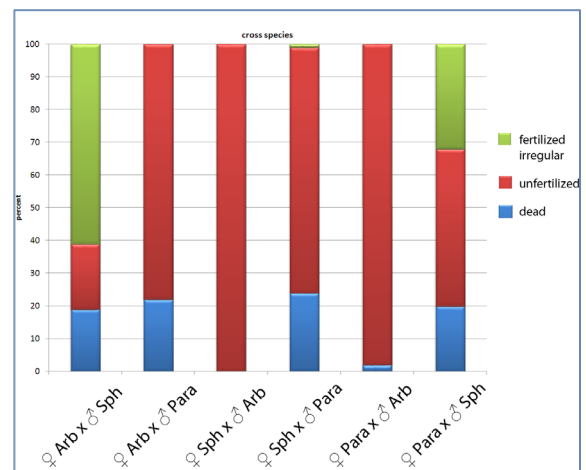


Fig.3: Development states 3h after the fertilization for all species combinations.

Arb= *Arbacia lixula*

Sph= *Sphaerechinus granularis*

Para= *Paracentrotus lividus*



Fig.4: The cross species fertilization of *Arbacia lixula* (♀) and *Sphaerechinus granularis* (♂) (A) showed unusually long cilia in comparison to a normal blastula stage of *Sphaerechinus granularis* (B). Other examples of irregular development stages at 3 hours after fertilization (C).

*Sphaerechinus granularis* (♀) x *Arbacia lixula* (♂)

3 hours after fertilization (see fig. 3):

Dead: 11%  
 Unfertilized: 87%  
 Fertilized irregular: 2%

After 20 hours of observation all of the cells were dead.

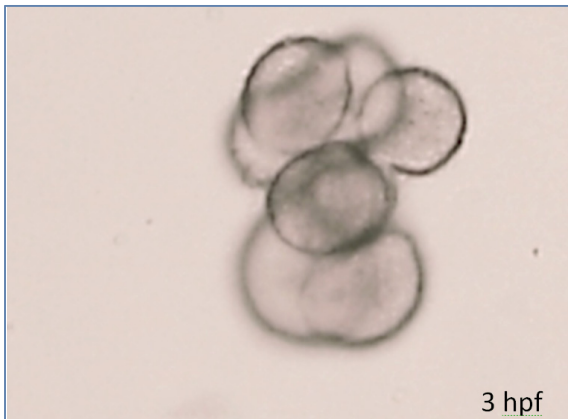


Fig.6: 8-cell stage of *Sphaerechinus granularis* (♀) and *Paracentrotus lividus* (♂) with blastomeres with an irregular configuration at 3 hours after fertilization (A).

*Paracentrotus lividus* (♀) x *Arbacia lixula* (♂)

3 hours after fertilization (see fig. 3):

Dead: 4%  
 Unfertilized: 96%

After 20 hours of observation all of the cells were dead.

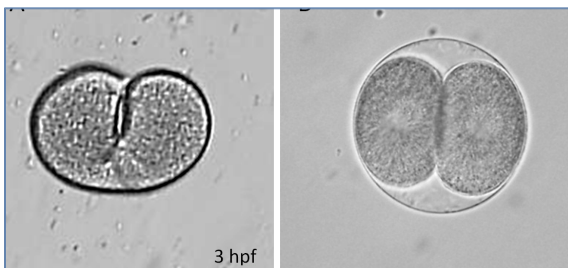


Fig.8: 2-cell stages of a cross species fertilization of *Paracentrotus lividus* (♀) and *Sphaerechinus granularis* (♂) (A), and a normal 2-cell stage by *Paracentrotus lividus* (B). During the cleavage it was observed an irregular development by *Paracentrotus lividus* (♀) and *Sphaerechinus granularis* (♂) (A).

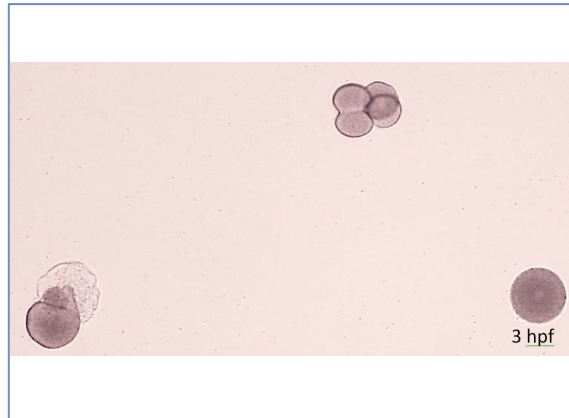


Fig.5: Overview of *Sphaerechinus granularis* (♀) and *Arbacia lixula* (♂) at 3 hours after fertilization (A). One egg is unfertilized (right, down), one is dead (left, down) and one has blastomeres (up) that are positioned abnormally in the four-cell stage.

*Sphaerechinus granularis* (♀) x *Paracentrotus lividus* (♂) 3 hours after fertilization (see fig. 3):

Dead: 43%  
 Unfertilized: 55%  
 Fertilized irregular: 2%

After 20 hours of observation none of the cells remained alive.



Fig.7: The fertilization did not work for *Paracentrotus lividus* (♀) and *Arbacia lixula* (♂). Here you see a dead embryo at 3 hours after fertilization (A).

*Paracentrotus lividus* (♀) x *Sphaerechinus granularis* (♂) 3 hours after fertilization (see fig. 3):

Dead: 7%  
 Unfertilized: 81%  
 Fertilized irregular: 10%  
 Fertilized regular: 2%

After 20 hours of observation some embryos reached the blastula stage.

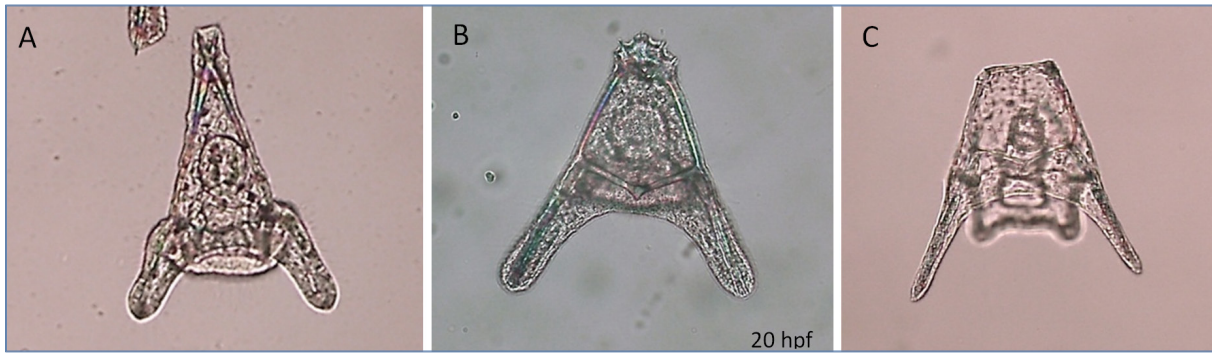
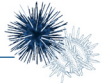


Fig.9 Some of the cross species fertilizations of *Paracentrotus lividus* (♀) and *Sphaerechinus granularis* (♂) developed into pluteus larvae as shown in B. To compare this pluteus larva with wildtype pluteus larvae, you see an elongated *Paracentrotus lividus* pluteus larva (A) and a cubical *Sphaerechinus granularis* pluteus larva (C).

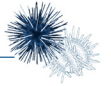
The most successful combination was the fertilization of female gametes of *Arbacia lixula* with sperm of *Sphaerechinus granularis* (more than 80% of the eggs were fertilized) (see also fig. 3). The other cross combinations were more or less inefficient. After 5 hours almost no regular development stage could be observed. Most of the fertilized eggs had stopped their development or developed irregularly like some blastomeres which are no longer able to arrange in a row, or blastomeres which stopped-cleavage, whereas others continue their development. During the time of observation (24 hours) all of the cells died before they reached the pluteus larvae stage. In addition to our experiments, which are shown in this protocol, another member of the group repeated the experiments under the same conditions (trypsin-concentration, length of the treatment, etc.). The pluteus larva from the cross species fertilization of *Paracentrotus lividus* (♀) and *Sphaerechinus granularis* (♂) as shown in figure 9B is a result from these repeat-tests. In this experiment, the hybrid embryo achieved the pluteus larva stage, despite the fact that in our tests the same cells stopped development at the blastula stage. Too long or too short treatment with trypsin or a frouzy, imprecise care are some of the possibilities, why the results show huge differences. The death of many eggs and the different fertilization results (see fig. 3) can also be a result of polyspermy, too high sperm concentration or a deficient maintenance.

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## 3) Manipulation of Vegetal-animal axis

Thomas Pomberger, Rene Mähr

### Introduction

During the first three rounds of cell divisions, all cells divide identically. With the start of the fourth cell division, cells begin to divide asymmetrically and form two hemispheres on the blastula embryo; the animal and the vegetal pole.

With the entry into gastrulation the embryo starts to invaginate on the vegetal pole, which makes it possible to visually recognize the primary body axis of the embryo for the first time.

The determination of the body axis starts much earlier in development. Actually it starts with the fourth cell division, where due to the asymmetrical division, maternal determinants get distributed into the daughter cell in an unbalanced way. This unbalanced distribution of maternal determinants is essential for the determination of cell fate. Maternal determinants can work as activators or inhibitors to intracellular signaling-pathways and so irreversibly determine cell fate.

There is a vast diversity of pharmacological substances, which are able to interact with those sorts of intracellular signaling-pathways or their regulation. As a result of those interactions, embryo development and the phenotype as well can be altered.

With knowledge of the mode of action of such pharmacological substances it is possible to design experiments able to examine the influence of a particular signalling pathway on certain developmental steps.

We conducted experiments with three different substances knowing to interfere with the establishment of the animal-vegetal-axis, Lithiumchlorid (LiCl), Alsterpaullone (Alp) and Valporic acid (VPA) plus combinations of LiCl-VPA and Alp-VPA on embryos of two different species, *Arbacia lixula*, *Paracentrotus lividus*.

**LiCl:** Lithiumchlorid ions inhibit GSK3 $\beta$  (Glykogen Synthase Kinase 3  $\beta$ ). This Kinase phosphorylates in the “off-state” of the Wnt-signaling pathway the transcription factor  $\beta$ -Catenin. This leads to the degradation of  $\beta$ -Catenin and therefore to no expression of target genes. During the “on-state” of the Wnt-signaling pathway GSK3 $\beta$  is inhibited, in consequence  $\beta$ -Catenin is stabilized and the target genes are expressed. LiCl therefore leads to an expression of Wnt-signaling target genes. These genes are supposed to influence the development of the vegetal pole of an embryo. As a result, treating the embryos with LiCl it should lead to a reduction of the animal pole and an enhancement of the vegetal pole.

**Alp:** Alsterpaullone also has an inhibiting effect on GSK3 $\beta$  and CDKs (Cyclin dependent Kinasis). It acts as competitive inhibitor by binding at the ATP position of GSK3 $\beta$  and therefore inhibits the transfer of phosphate from ATP to an amino acid residue. This should also lead to an enlargement of the vegetal pole of the embryos.

**Valp:** Valporic acid is believed to affect the function of the neurotransmitter GABA in the human brain, making it an alternative to lithium salts in treatment of bipolar disorder. Its mechanism of action includes enhanced neurotransmission of GABA (by inhibiting GABA transaminase, then GABA would increase in concentration). Valporic acid also blocks the voltage-gated sodium channels and T-type calcium channels. These mechanisms make valporic acid a broad-spectrum anticonvulsant drug. Valporic acid is an inhibitor of the enzyme histone deacetylase 1 (HDAC1). Inhibition of HDAC1 leads to a disturbance of epigenetic gene modification.

