This handbook was compiled to accompany the seventh edition of the study unit BIO3060 Field Biology with the theme ‘Coastal and Marine Biology’. The first edition of BIO3060 was generously supported by UNESCO, through the National Commission for UNESCO, by means of a grant under the UNESCO Participation Programme for 2002-2003 towards a project titled: ‘Setting up a practical marine biology course as a pilot project within the University of Malta’ reference 183 342 04 MAT.

UNESCO’s contribution is gratefully acknowledged.
CONTENTS

1. Study unit description
2. Introduction
3. General notes
4. Course contents
   General introduction
   Sample collection dive
   Briefing on course contents
   Introduction to Mediterranean marine ecology (with special reference to
   the Central Mediterranean).
   Topic 1 – Adlittoral habitats
   Lecture: Adlittoral macrophytic vegetation on rocky shores of the
   Maltese Islands
   Practical 1: Analysis of the spatial distribution of plants on a rocky shore
   Topic 2 – Rocky shores
   Lecture: Ecology and biota of rocky shores and other hard substratum
   shore types.
   Practical 2: Rocky shores
   Annex 1 Distinguishing between barnacles, limpets and top-shells of
   Maltese shores
   Annex 2 - The Thomas Exposure Index for Maltese shores
   Topic 3 – Sandy shores
   Lecture: Ecology and biota of adlittoral habitats and sandy shores.
   Practical 3: Sandy shores
   Topic 4 – The shallow sublittoral
   Lecture: Ecology and biota of sublittoral habitats
   Practical 4: Shallow infralittoral habitats
   Topic 5 – Offshore sampling of benthic biota and plankton, and physico-
   chemical characteristics of the water column
   Lecture: Mediterranean plankton, nekton and physico-chemical
   characteristics of the water column.
   Practical 5: Water chemistry and identification of plankton and benthic
   fauna (collected by remote sampling)
1. STUDY-UNIT DESCRIPTION

| Credit value: | 4 Credits |
| Department:   | Biology   |
| Faculty:      | Science   |
| Course:       | B.Sc. (Hons) and others. |
| When offered: | September before Semester 1 of Year III of BSc (Hons) course. |
| Method of teaching: | 12 hours of lectures; 5 fieldwork sessions of 3 hours each; 5 laboratory sessions of 3 hours each. |
| Assessment:   | 100% by practical reports. |
| Pre-requisites: | BIO1020, 1030, 2040; SOR1211, 1221 or equivalent units; pre-requisites may be waived with prior permission. |
| Tutors:       | V Axiak, J A Borg (Course Coordinator), A Deidun, E Lanfranco, S Lanfranco and P J Schembri. |

2. INTRODUCTION

With a coastline of some 190km and a submerged area within the 100m-depth contour of ca 1,940km², the Maltese Islands present practically all the different adlittoral, littoral and shallow sublittoral habitats characteristic of the central Mediterranean. Additionally, the islands’ environment, history, culture and economy are intimately connected with the sea. It is therefore appropriate that the coastal and marine environments of the Maltese Islands should be studied as important components of the islands’ ecosystem, while they are ideal natural laboratories for field biology in general.

This short, intensive course in practical field biology as applied to coastal and marine systems is designed to familiarise students with the methodologies used in the study of these environments and the practical application of these techniques, as well as the organisms and habitats of the Maltese coastal zone including the shore and the seabed and waters off it. This course is primarily aimed at undergraduate biology students, but may also be followed with benefit by others. Because the course is intensive and heavily fieldwork-based, it cannot be offered when the university is in session as it would interfere with other academic activities, and it is therefore offered during the summer recess, also because summertime is ideal for fieldwork for weather considerations.
Objectives

1. To give students the opportunity to supplement the theoretical component of their biology course with practical work using a variety of standard fieldwork techniques and equipment used to study, sample and monitor coastal and marine habitats and their biota.

2. To acquaint biology students with the range of habitats constituting the coastal and marine environments of the Maltese Islands, including the terrestrial maritime fringe, sandy beaches, rocky shores, sublittoral hard substrata, sedimentary bottoms and seagrass meadows, shallow coastal waters, and the biota they support.