## Material and Methods

The fertilized eggs were able to develop normally until a point between the 8-cell and 16-cell stages in untreated seawater. Then, the embryos were transferred into petri dishes with seawater and concentrations of the substances as indicated for six hours. The different substances and concentrations are listed in the following table:

**Table 1:** concentrations of substances

Substances	LiCl	Alp	Valp	Valp-LiCl		Valp-Alp	
				Valp	LiCl	Valp	Alp
	50 mM	0,5 $\mu$ M	5 mM	5 mM	50 mM	5,0 mM	0,5 $\mu$ M
		1,0 $\mu$ M	25 mM	25 mM	50 mM	12,5 mM	0,5 $\mu$ M
		5,0 $\mu$ M	50 mM	50 mM	50 mM	25,0 mM	0,5 $\mu$ M

After the treatment, the embryos were washed by putting them with a pipette into a new petri dish. Afterwards fresh seawater was given on them. This procedure was done three times consecutively. Further development was observed and the blastula, gastrula, prism and pluteus stages were documented.

### LiCl induced vegetalisation and generation of an Exogastrula

Figure 1A shows an *Arbacia lixula* embryo treated with 50 mM LiCl, 24 hpf. Figure 1B shows a wildtype embryo after 14 hpf in the gastrulation. Due to the enhanced vegetalisation of the embryo a characteristic exogastrula can be observed. The treatment with 50 mM LiCl decelerates the development strongly and the embryos go into gastrulation respectively exogastrulation after 20 to 24 hpf. There are no results for *Paracentrotus lividus*.

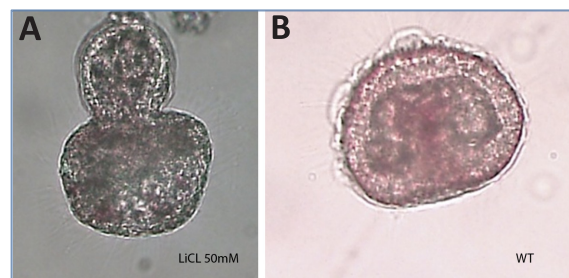


Fig.1: (A) Exogastrula of *Arbacia lixula* at 50 mM LiCl. (B) WT Gastrula of *Arbacia lixula*

### Alp induced reduction of the animal pole

*Arbacia lixula* embryos were treated with three different concentrations of Alp, 0,5  $\mu$ M, 1  $\mu$ M and 5  $\mu$ M. The embryos showed a slight deceleration of development when treated with 0,5  $\mu$ M Alp, whereas on the treatment with 1  $\mu$ M and especially 5  $\mu$ M they responded with a decelerated development and a reduced animal pole. The deceleration seems to be caused by the interaction of Alp with certain CDKs. Figure 2B shows an embryo in the prism-stage, where due to the reduced animal pole the apex is flattened in comparison to the wildtype (fig.2C). Similar to the treatment with LiCl, exogastrulae can be observed (fig.2A).

At an amount of 0,5  $\mu$ l of Alsterpaullone the *Paracentrotus lividus* embryos don't show any transformation of the vegetal pole at the late blastula and gastrula stage. However, the pluteus larvae show an explicit deformation (fig.3A). The red arrows point at the two appendices which aren't able to found on the wildtype pluteus (fig.3B). 1  $\mu$ M of Alsterpaullone affected in an exogastrula at the beginning of the gastrula stage. Figure 4A shows an embryo at the beginning of the gastrula stage where the vegetal pole begins to develop outside. In the middle of

the gastrula stage, the vegetal pole is clearly separated from the animal pole – the embryo isn't able to develop further (fig.4B). Figure 4C shows a gastrula stage of a wildtype of *Paracentrotus lividus*. At an amount of 5  $\mu$ M of Alsterpaullone the embryos aren't able to develop over the blastula stage. They begin to disaggregate (fig.3C).

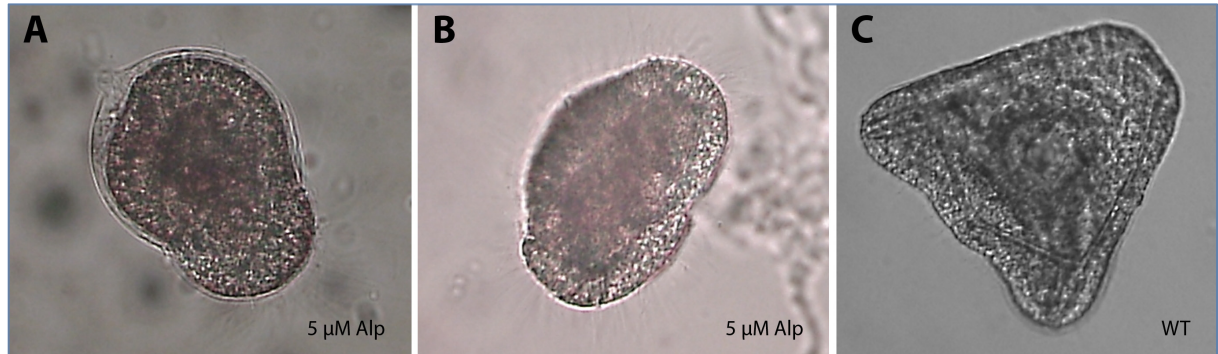


Fig.2: (A) 5  $\mu$ M Alp 18 hpf exogastrula of *Arbacia lixula*. (B) 5  $\mu$ M Alp 18 hpf early Prism-Stage of *Arbacia lixula*. (C) Prism-Stage wildtype of *Arbacia lixula*

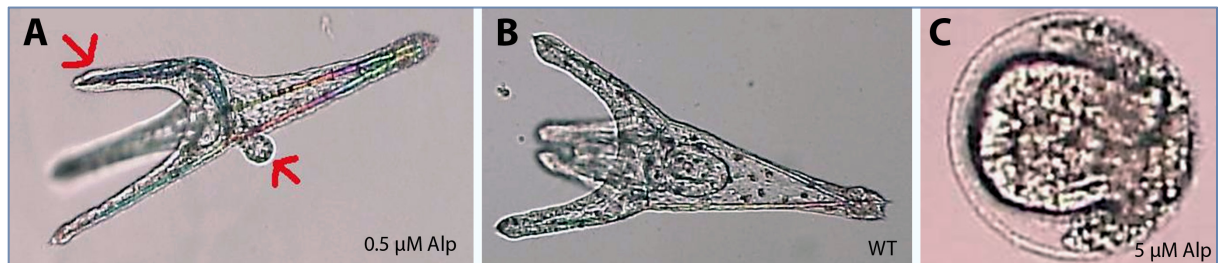


Fig.3: (A) Pluteus larvae of *Paracentrotus lividus* after treatment with 0,5  $\mu$ l Alp with only one apical arm; the red arrows mark the deformed parts. (B) Pluteus larvae of *Paracentrotus lividus* wildtype. (C) Disaggregation of a beginning gastrula 5  $\mu$ M of *Paracentrotus lividus*.



Fig.4: (A) Gastrula of *Paracentrotus lividus* after treatment with 1  $\mu$ l Alp; the red arrow marks the beginning exogastrula. (B) Gastrula of *Paracentrotus lividus* after treatment with 1  $\mu$ l Alp. (C) Gastrula of *Paracentrotus lividus* wildtype

### VPA showed primarily toxic effects

The treatment with 5 mM Valporic acid seems to have no impact on the speed of development or the animal-vegetal-axis of *Arbacia lixula* (fig.5A). Higher concentrations of 25 mM lead to ensuing disaggregation (fig.5B). At a concentration of 50 mM the development stopped (fig.5C). There are no results for *Paracentrotus lividus*.



Fig.5: (A) Valp 5mM Prism-Stage of *Arbacia lixula*. (B) Valp 25 mM Exogastrula of *Arbacia lixula*. (C) Valp 50 mM of of *Arbacia lixula*.

### Double treatment of ALP and Valp

The counter acting effects of Alp and Valp don't neutralize their mode of actions neither at *Arbacia lixula* nor at *Paracentrotus lividus*. Exogastrulae can be observed and an increase of sensitivity for Valp due to the treatment with Alp (fig. 6A and 6C). At higher concentrations than 5 mM of Valp, the embryo stops to develop and disaggregates before gastrulation (fig.6B).



Fig.6: (A) Exogastrula of *Arbacia lixula* at 0,5 μM Alp and 5 mM Valp. (B) Disaggregation of *Arbacia lixula* at 0,5 μM Alp and 25 mM of Valp before gastrula. (C) Beginning exogastrula of *Paracentrotus lividus* at 0,5 μM Alp and 5 mM Valp; the arrow points at the animal pole

### Double treatment of LiCl and Valp

Double treatment with LiCl and Valp shows similar results as the one with Alp and Valp at *Arbacia lixula*. There are no results for *Paracentrotus lividus*. The amount of 5 mM of Valp doesn't neutralize the effect of LiCl therefore it comes to an exogastrula (fig.7A). At a concentration of 25 mM of Valp, exogastrulae can still be observed although the vegetal pole seems to disaggregate (fig.7B). Higher concentrations of Valp lead also to a stop of development at the blastula stage (fig.7C).

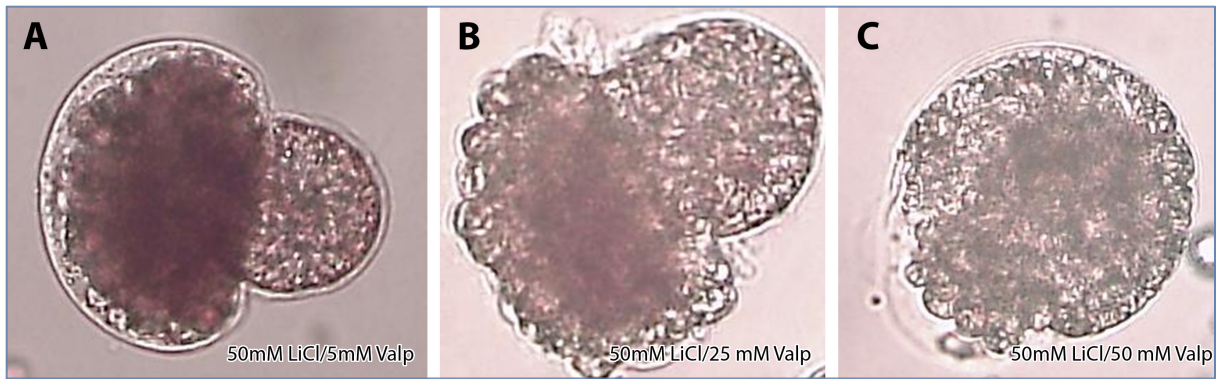
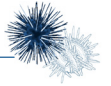


Fig.7: (A) Exogastrula of *Arbacia lixula* at 50 mM LiCl and 5 mM Valp. (B) Exogastrula of *Arbacia lixula* at 50 mM LiCl and 25 mM Valp. (C) Disaggregation of *Arbacia lixula* at 50 mM LiCl and 50 mM Valp

## Discussion

This year's experiments with LiCl show a similar deceleration of development as it was observed in the years before. Exogastrulae could also be observed. Due to the inhibiting influence of LiCl and Alp on GSK3 $\beta$ , the transcription factor  $\beta$ -Catenin is stabilised, which leads to an increased vegetal pole. This phenomenon seems to have its origin in the fact, that LiCl increases the vegetal area on costs of the animal area, so there is no place left for the embryo on the animal area to invaginate correctly and it comes to an exogastrula.

Experiments with Alp showed the expected deceleration of development. This deceleration could be caused by the interaction of Alp with CDK's, as it was already assumed in the report of 2010. More difficult is to explain the flattening of the apex of the prism-stage. Most likely this is simply caused by the increase of the vegetal area on cost of the animal area. In contrast to the data of 2010, we examined alterations of the phenotype by 0.5  $\mu$ M Alp. Concentration higher than 1  $\mu$ M Alp seem to be toxic.

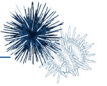
In comparison to the report of 2010, where the treatment with VPA led to flattened embryos, we weren't able to observe anything like this. On the other hand we observed a sort of exogastrula, which was unexpected, due to the fact that VPA was intent to enhance the animal area. We couldn't find a consisting explanation for that, maybe there was simply a mishap by the handling or renaming of the pictures. We suggest that this result should be seen with suspicion and be repeated at the next course.

As already seen 2010, Alp and VPA don't neutralize their effects, which can lead to the conclusion that those two pharmacological substances interact with two completely separate signaling-pathways.

Due to the lack of new information on the mode of action of VPA, it is still unclear, as it was in the report 2010, if the LiCl-VPA double treatment is a rescue-effect. Therefore the two mechanisms have to interact with the signal-network pretty directly. The mode of action of VPA is still unknown and so the exact interaction of the two substances with each other and the organism stays unrevealed.

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## 4) Actin-staining using Phalloidin

Julia Offer and Julia Wunderer

### Introduction

Phalloidin is an isolated bicyclic heptapeptide from the death cap (*Amanita phalloides*) and it is able to bind F-actin, preventing its depolymerization and poisoning the cell. It binds specifically at the interface between F-actin subunits, locking adjacent subunits together.

Furthermore Phalloidin is found to inhibit the ATP hydrolysis activity of F-actin.

The properties of Phalloidin make it a useful tool for investigating the distribution of F-actin in cells by labeling Phalloidin with fluorescent analogs and using them to stain actin filaments for light microscopy. Fluorescent derivatives of Phalloidin have turned out to be enormously useful in localizing actin filaments in living or fixed cells as well as for visualizing individual actin filaments *in vitro*. Correctly designed fluorescent Phalloidins only bind to the native quaternary structure of F-actin and therefore have a low background. To create the correct fixation conditions for Phalloidin binding, Paraformaldehyde (PFA) should be used as the fixative because it retains the quaternary protein structure which is necessary for high affinity. Methanol destroys the native conformation and hence is not suitable for actin-staining with Phalloidin (Wulf et al., 1979).

The amount of fluorescence visualized can be used as a quantitative measure for the amount of filamentous actin in cells, if saturating quantities of fluorescent Phalloidin are used. Consequently, immunofluorescence microscopy along with microinjection of Phalloidin can be used to evaluate the direct and indirect functions of cytoplasmic actin in its different stages of polymer formation.

Phalloidins do not permeate cell membranes, making them less effective in experiments with living cells. Cells treated with Phalloidins exhibit a number of toxic effects and frequently die.

### Materials and Methods

First, a test was made to find out which fixation method would work best and to see, if it is generally possible to stain the actin filaments in different developmental stages of the sea urchin.

For this test, gastrula (14 hpf), prism stage (18 hpf) and pluteus larvae (24 hpf) of the sea urchin *Arbacia lixula* were fixed with three different methods for respectively one hour. The different fixation mediums were following:

1. 4% PFA (Paraformaldehyd) in PBS- buffer
2. Methanol + Sucrose
3. 4% PFA in Seawater

After fixation the developmental stages of *Arbacia* were washed four times for ten minutes in PBS-buffer and incubated in Phalloidin solution (concentration 1:100) for one hour in a dark place. After the Phalloidin incubation, the samples were washed again four times for ten minutes in PBS-buffer. While washing they were kept in a dark place to protect fluorescence.

Only the fixation with 4% PFA in PBS worked, while the Methanol and the PFA in Seawater flocculated too strongly. Besides it was nearly impossible to wash the gastrula and prisma stage, as they were too small and got lost nearly completely during the different washing steps. The fluorescent signal in the pretest samples was very slight, only a light staining in the pluteus larvae could be detected. For this reasons the proper experiment was done only with pluteus larvae (24 + hpf) and a higher number of pluteus were used. Fixation was done with 4% PFA in PBS. In addition two different Phalloidin solutions (1:100 and 1:50) were taken to see, if fluorescent signal could be improved with a higher Phalloidin concentration.

1. Fixation of *A. lixula* pluteus larvae in 4% PFA for one hour
2. Washing (four times in PBS buffer for respectively ten minutes)
3. Incubation in Phalloidin solution (C= 1:100 or 1:50) in a dark place for one hour
4. Washing (four times in PBS buffer for respectively ten minutes), samples were kept in the dark
5. Cover with Vectashield

## Results

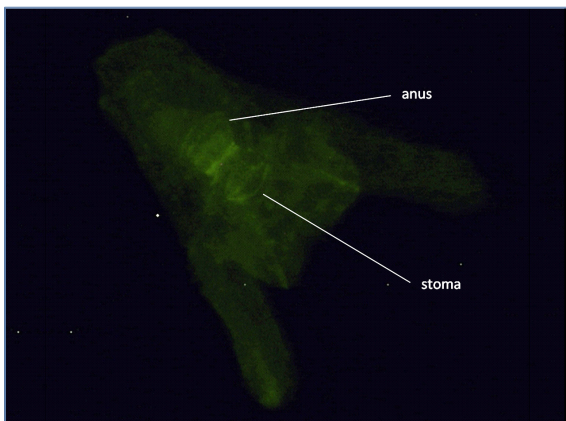


Fig.1: Phalloidin Staining (concentration 1:100) of the pluteus larvae of *Arbacia lixula* (24 hpf +). A clear fluorescent signal is visible around the digestive system. The actin filaments are prominent around the mouth and the anus. This picture has been taken with a optical magnification of 400.

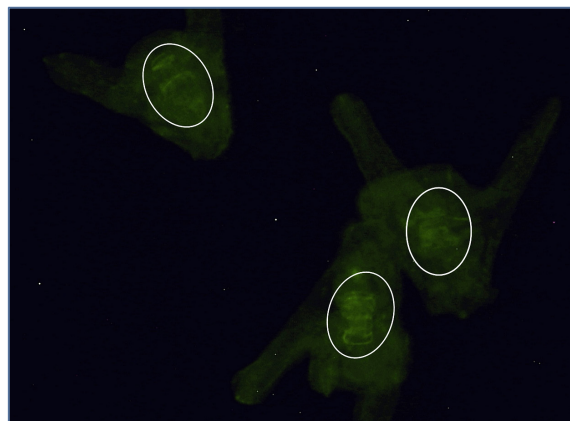


Fig.2: Phalloidin Staining (concentration 1:100) of three pluteus larvae of *Arbacia lixula* (24 hpf +). A clear fluorescent signal is visible around the digestive system (circled in white). This picture has been taken with a optical magnification of 400.

A clear fluorescent signal could be detected around the digestive system of the pluteus larvae. The most prominent staining of actin filaments could be seen around the stoma and the anus of the larvae (fig.1 and fig.2).

Besides the fluorescent signal from the Phalloidin-staining an auto fluorescence of the spiculae has been detected (fig.3).

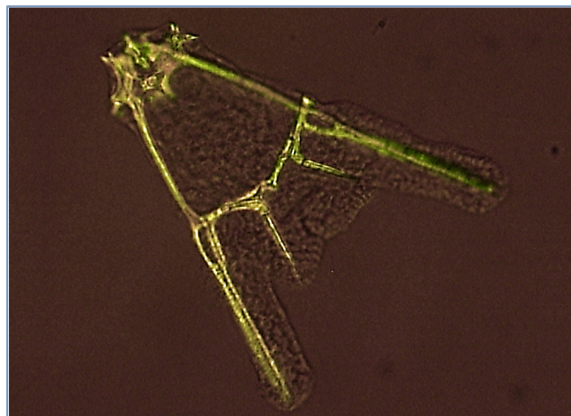
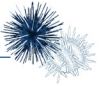


Fig.3: Auto fluorescence of the spiculae in the pluteus larvae of *Arbacia lixula* (24 hpf +). This picture has been taken with a optical magnification of 400.





## Discussion

With the Phalloidin staining method a clear fluorescent signal could be found in the pluteus larvae. The staining was not successful with the earlier developmental stages, as they were much too small and got lost during the washing steps. Test samples showed that a fixation with methanol or seawater is not possible as the media flocculated strongly. The best results could be seen with a phalloidin concentration of 1:100 whereas a higher Phalloidin concentration brought no improvement of the fluorescent signal. As shown in fig.1 and fig.2, the fluorescent signal was concentrated around the digestive system, so it is clear that intestinal muscles already start to form in this developmental stage. A high concentration of actin filaments was found around the anus and the stoma of the larvae.

Pluteus larvae are a free swimming stage and can be considered as temporarily feeding devices. Indeed, the entire repertoire of larval behavior seems to center on feeding.

Burke (1981) describes the muscular structure of the digestive tract of the pluteus larvae as follows:

*“The esophageal muscles of the pluteus larva of many Echinoids are similar to the smooth muscle of the adults, but there are several differences. The arrangement of the thick and thin filaments in esophageal muscle is sufficiently random that the fibers appear smooth; however, the periodic arrangement of the dense bodies can result in indistinct Z-lines. The alignment of the dense bodies is not consistently present and may represent differences in the state of contraction of individual fibers.*

*The cardiac sphincter consists of an 'hourglass-like' constriction formed from simple, striated myoepithelium.*

*The pyloric sphincter separates the stomach and intestine. It consists of a constrictable opening at the posterior end of the stomach made up of a ring of stomach cells that have in their basal regions a single band of circumferentially oriented thick and thin myofilaments.*

*The anus is a thickened ring of ciliated intestinal epithelium that forms a junction with the epidermis. The cells that form the anus have in their basal regions thick and thin filaments arranged in a manner similar to that described for the pyloric sphincter.”*

Next step would be to improve the staining method by trying out other fixation methods or phalloidin concentration and have other trials with earlier developmental stages to see, if actin filaments are already present in an earlier stage of development. It has also to be mentioned, that the microscope used was an older type. This experiment was a first try out, to see if it is even possible to stain actin filaments in pluteus larvae, so the results are rather satisfying.

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# Projects



## Fish Transect

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## Introduction

When entering the water near STARESO it's evident that the fish diversity is vast. This is partly because of the good water quality and a protected area nearby. It was noticeable that in the bay of Stareso the richness in fish was even greater than outside the bay. This could be due to the protection of the harbour as well as the food supply, partly consisting of organic waste from the station.

The two major fish groups abundant in the mediterranean sea are the wrasses and the sea breams. To get a survey of the fish diversity as well as activity patterns we did various transects on different substrates, as not the depth but the substrate type is a significant factor in fish density and diversity. For instance the distribution of *Coris julis* and *Symphodus ocellatus* is in corellation with the cover of macroalgae on rubble and small blocks. The general factors influencing distribution of fishes are classified in three main groups (Letourneur, Ruitton, Sartoretto, 2003):

- Biotic factors like the composition of the benthos, competition between fish, predation and recruitment.
- Abiotic factors like depth, salinity, temperature and exposure to trade winds.
- Historical factors like unusually violent storms or unusually high seawater temperatures.

In general fish activity is dependent on many factors like food availability, predation, visibility, water temperature or lighting conditions. To observe activity patterns of fish during day and night time we did a 24 hours line transect.

## Materials and Methods

In this year two different visual census techniques were used. For quantitative estimates of fish abundance the strip transect technique (STT) was used, for qualitative estimates of the fish assemblages on different substrate types the rapid visual technique (RVT) was used (Sanderson and Solonsky, 1986). The STT was conducted along the right site of the Harbour of STARESO and ended on the red buoy marking the harbour entrance.