3. To instruct students in the use of such equipment as quadrats, corers, grabs, dredges, meters for in-situ measurement of physical and chemical parameters, and water samplers, and to give students hands-on experience using such equipment in the field as well as analysis of samples in the laboratory.

4. To familiarise students with the taxonomic identification of various groups of coastal and marine plants and animals, including terrestrial coastal biota, benthos, nekton and plankton.

Programme

The course will run for a period of 6 days. The first day will consist of a briefing on the whole course and introductory lectures. Following that, each day will start with a short briefing on the practical session of the day, followed by fieldwork and subsequent laboratory processing. Each day will end with a short discussion session to review and integrate the day’s academic work, followed by an introductory lecture to set the next day’s practical sessions in context. Fieldwork will mainly focus on exposing students to different coastal and marine habitats and the techniques used to study and sample them. Laboratory work will concern processing of samples, analysis of environmental samples, and species identification. The table below gives an outline of the course components for the session running during the period 16-24 September 2010.
<table>
<thead>
<tr>
<th>Date</th>
<th>Morning</th>
<th>Lunch break</th>
<th>Lectures</th>
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<td>14.00 – 15.00 Introduction to the course Lecturers:  Prof P J Schembri &amp; Dr J A Borg</td>
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<td>15.00 – 16.00 Introduction to Mediterranean marine ecology (with special reference to the Central Mediterranean) Lecturer: Prof P J Schembri</td>
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<td>16.00 - 17.00 Adlittoral macrophytic vegetation on rocky shores of the Maltese Islands Lecturer: Dr S Lanfranco</td>
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<td>Briefing</td>
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<td>17-9-10</td>
<td>9.00 - 9.30 Fieldwork in the terrestrial</td>
<td>9.30 - 12.30</td>
<td>14.00 – 15.00 Ecology and biota of rocky shores and other hard substratum shore types Lecturers:  Prof P J Schembri &amp; Dr J A Borg</td>
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<td></td>
<td>environment (Dr  S Lanfranco)</td>
<td>13.00 to 14.00</td>
<td>15.00 - 17.30 Identification of specimens; data processing (terrestrial ecology) (Dr S Lanfranco)</td>
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<td></td>
<td>9.00 - 9.30 Fieldwork on rocky shores</td>
<td>9.30 - 12.30</td>
<td>14.00 – 15.00 Ecology and biota of adlittoral habitats and sandy shores Lecturer: Dr A Deidun</td>
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<td></td>
<td>(Prof P J Schembri)</td>
<td>13.00 to 14.00</td>
<td>15.00 - 17.30 Identification of specimens; data processing (rocky shore ecology) (Prof P J Schembri &amp; Mr E Lanfranco)</td>
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<td>20-9-10</td>
<td>9.00 - 9.30 Fieldwork in the adlittoral</td>
<td>9.30 - 12.30</td>
<td>14.00 – 15.00 Ecology and biota of shallow sublittoral habitats Lecturers: Mr E Lanfranco &amp; Dr J A Borg</td>
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<td>and on sandy shores (Dr A Deidun)</td>
<td>13.00 to 14.00</td>
<td>15.00 - 17.30 Identification of specimens; data processing (sandy shore ecology) (Dr A Deidun)</td>
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<td>22-9-10</td>
<td>9.00 - 9.30 Fieldwork in the shallow</td>
<td>9.30 - 12.30</td>
<td>14.00 – 15.00 Mediterranean plankton, nekton, and chemo-physical characteristics of the water column Lecturer: Prof V Axiak</td>
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<td>sublittoral (Dr J A Borg)</td>
<td>13.00 to 14.00</td>
<td>15.00 - 17.30 Identification of specimens; data processing (sublittoral ecology) (Dr J A Borg)</td>
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<td>23-9-10</td>
<td>9.00 - 9.30 Plankton, nekton and water</td>
<td>9.30 - 12.30</td>
<td>14.00 - 16.30 Identification of specimens; water chemistry; data processing (remote sampling of benthos, and plankton and water physico-chemical parameters) (Prof V Axiak, Dr J A Borg &amp; Prof P J Schembri)</td>
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<td></td>
<td>chemistry (Prof V Axiak)</td>
<td>13.00 to 14.00</td>
<td>16.30 - 17.30 Conclusion (forum) (Dr J A Borg &amp; Prof P J Schembri)</td>
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<td>24-9-10</td>
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Notes on the fieldwork sessions