### Strip transect technique (STT):

First of all, the snorkeler swam through the research area in order to find the appropriate route with different substrate types and also changing depths. Following, the chosen 134.1 m long route was marked with a nylon



Fig.1: Diver fixing the transect line on rocky substrate

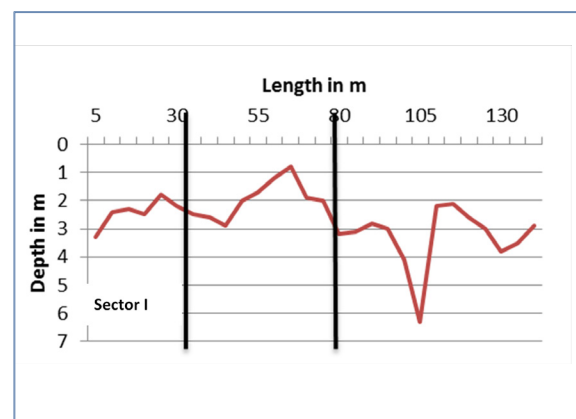


Fig.2: Strip transect length- and depthprofile

line, anchored on the substratum (fig. 1), one the hand for orientation and on the other hand to make the results comparable and reproducible. To fix the width of the transect, marked stones were placed regularly in 3m distance left and right of the line. The transect depth was measured every 5m with an acoustical depth finder (fig.2). After that the STT was divided into 3 different sectors. The end of every sector was marked with withe buoys. The first sector had a length of 41 meters and an average depth of 2.5 m. This section consisted out of two substrate types on the left handed site was *posidonia oceanica*, on the right handed site was the Blockfield (area with bigger rocks). The second sector was shallower (1,5 m) and had a length of 36.1 m. Also the substrate changed, much smaller stones and even sand could be found in this sector. The 57m long third sector included the deepest spot (6,3 m). This sector left the port basin and entered the next greater bay; due to this fact the substrate changed from smaller stones to a cliff.

The strip transect was conducted over a 24 h period, in which the transect was observed every two hours by two men teams. The first 24h hour run started on the 4th of September 2012, but it had to be aborted precociously because of bad weather conditions and missing data material (22:00). The second run started at 2 pm on the 5th September.

Brock (1954) suggested that the two observers swim on either site of the line. The experienced census divers swam slowly and quietly, without diving, counting, identifying and recording all fishes on and ahead the substrate for each sector and in total (Bobsien and Brendelberger, 2006). Fish which crossed the line where recorded only on the side where they originated. The observers began the census simultaneously at the beginning of one sector and didn't stop until the census was completed at the end of each sector. To achieve the total number of individuals of each species seen in the sector the two observers combined their data, categorized the abundance and wrote it down immediately. The used categories were:

- Cat A = 1 Individual
- Cat B = 2 to 5 Individuals
- Cat C = 6 to 30 Individuals
- Cat D = 31 to 100 Individuals
- Cat E = over 100 Individuals

**Rapid visual technique (RVT):**

The rapid visual transect took place in two locations (“Sandy Beach” 1+2) in which the species composition of two different habitat types were observed (fig.3; fig.4). The compared habitat types were on the hand *posidonia* fields



Fig.3: “Sandy Beach” 1 (+42° 34'; +8° 43'); red mark: *posidonia* field; yellow mark: sand bank, (Google Earth)



Fig.4: “Sandy Beach 2” (+42° 33', +8° 43'); red marks: *posidonia* field, yellow mark: sand bank, (Google Earth)



and on the other sand banks. Seagrass beds of shallow water are important habitats because of their trophic and nursery functions for many fish species all over the world (Bobsien and Brendelberger, 2006). Contrary to this ecosystem diversity, the sand banks represent a more monotone habitat type, offering just a few ecological niches.

Using the RVT the observer was allowed to swim randomly over each substrate type. The observers (2 men teams) swam for 5 min intervals and each species seen was categorized in the same way done like in the strip transect (see above). After the first interval the teams changed the substrate type and counted again for 5 minutes. This procedure was repeated once.

#### **Statistical Methods:**

The collected data material of the strip transect was analyzed in Microsoft Excel 2010. To interpret the abundance of the different families and species the nominal data were summarized with the mode and set in reference to daytime and sector appearance. It has to be mentioned that the mode is a way of expressing important information in a single number about a random variable or a population. According to this, a missing in the abundance doesn't imply that the fish hasn't been observed over this sector or in this time period, it just represents the majority of the categorization.

The rapid visual transect data were also analyzed and are shown in an occurrence list.

## Results

### 1) Strip transect

#### Sparidae

The breams were observed at every timepoint during the 24 hours with exception of *Diplodus puntazzo*, but the activity level shown by the fish was higher during the day, overnight they were often seen motionless/inactive on or over the ground.

*Diplodus puntazzo* shows a real day activity and couldn't be observed during the night time. The other breams have sometimes very different activity patterns, e.g. *Sarpa salpa* abundance varied between category 1 at 4 am and category 4 at 2 am and 4 pm. Also *Diplodus sargus* abundance differed significantly between the time period from midnight to noon and 2 pm till 10 pm. Contrary the abundance of *Diplodus annularis*, *Diplodus vulgaris* and *Oblada melanura* was more constant, ranged between categories 1 and 3, except some outliers (see figure 5).

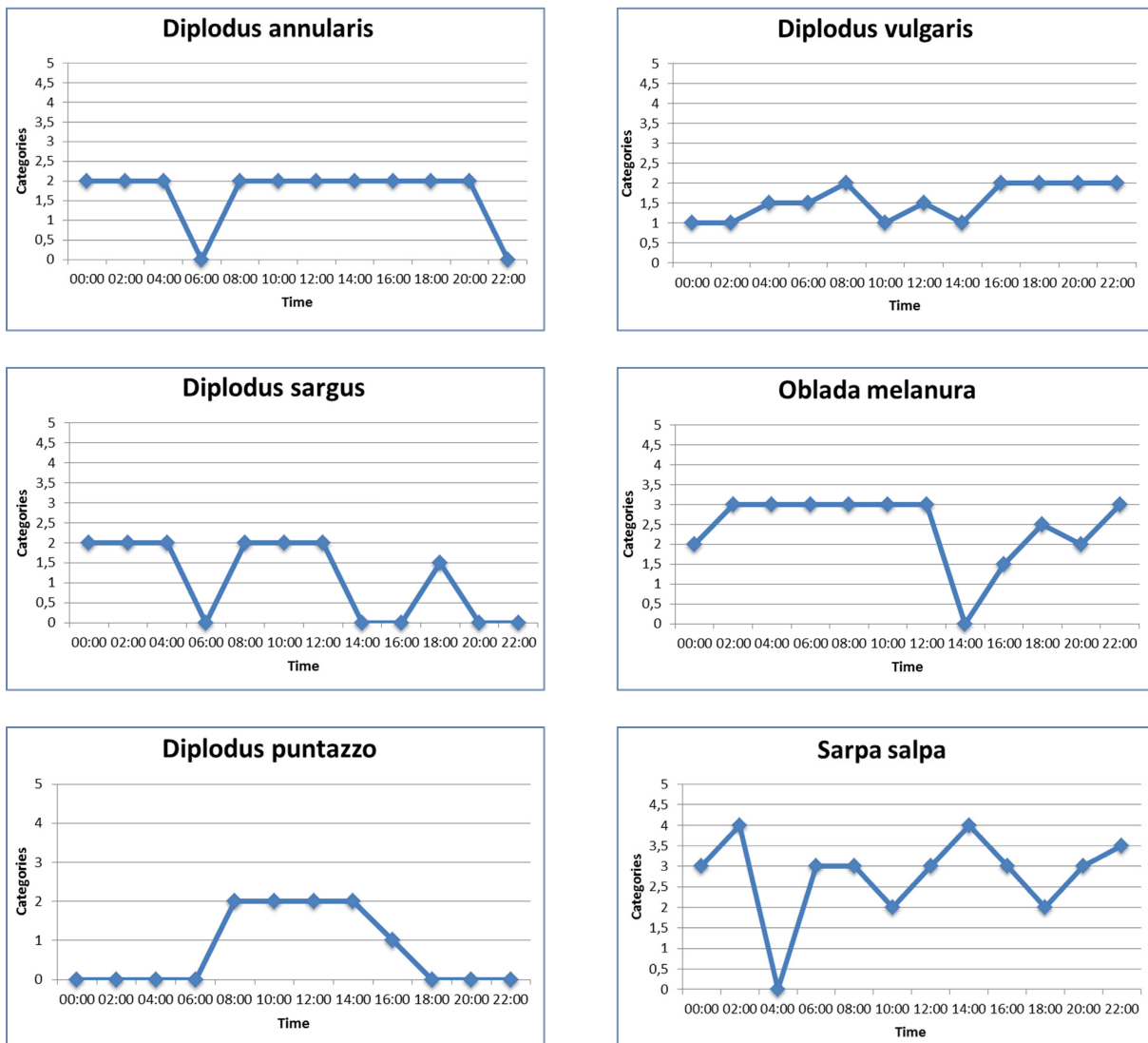


Fig.5: Abundance of the different Sparidae species





*Diplodus annularis*, *Diplodus vulgaris*, *Oblada melanura* and *Sarpa salpa* didn't show any substrate preferences. All 4 species could be observed quite frequently over all three sectors. Despite these four generalists there were also two species with a preferred habitat type. *Diplodus sargus* showed a clear preference to the shallower water of sector 2. A clear activity pattern of *Diplodus puntazzo* could only be observed in sector 1 (boulder field) (see fig.6).

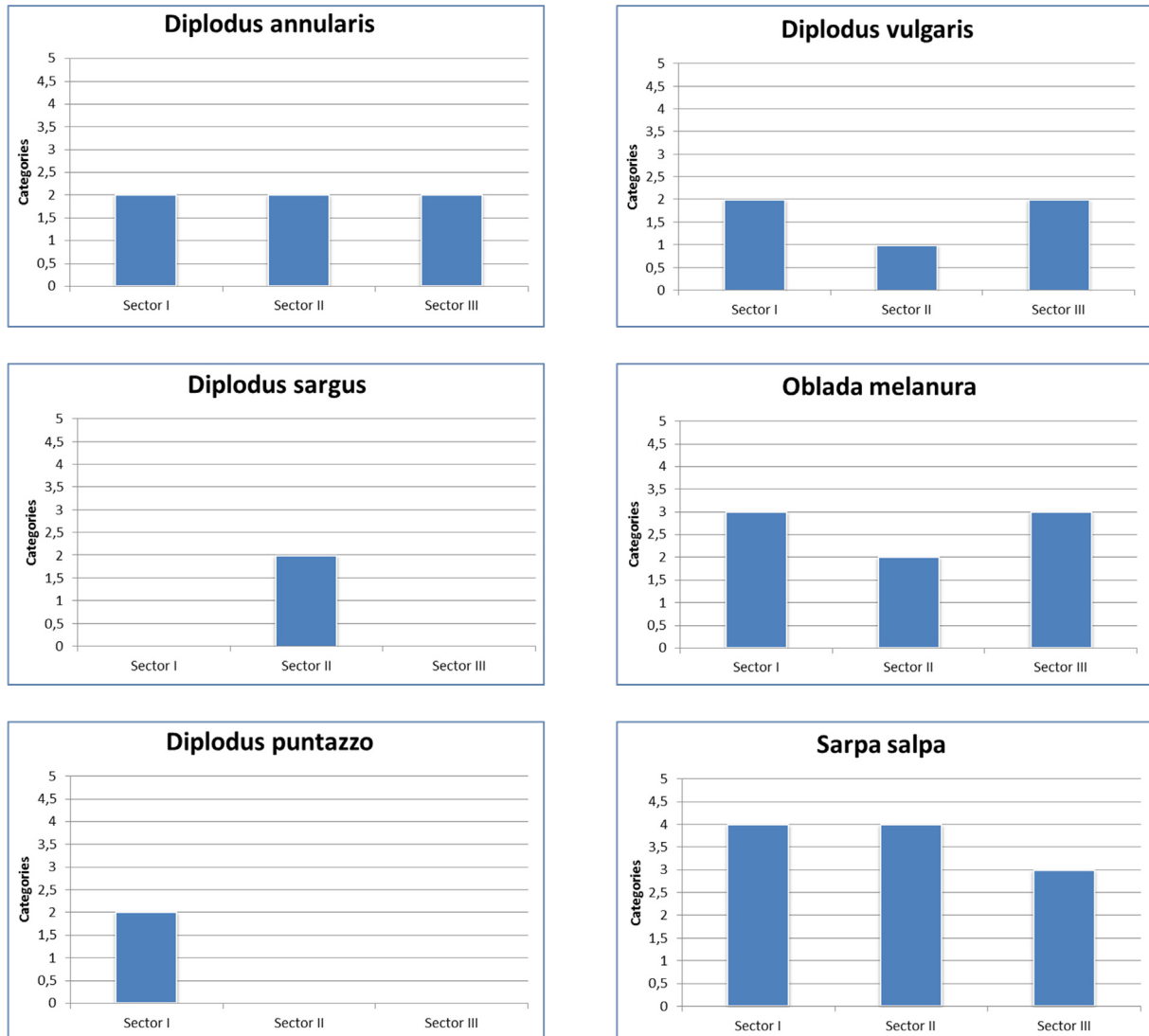


Fig.6: Sector abundance of the Sparidae species

### Labridae

Nearly all wrasse species, excepted *Symphodus tinca*, could be observed only during the day. In common the activity period started between 6 and 8 am and ended between 6 and 8 pm. The highest abundance of all species could be observed in the afternoon. During the afternoon up to 30 individuals per sector (especially *Symphodus tinca*) were observed (fig.7). *Symphodus melanocercus* and *Thalassoma pavo* were sighted only sporadically.

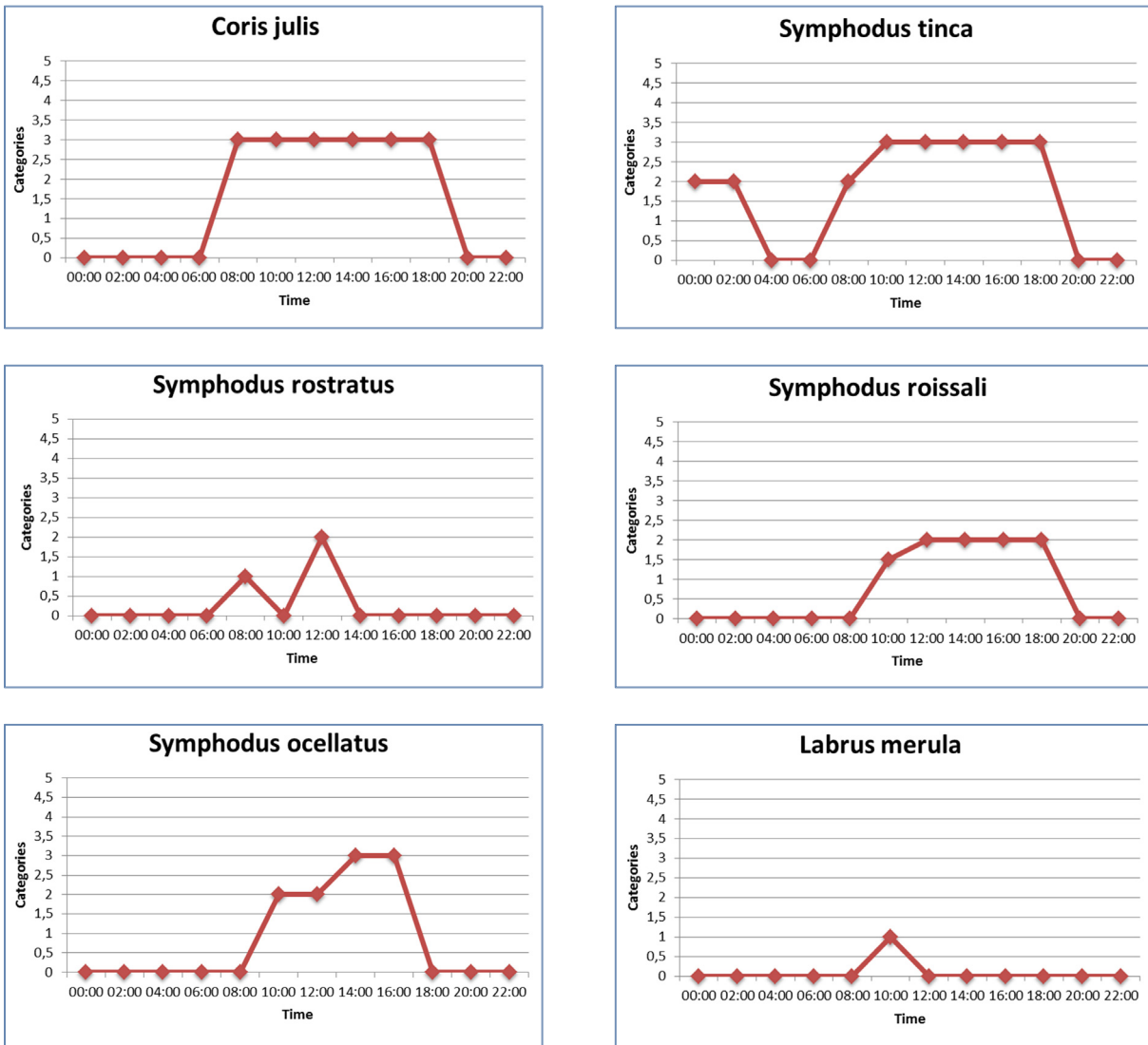


Fig.7: Abundance of the different Labridae species

No sector abundance could be determined for all wrasse species, depending on this statistical method. Exclusion is *Symphodus tinca*, which had a high abundance over all three sectors (see fig.8).

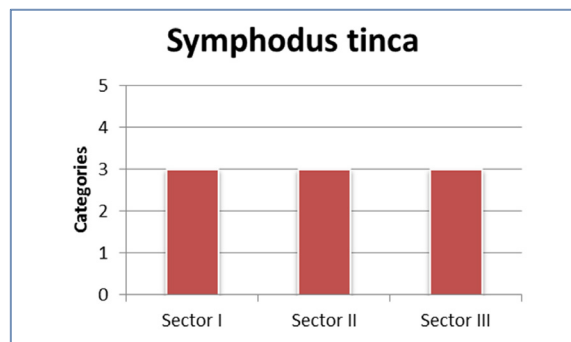


Fig.8: Sector abundance of *Symphodus tinca*



### Other fish species related to the seaground

*Apogon imberbis* represents a night active species which has his action period during the night between 10 pm and 6 pm. Other species like *Atherina hepsetus*, *Oedalechilus labeo*, *Mullus surmuletus* and *Chromis chromis* were present nearly all the time but inactive during the night. *Chromis chromis* lost its covey behaviour at night and appeared only scattered. The species with the most general activity pattern was *Serranus scriba*, no specific recovery time could be estimated (fig.9).

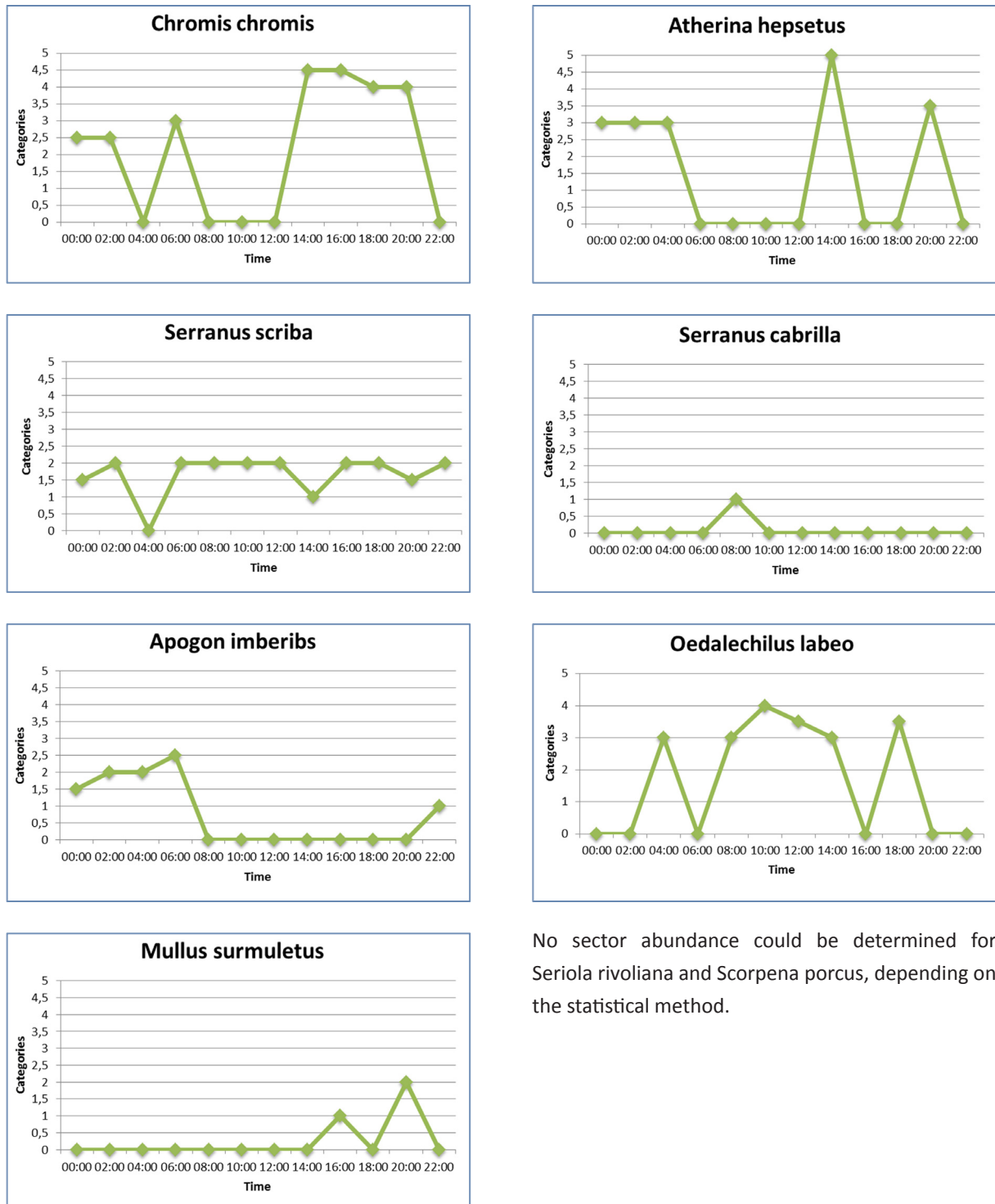


Fig.9: Abundance of the other fish species related to the seaground

No sector abundance could be determined for *Seriola rivoliana* and *Scorpena porcus*, depending on the statistical method.

*Atherina hepsetus* showed a very special sector preference. During the night it appeared only in the more protected sector 1, in the other sectors observation was sporadically during daytime. *Chromis chromis* showed a clear preference to open sea water (sector 3). In this sector it appeared in big coveys with over 100 individuals (see fig.10).