Day 2 – Adlittoral habitats
Students will study the terrestrial plants of the maritime fringe in order to familiarise themselves with the species. They will use quadrat sampling to determine the spatial distribution of plants on rocky terrain in the adlittoral zone. In the laboratory they will analyse data collected in the field to obtain an estimate of the abundance of a selected species and to estimate resource usage of this species.

Day 3 – Rocky shores
This will consist of a transect study of the zonation of biota on local rocky shores with different characteristics. Quadrats will be used to study the distribution and abundance of the biota on the shore while representative samples will be collected for laboratory identification.

Day 4 – Sandy shores
Students will collect core samples from stations along the shore and will sieve out the biota for later identification in the laboratory. They will also investigate the biota of the upper infralittoral and will take sediment samples for granulometric analysis in the laboratory.

Day 5 – Shallow water sublittoral habitats
Students will be required to map the spatial distribution of shallow sublittoral benthic habitats along shore-normal transects using snorkelling techniques. Quadrat samples of infralittoral biota will also be collected for sorting and identification in the laboratory.

Day 6 – Sampling of offshore plankton, benthic biota and water. Students will go on a boat and will collect samples of plankton and benthic biota using appropriate remote sampling equipment. The samples will be later analysed in the laboratory. They will also collect water samples for later chemical analysis in the laboratory as well as measure physico-chemical parameters 'on site' using appropriate meters.

Notes for participants

This study-unit is intended for students who must be prepared to attend all sessions of this intensive course and who must be physically fit and preferably good swimmers. Special arrangements can be made to accommodate non-swimmers or those persons with special needs but such persons need to identify themselves when they register for the course.

Students must provide themselves with appropriate clothing especially boots and plimsolls for use on the boat and a light waterproof for use on the boat and on the shore if necessary. Students must also take precautions to protect themselves from the sun. Lifejackets will be provided by the Department of Biology for use on the boat.

Students also need their own masks, snorkels and fins for use during the sublittoral practicals.

Students who are SCUBA divers need to be in full conformity with national and University requirements for diving, including the compulsory purchase of diving insurance, and need to bring their own equipment.
It is strongly recommended that participants bring with them to the laboratory sessions a copy of the following work:


As the above text is in Italian, participants may want to acquire the following work in English, which although it does not focus specifically on Mediterranean fauna and does not cover flora, it nonetheless provides excellent keys for the identification of the major groups of fauna that are expected to be encountered during fieldwork.


Students will also need notebooks appropriate for use on the shore and at sea (i.e with waterproof covers), writing implements, dissecting kit, dividers, a strong knife or penknife, and a calculator. A detailed list of items required is provided in the practical schedules that form part of this document and well as a separate list on the Department of Biology's website.

Students need to take out insurance cover to participate in this study-unit, (separately from any diving insurance that will be required if they plan to SCUBA-dive). This insurance will be facilitated by the Department of Biology but must be paid for by the students.

3. GENERAL NOTES

Make sure you bring the following items for the fieldwork/laboratory practicals:

- Field notebook & waterproof bag.
- Laboratory notebook.
- Any identification guides you may have.
- Pens/pencils.
- Shoes or old trainers that you don’t mind getting wet (but do not wear sandals and the like which do not offer good protection while walking on rocky shores).
- Light waterproof jacket (& trousers if you have them) for use on board the vessel during the offshore fieldwork and if it rains.
- Appropriate clothing, depending on weather conditions (e.g. light clothing in hot weather) and a swimming costume which you should bring to ALL fieldwork sessions, but especially those that involve snorkelling.
- Mask, snorkel and fins, and a diver writing slate for the sublittoral field sessions.
- If you plan to dive, full diving gear, including a 10-12 L cylinder.
- Hat/cap and sun-screen cream.
• Water/drinking fluids EXCEPT alcoholic drinks.
• Sea sickness tablets (for use on board the vessel during the offshore fieldwork session if necessary)

However, don’t bring more than you can carry!