In contrast to the habitat preferences of *Atherina hepsetus* and *Chromis chromis*, *Serranus scriba* showed a very general sector abundance.

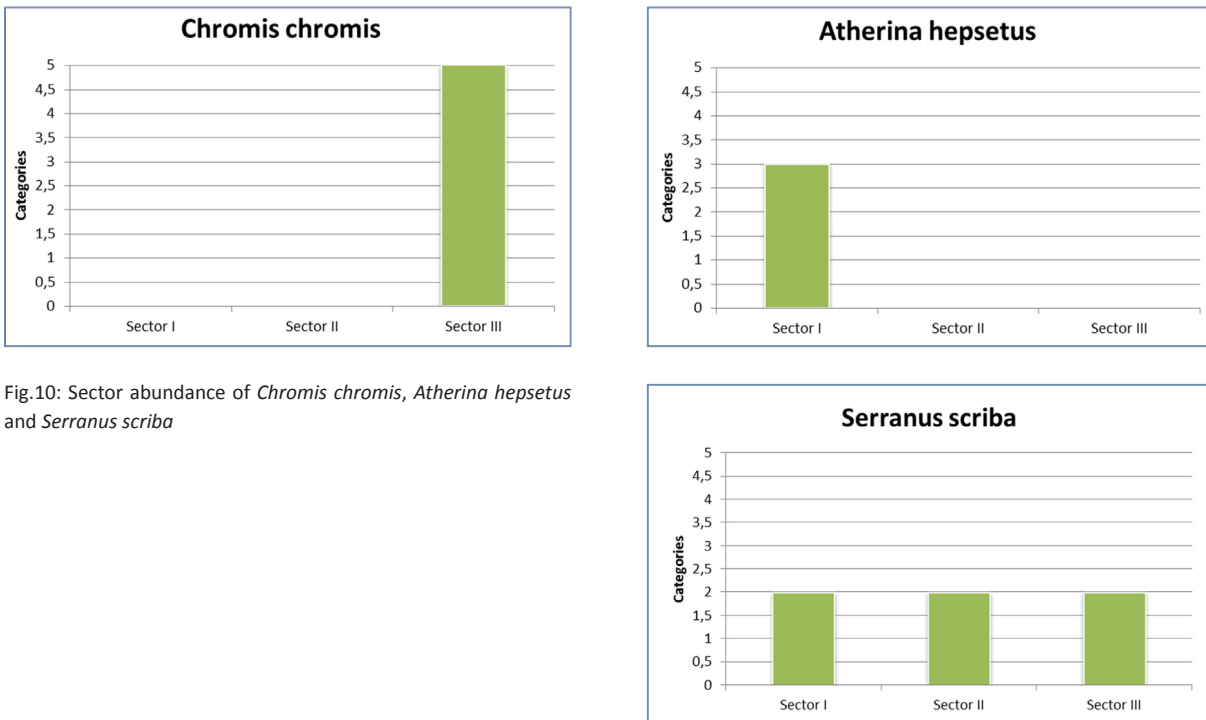


Fig.10: Sector abundance of *Chromis chromis*, *Atherina hepsetus* and *Serranus scriba*

**Species, not statistically analyzed**

Beside the sparidae, labridae and the species mentioned in the section above, also *Muraena helena*, *Dicentrarchus labrax*, *Octopus vulgaris*, *Belone belone* and *Mycteroperca rubra* could be observed sporadically, but they had a non-significant appearance.

**2) Substrate transect**

RVT-Observations on different kinds of substrate showed that there were on the one hand species with a preference to sand or seagrass and on the other hand generalists which occurred on both substrate types (see fig.11). Typical fish linked to sandy habitat were *Lithognathus mormyrus*, *Trachinus draco*, *Bothus podas* and *Mullus surmuletus*. Their morphology and behavior show typical adaptations to this kind of substrate.

Nearly all wrasses prefer heterogenic habitat types. Some of them developed special behavior adaptations to seagrass (e.g. breeding in *Posidonia* fields). Beside the wrasses *Chromis chromis* and *Serranus scriba* were observed only over seagrass.

Most of the wrasses showed a general distribution and could be observed on both substrates. Exception is *Lithognathus mormyrus*, mentioned above as a sand specialist.



Species	Abundance			
	on Sand	on Seagrass		
<b>Sparidae</b>				
<i>Diplodus annularis</i>	+	+		
<i>Diplodus vulgaris</i>	-	+		
<i>Diplodus puntazzo</i>	-	+		
<i>Diplodus sargus</i>	+	+		
<i>Oblada melanura</i>	+	+		
<i>Sarpa salpa</i>	+	+		
<i>Lithognathus mormyrus</i>	+	-		
<i>Pagellus erythrinus</i>	+	-		
<i>Spicara maena</i>	-	+		
<b>Labridae</b>				
<i>Coris julis</i>	-	+		
<i>Symphodus tinca</i>	+	+		
<i>Symphodus ocellatus</i>	-	+		
<i>Symphodus rostratus</i>	-		+	
<i>Symphodus roissali</i>	-		+	
<i>Thalassoma pavo</i>	-		-	
<i>Labrus merula</i>	-		-	
<i>Chromis chromis</i>	-		+	
<i>Atherina hepsetus</i>	+		-	
<i>Oedalechilus labeo</i>	-		+	
<i>Serranus scriba</i>	-		+	
<i>Serranus cabrilla</i>	+		+	
<i>Mullus surmuletus</i>	+		+	
<i>Apogon imberbis</i>	-		-	
<i>Sphyaena viridensis</i>	+		+	
<i>Trachinus draco</i>	+		-	
<i>Bothus podas</i>	+		-	
<i>Seriola sp.</i>	-		+	

Fig.11: Species occurrence on seagrass and sand

## Discussion

Since 1996 about 100 different species of fish were registered in this area, whereas the maximum of observed species in one year was 65. This maximum was reached in the years between 2004 and 2010 practically every two years. This year we only saw 45 species, which could be due to heavy weather conditions, firstly preventing us from spending much time in the water diminishing observation time and secondly leading to a cooling of the water from 26°C to 18°C in the timespan of a few days. While snorkeling the difference in fish abundance and activity on hotter and cooler days was evident, as we saw considerably less fish which were furthermore calmer in their behaviour on days with lower temperatures.

Before comparing the observed species on different substrates it has to be said, that the rocky substrate was much more carefully observed as we spent most of our time there and did the 24h-transect, thereby registering diurnal and nocturnal fish. The sandy substrate and posidonia-fields were only observed in the morning and in the afternoon for a relatively short amount of time to get a general idea of the species composition. Therefore we may not have observed all species abundant on those two substrates.

Even though we may not have registered all species we can see that there are generalists and specialists among the fish. Some fish like *Lithognathus mormyrus*, *Trachinus draco* and *Bothus podas* are specially adapted for sand because of their feeding habits and colouring, others like *Apogon imberbis*, *Scorpaena porcus*, *Seriola rivoliana*, *Muraena helena*, *Belone belone* and *Mycteroperca rubra* were only registered above rocky substrate. *Apogon imberbis*, *Scorpaena porcus*, *Muraena helena* and *Mycteroperca rubra* need coves and holes in rocks to retreat to during the time they are not hunting, explaining that they were only observed on rocks. *Apogon imberbis* and *Scorpaena porcus* could not have been observed above the other substrate types, as they hide during the day and only come out at night.

The higher abundance of predators during the night is one reason for the absence of the Labridae at nighttime. Furthermore Labridae need light to feed, as they have to distinguish algae partly containing feeding organisms from the substrate. This applies also for the Mugilidae as they dabble through the sediment or browse algae from rocks thereby needing light.

*Atherina hepsetus* and *Chromis chromis* which show swarming behaviour during the day show different behaviour during the night, as we observed single fish standing in the water column or under rocks.

By performing these transects we gained data, but it has to be viewed very critically as so many people took part in gathering this data and the knowledge of the species and the execution time of the observation varies between different persons. Secondly none of us have ever performed a transect before and to recognise all species proved to be difficult especially during the night.

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# Projects



Module: Current Topics in Fish Ecology and Aquaculture 2012

## Diet composition and weight length relationship of four common wrasse species (Labridae)

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Joao Vicente Gladek, Balsam Al-Janabi  
- Veronica Prantl, Reinhold Hanel -







## Introduction

Research into food webs is an important part of marine ecology, as it gives valuable information of the relationships between organisms and how they interact. It is also important to understand the ecology and behavior of single fish species. Analyzing gut contents can efficiently do this. Furthermore, it has the advantage of also giving important information about the feeding success and general fitness of fish in certain areas and enables a comparison between species. Gut content analyses also allow differentiating between selective predators or non-selective feeders (Bell & Harmelin-Vivien 1983) and can show overlaps between ecological niches between sympatric species. Studying the nutritional composition of key species is an important part of a food-web analysis (Gaedke 1995). The different food types and the frequently consumed prey can help to determine feeding habits (Zacharia 1974). In some species, these habits include interaction with other species. It is common to find *S. ocellatus* schooling with individuals of other species, like *S. tinca*, *S. quinquemaculatus*, *S. rostratus* and *Coris julis*, to form feeding aggregations. To prey small crustaceans, *S. tinca* chunks of big pieces of algae, making other small crustaceans and organisms available for *S. ocellatus* prey upon (Pallaoro & Jardas 2003, Taborsky et al. 1987). *S. rostratus* is an exclusive predator of evasive prey and it is expected that it exploits the concealment given by the school of *S. ocellatus* and used it to prey smaller fishes (Westneat 1995).

This study focused on species of the family Labridae and tried to examine the interactions between some sympatric species in a rocky coastal habitat. It was conducted during one week in September 2012 at the research station STARESO near Calvi, Corsica. Individuals of four species, *Symphodus tinca*, *S. ocellatus*, *S. rostratus* and *Coris julis*, were caught while snorkeling and the gut content was examined to answer the following questions:

1. What is the diet composition and trophic level of the species?
2. Is the diet composition significantly different among the species?
3. Does it change with body length?
4. How is the length and weight of the fishes correlated?

## Material and Methods

Gut contents of four common species of the family Labridae (*Symphodus tinca*, *S. ocellatus*, *S. rostratus* and *Coris julis*) were analyzed. The *Symphodus* species are interacting in feeding groups. *Coris julis* was taken as an out-group.

Fishes were sampled from 26 August until 06 September 2012 along the Revellata peninsula, Gulf of Calvi, Corsica (Latitude: 42°34'49.39"N, Longitude: 8°43'26.93"E), at depth of up to 6 m. Specimens were collected by snorkeling at random times during the day, between 10:00 a.m. and 18:00 p.m., with the help hand nets. After catch they were immediately killed in iced sea water and stored frozen until further analysis.

The total length and weight was measured of each individual (Zacharia 1974). The gut was removed by a longitudinal incision starting from the anus. The gut content was put in a petri dish and weighted with an accuracy of 0.01 g using a micro scale.

Gut analyses were performed using stereo microscopes. As several species damage their prey with their pharyngeal jaws and there was little time to train the different researchers in identification skills, food items were classified by large taxonomic group, and the percentage composition by volume of each food item was recorded.

Statistical analysis of the difference of diet composition among the four species was done by an ANOSIM. It tested for the differences in community composition (gut content) among groups (fish species). The method uses the Bray-Curtis measure of similarity. In those data often species are not normally distributed because some species

are absent. Therefore, we applied a simple non-parametric permutation procedure (analysis of similarity). This method tests the null-hypothesis that there are no differences in community composition (gut content taxa) between treatments (fish species). The R value is 0 if the null-hypothesis is true and 1 if all replicates within groups are more similar to each other than any replicates of different groups. The p-value gives the likelihood of rejecting the null-hypothesis though it's true. Additionally we applied a frequency of occurrence. Here, the number of guts in which single taxa occurred, is recorded and expressed as a percentage of the total number of gut examined:

$$O_i = J_i / P$$

In which, J is number of fish containing prey i and P is the total number of fish with food in their gut.

The length weight relationships is described by

$$W = a * L^b$$

where w is the weight in gram, a is the intercept, L is the total length in cm and b is the slope of the regression line.

## Results

### Gut content

The results of the gut content analyses are shown in the following graphs. Figure 1 shows the content of all guts of *Symphodus tinca* that were analyzed. The individuals are ordered according to length. The smallest individual was only 5.6 cm and the longest 21.0 cm. There is no obvious connection between fish length and gut content. *S. tinca* consumed a wide range of food sources from other fish species, over many different invertebrates to algae. Crustaceans made up up to 80% of the gut content. Molluscs were present in most guts, but represented mostly

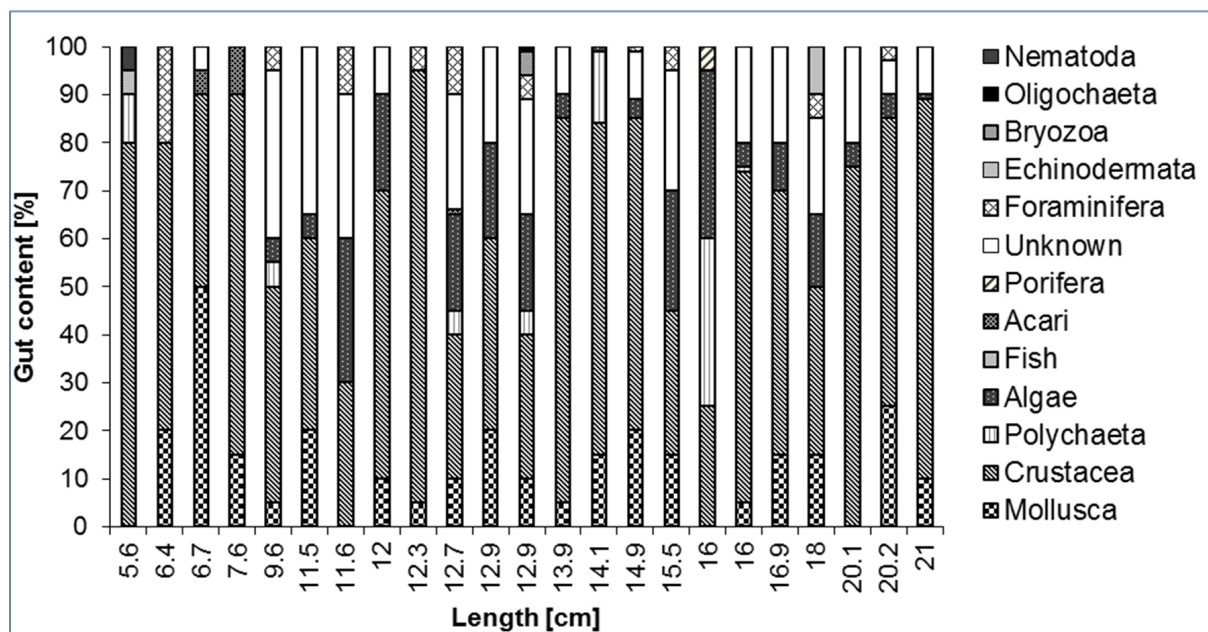


Fig.1: Gut content of all individuals of *Symphodus tinca* examined, ordered by their length



only between 5 and 20% with one individual's gut containing about 50% molluscs. Algae were only found in some specimens, but sometimes constituted for nearly 30%. Other fishes and all other groups made up the remaining percentages together with some unknown or unidentifiable food items.

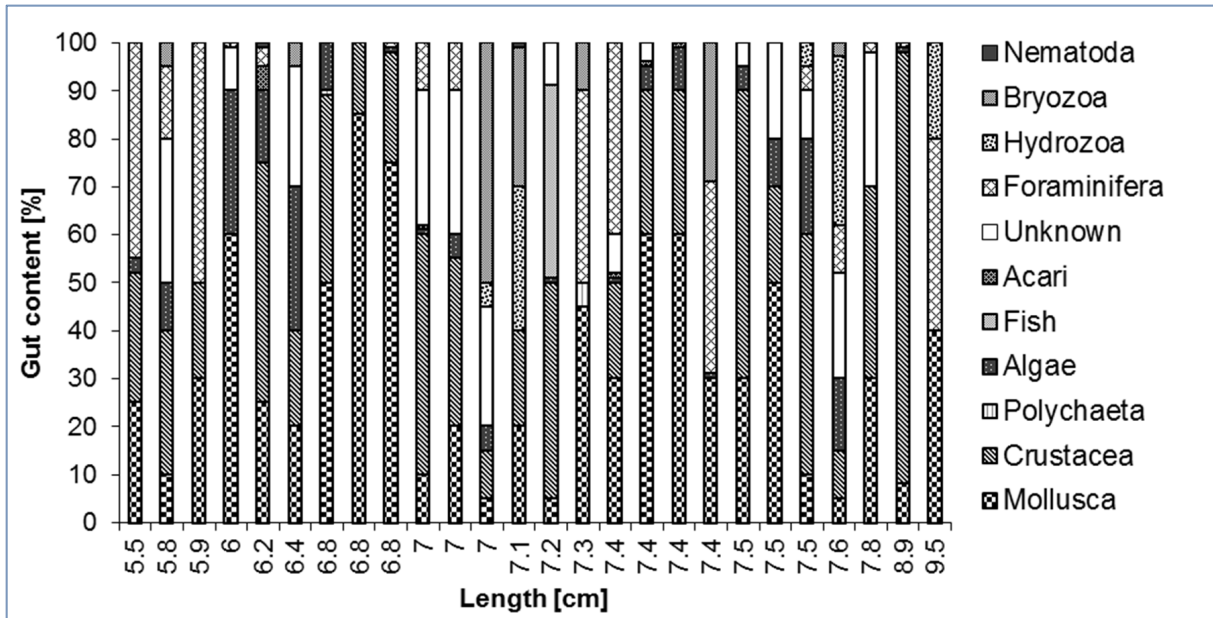


Fig.2: Gut content of *Symphodus ocellatus*

The results of the gut content analysis of *Symphodus ocellatus* are shown in figure 2. The gut content is less diverse here than in *S. tinca*, but still shows quite a range of organisms. Molluscs represent a much larger proportion of the gut contents, with up to 85% in one individual. In *S. ocellatus* hydrozoa were found and made up 35%. Otherwise foraminifera, algae and crustaceans were found regularly and in high numbers. There is no trend in gut content with increasing length of the fish but the composition of the gut content changes substantially between individuals.

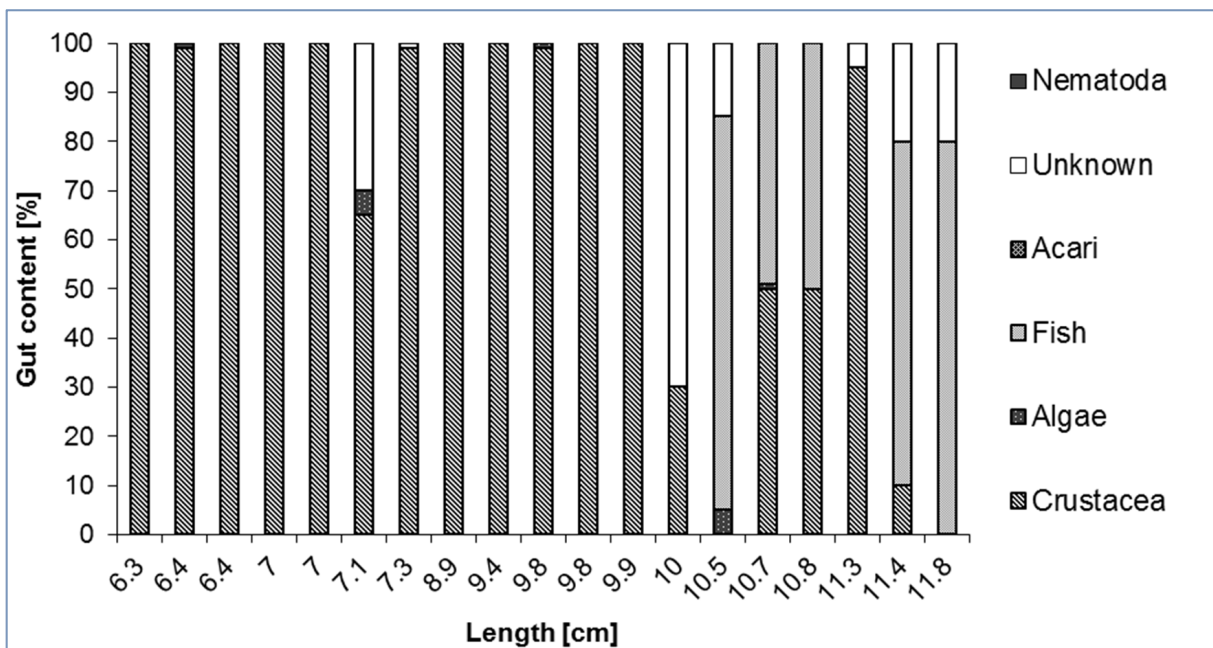


Fig.3: Gut content of *Symphodus rostratus*

The gut content of *Symphodus rostratus* is much less diverse. It is shown in figure 3. Many and especially smaller individuals only consumed crustaceans. The gut content of the largest individuals also contained substantial numbers of fish with up to 80%.

The remaining content was unidentifiable or contained also crustaceans and in very small amounts algae, mites (acari) and nematods.

The fourth species investigated was *Coris julis*. The results are shown in figure 4 and show a similar diversity to *S. rostratus*, but with different percentages. There was again no clear size-content relationship and percentages varied greatly between individuals. Often found were crustaceans, sometimes with up to 100% and molluscs with up to 80%. Algae and bryozoa were found regularly in smaller percentages. Foraminifera and polychaetes were less common.

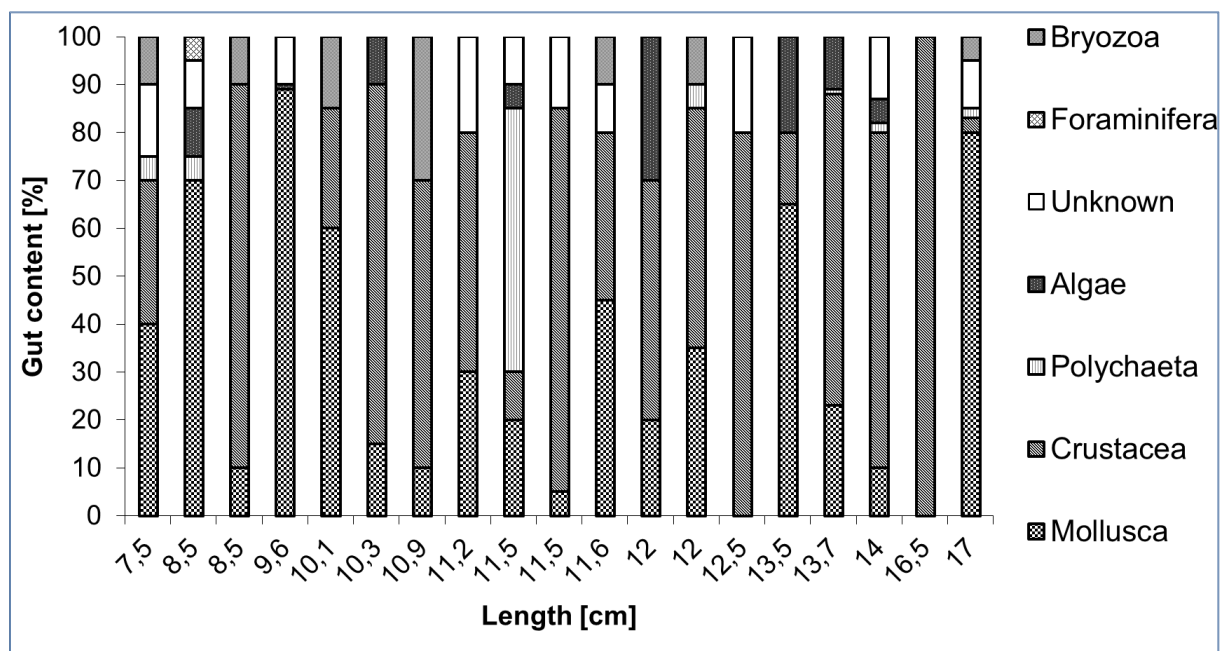


Fig.4: Gut content of *Coris julis*

The differences between gut contents were statistically compared using an ANOSIM. The results ( $R= 0.2479$ ,  $p= 0.001$ ) show that there is no significant difference between the species. Secondly, the percentage composition by volume data was analyzed by a frequency of occurrence method (table 1).