Since in recent years there has been an abundance of jellyfish in local inshore waters, it is strongly recommended that, apart from taking precautions not to get stung in the first place, students bring with them medication against jellyfish stings, especially if they are susceptible to their toxin. If you have a particular allergy towards jellyfish and insect stings, please inform the course coordinator before the course.

Participants are expected to respect living organisms. Collection of specimens is to be undertaken strictly under the supervision of a demonstrator/academic member of staff. Particular care should be taken not to damage or kill species that are rare or endangered and protected species are not to be collected or damaged in any way.

Although fieldwork will obviously be outside the University, nonetheless, this is part of a formal University course and all rules of conduct that apply to activities within the University precincts apply to fieldwork as well. Please remember that you are representing the University of Malta and therefore safeguard the good name that the University enjoys in the community.

Health and safety
Participants are requested to read the Department’s ‘Safety Precautions and Code of Practice’, be fully aware of its contents, and follow the guidelines scrupulously during field and laboratory work.

Misconduct
Serious misconduct will be subject to the Student Disciplinary Procedure of the University.

Transport
Participants are expected to provide their own transport. Those who do not have their own transport for fieldwork sessions or cannot arrange a lift are requested to inform the course coordinator in good time.

Support from Demonstrators and Laboratory Officers
All work related to this study unit is to be carried out by the participants. Therefore, participants should not expect Demonstrators and Laboratory Officers to do work for them. Demonstrators are available to supervise field and laboratory work, and to demonstrate the use of specialized equipment and techniques. Laboratory Officers are available to provide advice on resources during field and laboratory work, technical support for provision and use of equipment, and when faulty apparatus is encountered.
4. COURSE CONTENTS

Optional sample collection SCUBA dive
Dr Joseph A Borg

SCUBA diving is an invaluable technique for biological studies in the marine (and sometimes freshwater) environment. Using SCUBA techniques, the biologist can gather:

(i) qualitative data, e.g. using direct observation, and possibly record such observations permanently on video or photographic film;
(ii) semi-quantitative data, e.g. recording percentage cover of flora and sessile fauna, and density counts of motile fauna (e.g. fishes); and
(iii) quantitative data, e.g. by collecting samples using quadrats and corers.

However, SCUBA diving has considerable limitations, since the safe diving limit using sports diving equipment and normal air is around 40m. Using special air mixtures and technical diving equipment, the limit of safe diving can be extended to around 70-80m. To be able to practice sports diving, an individual is required to attend a SCUBA diving course organized by a professional diving school or a non-profit diving club. In any case, biologists using SCUBA diving should be fully aware of the limitations and dangers that are associated with this technique. Furthermore, the use of technical diving requires a high level of skill, training and experience, and should certainly not be attempted by the novice diver.

The aim of this session is to allow students who are qualified SCUBA divers to participate in a scientific dive on Thursday 23-9-10 (towards the end of the sublittoral field session). During the session, a demonstration of the applications of scientific diving to field biology will be made.

BIBLIOGRAPHY


BRIEFING ON COURSE CONTENTS

Dr Joseph A Borg & Prof Patrick J. Schembri

This briefing is intended to give final details of the course programme and to answer any queries brought forward by the participants. Emphasis will be made of safety aspects and material and equipment which the participants need to bring with them.

INTRODUCTION TO MEDITERRANEAN MARINE ECOLOGY (WITH SPECIAL REFERENCE TO THE CENTRAL MEDITERRANEAN).

Lecturer: Prof Patrick J. Schembri

THE MEDITERRANEAN SEA

The Mediterranean Sea covers an area of some 2.5 million square kilometres, excluding the Black Sea, and has an average depth of about 1500m. As a sea, it is rather unique in that it is almost completely surrounded by land: Europe to the north, Africa to the south, and Asia Minor to the east.

The Mediterranean as a whole is a silled basin, separated from the Atlantic Ocean by a shallow sill of ca. 320m depth at the Straits of Gibraltar. It is itself composed of a series of basins separated by sills, the principal ones of which are: the two sills separating the Black Sea from the Aegean Sea; a sill at the Straits of Otranto separating the Adriatic Sea from the East Mediterranean, and the Siculo-Tunisian sill close to which lie the Maltese Islands, dividing the Mediterranean into an East and a West Basin.

The Mediterranean Sea has three principal kinds of water: Atlantic surface water of normal salinity (36.5 psu)\(^1\) enters the West Basin through the Strait of Gibraltar and moves eastwards in an anti-clockwise direction; this is the MEDITERRANEAN SURFACE WATER (0 - 200m deep). The surface circulation is greatly influenced by the topography of the coastline and seabed and is not simple, but gives rise to a number of gyres\(^2\). In the East Basin, the high air temperature and the low humidity cause a high evaporation from the Mediterranean Surface Water resulting in increased salinity (in excess of 38 psu). During summer, a thermocline forms preventing this dense saline water from sinking, however, in winter, a combination of cold air temperature and mixing of the surface water due to storms, destroys the thermocline. The Mediterranean surface water thus sinks to become the MEDITERRANEAN INTERMEDIATE WATER (200 - 600m deep), which flows westwards and passes back into the Atlantic over the Gibraltar sill. The turnover time from entry as surface water until it is expelled back into the Atlantic as the MEDITERRANEAN OUTFLOW is of the order of 80-100 years.

\(^1\) psu = practical salinity unit; for ordinary use, more or less equivalent to parts per thousand.
\(^2\) gyre = a closed circulatory system.
In certain places (Gulf of Lions in the West Basin and southern Adriatic in the East Basin), the low winter air temperatures combined with very cold, dry winds that blow in the area, cool the surface water and increase its density. This water then sinks to depths greater than 600m to give the MEDITERRANEAN DEEP WATER. Mixing of Mediterranean Deep Water from the West and East Basins is prevented by the Siculo-Tunisian Sill, which is only just over 400m deep at its deepest.

The Mediterranean generally has tides of very low amplitude (on average, about 30cm). Two exceptions are the Gulf of Gabes and the Adriatic, where the tidal amplitude is between 1.5m and 2m. Surface water temperature varies with the season and ranges from 12-16°C in the East Basin. Surface salinity increases from east to west as already mentioned and may be as high as 39 psu in the Levantine basin\(^3\) - the Mediterranean being one of the most saline seas known. The Mediterranean is well oxygenated but poor in nutrients since over 95% of its waters are derived from the already impoverished Atlantic surface water. Except for areas of local enrichment, such as the mouths of rivers, coastal waters receiving terrestrial run-off, and where sewage is discharged into the sea, primary production\(^4\) is low. For this reason, there is little plankton in the Mediterranean, which gives the sea its characteristic transparency and blue colour.

GEOLOGICAL HISTORY OF THE MEDITERRANEAN

To understand the geography and biogeography of the Mediterranean, knowledge of its geological evolution is essential. The origins of the present day Mediterranean can be traced to the Triassic (ca. 200 million years before present = 200 My BP).

The first ancestor of what we call today the Mediterranean was the TETHYS SEA consisting of an open shallow water basin in the supercontinent PANGAEA. Typical shallow water sediments of that age are still found in places around the Mediterranean Sea. In the Jurassic (ca. 150 My BP), there was a period of intense ocean rifting in an East–West direction along the Tethys, which produced the first outlines of Europe and Africa. Later in the Jurassic (ca. 135 My BP), initiation of rifting between Africa and S. America produced the first South Atlantic sea floor and started the anticlockwise rotation of Africa toward Europe. This reversed the rifting process and converted the PALAEOMEDITERRANEAN\(^5\) into a subduction zone. The original Tethys ocean floor gradually began to be consumed as it was subducted under the Eurasian plate. Much volcanism was produced and most of today's volcanic activity in the Mediterranean is associated with this subduction process, as is most seismic activity.