**Table 1:** Frequency of occurrence of taxa in the gut content

	Mollusca	Crustacea	Polycheta	Algae	Fish	Acarea	Porifera	Unknown	Foraminifera	Echinodermata	Bryozoa	Oligochaeta	Nematoda
<i>S.tinca</i>	0.826	1.000	0.304	0.783	0.043	0.130	0.043	0.739	0.435	0.043	0.043	0.043	0.043
<i>S.ocellatus</i>	1.000	0.846	0.077	0.769	0.038	0.192	NA	0.538	0.538	0.231	0.269	0.000	0.077
<i>S.rostratus</i>	0.000	0.895	0.000	0.211	0.263	0.000	0.000	0.263	0.000	0.000	0.000	0.000	0.053
<i>C.julis</i>	0.895	0.895	0.368	0.421	0.000	0.000	0.000	0.526	0.053	0.000	0.368	0.000	0.000



It was also interpreted how often a certain food source was found in the gut of each fish species. The results of this are shown in table 1. Food sources that were never found in the species are highlighted in blue and sources that were found in 100% of the individuals are highlighted in red. It can be seen that *Symphodus rostratus* and *Coris julis* have a narrower preference than *Symphodus tinca* and *S. ocellatus*, in whose guts nearly every observed food source was found. However, these two species also each have two food sources, which were always found, Crustacea and Mollusca, respectively.

### Length-weight relationship

The length-weight relationships for all four species were tested and the results are shown in tables 2 to 2. Mean weight and length varied greatly between species. The greatest variance is shown by *Symphodus tinca*, which also had the highest values for both length and weight. For all four species the length very well explains weight. *S. rostratus* has the highest values for this (0.99) and *S. ocellatus* the lowest (0.85) (tab.2).

**Table 2:** Lengths and weights of all species with their corresponding minimal and maximal value and standard error as well as results of the length to weight comparison

Species	N	Mean TL (cm)	SE TL	Min TL (cm)	Max TL (cm)	Mean weight (g)	SE weight	Min weight (g)	Max weight (g)	a	b	se(b)	r <sup>2</sup>
<i>S. tinca</i>	23	13.4	0.91	5.6	21	41.1	7.53	2.13	135	0.01	2.99	0.17	0.94
<i>S. ocellatus</i>	26	7.1	0.17	5.5	9.5	4.4	0.32	2.05	9.54	0.02	2.73	0.23	0.85
<i>S. rostratus</i>	19	9.0	0.44	6.3	11.8	9.9	1.40	2.40	20.5	0.00	3.43	0.07	0.99
<i>C. julis</i>	19	11.7	0.57	7.5	17	16.6	2.93	4.10	50.8	0.01	3.20	0.10	0.98

## Discussion

### Gut content

This analysis only included species of the family Labridae, which are generally regarded as diurnal mesophagic carnivores. They show a very diverse diet. This agrees with our results, because in the guts of all four species we found at least small numbers of molluscs. Furthermore, the analysis of frequency of occurrence just agrees with our perception of the importance of crustaceans in the diet of these fishes. Although we did not find significant dissimilarities between the gut content compositions among the four species, a trend was visible. *Coris julis* is an interesting example of a fish that preys on molluscs, because it dashes them against stones to break their shells (Salla 1997). Thereby it is also able to eat larger species.

*Symphodus tinca* is the key species of a feeding aggregation of different fishes. It scratches algae and also animals from stones or out of the sediment, filters them through its gills and spits out the remains. Other sympatric wrasse species, like *Coris julis*, *Symphodus rostratus* and *S. ocellatus*, will feed then on the ejection (Zander & Sötje 2002). *S. tinca* can thereby also feed upon bigger and armored organisms, which have to be chewed. The majority of ejected organisms are smaller organisms and meiofauna. This may also be the reason why we found algae in the gut of all four species.

The value of eaten substrate is increased in summer. Kabasakal (2001) suggests that *Symphodus ocellatus* is an omnivorous species with a tendency to herbivory. But we never found more than 20% of algae in a gut and also

the feeding behavior indicates no algae uptake on purpose. Kabasakal (2001) explains the herbivory also with the morphology of the jaw dentition, the gut length and the gut coiling patterns.

We did not examine any physiological or anatomical features, but it is highly questionable, if predominantly carnivorous fish would have a benefit from deliberately ingesting minor amounts of algae, since the digestion of plant material requires specific modifications of the alimentary tract in fishes. So it can be regarded as doubtful to categorize *S. ocellatus* a herbivorous species. Bell & Harmelin-Vivien (1983) found, that mostly crustaceans dominate the diet of wrasses; this pattern is also visible in our study. All fishes except *S. ocellatus* had crustaceans as a main diet (up to 100%). Bell & Harmelin-Vivien (1983) further mentioned that copepods, decapods, echinoderms and polychaetes are significant food source for some wrasse species. Unfortunately we had not enough time to determine more than subphyla like crustacea. In order to compare the here gathered data with previous studies it would be useful to further classify prey items at least to subclasses and therefore test, whether some species are specialized predators especially concerning distinct crustacean groups. *Symphodus rostratus* is a specialist for crustaceans, which fits our findings (Quignard 1966, Bell & Harmelin-Vivien 1983). Quignard (1966) identified three different feeding groups within the genus *Symphodus*: principal crustacean feeders (*S. ocellatus*, *S. rostratus*), primarily mollusc feeders (not in our samples) and one echinoderm specialist (*S. tinca*). We also found this pattern: *S. ocellatus* and *S. rostratus* had 80%- 100% (except some single individuals) crustaceans in their gut while *S. tinca* was the only species, which had echinoderms in its gut. However, rather small numbers of echinoderms don't confirm the hypothesis considering the East Atlantic peacock wrasse an echinoderm specialist. Another noticeable pattern is visible in the gut content of *Symphodus rostratus*. The smaller individuals, until 10 cm, only feed upon crustaceans like expected, but above 10 cm we found a high percentages of fish remainings in their gut. When *S. rostratus* is getting older it obviously changes its diet. This is not mentioned in any paper before as far as we know and it is not explained with their synergistic behavior, because normally they feed on the ejected food of *S. tinca*. One explanation could be, that they feed, if they are big and fast enough, on other small fishes, which are attracted by the ejected food.

### Length-weight relationship

Length-weight relationships provide useful information for fisheries biologists to estimate stock biomass and to calculate the weight at a certain age.

We can also demonstrate seasonal variations in fish growth and they allow us to compare life history and morphological aspects of populations inhabiting different regions (Ilhan et al. 2008). In our study we found *Symphodus tinca*, which had a size range between 5.6-21 cm, with an mean of 13.4 cm. Pallaoro et al. (2003) found individuals with about double the size (up to 42 cm), but only some single individuals in this studies had a size above 25 cm and in this paper, it is also mentioned, that other authors didn't find individuals longer than 35 cm. But also the mean of the length range is much bigger (21.17 cm) in these studies. The length-weight relationship on the other hand is comparable to other studies (exponent now:  $b=2.98$ , Pallaoro et al. (2003): 2.81, Ilhan et al. 2008: 2.90). That is maybe due to the fact, that we did not measure a sufficient number of individuals. Also the season can play a role. We caught the fishes in the end of summer; Pallaoro et al. (2003) caught the fishes in the beginning of the reproductive season (April-May). This can affect the length-weight relationship insofar, that there are differences in condition and maturity. In addition, comparable studies are so far only published from the Adriatic Sea off the coast of Croatia and not yet from the Ligurian Sea. The temperature of different years can play a role, because it affects the physiology of the fish and biological production (food availability).

Since we sampled outside the spawning season we could not easily separate males and females, what may influence the results, because males can grow significantly bigger than females and maybe we caught more females than males. Gut fullness, disease and parasite load can also affect the b-value, but these factors were not considered. A parasite was found on most *Coris julis* in different severities, but this was not affecting the b-value



in this case. Another factor that could lead to a smaller mean size is that by catching fishes with hand nets, larger individuals are generally more difficult to get. All of the length-weight relationship results are supported by an excellent effect size ( $r^2$  from 0.85 – 0.99).

The size range from *Symphodus ocellatus* was much smaller than those of *Symphodus tinca*. It was between 5.5 -9.5 cm. Furthermore, this size range agrees very well with those found in previous studies (3.9-9.1 cm). The length-weight relationship ( $b= 2.73$ ) is also comparable to other studies like Dulcic (1996) ( $b=2.93$ ). For the ocellated wrasse, we examined more individuals than for all other species, what led to the highest accuracy.

The size range of *Symphodus rostratus* (6.3-11.8 cm) agrees very well with other studies (Dulcic 1996): 7.0-11.4 cm). It is comparable to the size range of *S. ocellatus*. The length-weight relationship is higher ( $b= 3.43$ ) than those of *S. tinca* and *S. ocellatus*, but a similar number can be found in previous publications (Ilhan et al. 2008:  $b= 3.292$ ).

The last species analyzed was *Coris julis*. Its size is between the other species with a mean of 11.7 cm. Fishes found in previous analyses were smaller than our samples (8.29 cm), and the size range is shifted (this study: 7.5-17 cm, Dulcic (1996): 4.3-15.3 cm). But the length-weight relationship is again, like in all other cases very similar to previous studies ( $b= 3.19$ ; Dulcic (1996): 3.23).

Characterizing predator-prey interactions is a very important component of ecosystem-level studies, particularly because some species will modify their diet in response to environmental change or perturbation. For an accurate interpretation of fish feeding patterns, a more detailed classification of the food items to a species level should be considered.

The different fish species could feed upon different prey species or functional groups within the same taxonomic unit without competing directly, as the classification by family could lead us to assume.

Furthermore, in order to be able to compare our data and the literature, and to increase the robustness of the relative importance of the prey, more parameters should be recorded and analyzed. These parameters are percentage by number, percentage by volume or weight, percentage of frequency of occurrence and IRI (Index of Relative Importance). The identification should be done to the lowest level possible. Another way to improve the data extraction is the use of molecular approaches to identify the food items. Using DNA amplification techniques will decrease the necessity of highly skilled and experienced personell to identify the organisms. This will decrease the time of analysis, since taxonomic identification is very time consuming and it will increase accuracy, since some body parts cannot be identified optically. The disadvantage is that the molecular approach is much more expensive and requires a laboratory infrastructure. Furthermore, some previous knowledge is required to use the right molecular markers to identify the expected groups (Carreon-Martinez & Heath 2010).

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