By the Cretaceous (ca. 65 My BP), tectonic movements progressively pushed the African landmass towards the Eurasian landmass until almost all the Tethys ocean crust was destroyed. A pocket of water was gradually pinched off - the forerunner of the Mediterranean, or Paleomediterranean - and the Paleomediterranean became a zone of continent-continent collision. The collision front between Africa and Europe followed roughly a line passing through the present N. African coast, N. Sicily, the Apennines,

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\(^{3}\) Levantine Basin: the easternmost part of the Mediterranean.

\(^{4}\) primary production: the synthesis of organic material starting from inorganic substances.

\(^{5}\) Palaeomediterranean: the forerunner of the present day Mediterranean.
down through the Dinaric Alps and the Hellenic chain. This collision initiated the main phase of Alpine orogeny (mountain building) around the Mediterranean, with great fold mountains composed of marine sediments and parts of African and European crust. The collision process continues to the present day.

Subduction of part of the African plate under the European plate in the Eastern Mediterranean produced the Greek volcanic arc, and thinning of the crust on the European side probably led to subsidence of the Aegean plate, forming today’s shallow Aegean sea.

In the late Oligocene and Early to Middle Miocene (28-10 My BP), there was development of new oceanic type basins in the Western Mediterranean - the Balearic and Tyrrenhian Basins. The opening of the Tyrrenhian basin produced the anticlockwise rotation of the Italian peninsula to its present position.

Deposition of the sediments making up the Maltese islands took place during this period (Late Oligocene – Miocene). The islands were uplifted above sea level about 10 My ago, probably coincident with opening of the PANTELLERIA RIFT, southwest of the islands.

During the Late Miocene (ca. 6-7 My BP), closure of the Strait of Gibraltar led to almost complete evaporation of the water in the Mediterranean Basin (the Messinian Salinity Event). This precipitated a layer of evaporitic sediments, which is found at about 100m below the surface of today’s sediments. The increased salinity led to the extinction of the marine biota in the Mediterranean and to the incision of deep gorges at river mouths due to increased velocity of the river waters falling over the steep sides of the dry basin.

In the Pliocene (ca. 5.5 My BP), re-opening of the Straits of Gibraltar caused the re-flooding of the Mediterranean. A new population of marine biota from the Atlantic was introduced. Thus, the pre-evaporitic fossils (including Maltese fossils) are different from the post-evaporitic ones.

During the Pleistocene (1.8 My to 10,000 y BP) the sea-level in the Mediterranean was affected by the European glacial/interglacial cycles. Glacials corresponded to lowering of the sea level (regression) with probable land connections established between Malta and Sicily, and migration of terrestrial Pleistocene biota to Malta.

THE DIVERSITY OF MEDITERRANEAN MARINE LIFE

Since ancient times, the rich marine life of the Mediterranean has attracted the attention of both coastal people and of scholars. Coastal people were interested in the sea life for practical reasons: fisheries were (and in many cases still are) important in the economy of the coastal states and islands of the Mediterranean. Students of nature found the variety of Mediterranean marine plants and animals, and their adaptations, fascinating. Aristotle of Stagira, who lived between 384 BC and 322 BC was the first great observer of Mediterranean marine life and he not only described hundreds of marine organisms but also understood many biological phenomena. For example, he studied the anatomy of dolphins and realised that they were mammals, not fish; he described vivipary in

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6 vivipary: the bearing of fully formed young as opposed to ovipary, the laying of eggs.
certain sharks (e.g. the Smooth-hound *Mustelus* [Mazzola]); he discovered that some fish (e.g. the Comber *Serranus* [Burqax]) initially only occur as females and that some of the older ones then transform into males; he also realised that in the octopuses, one arm of the male is specialised for use as a copulatory organ.

**ORIGINS AND AFFINITIES OF THE MEDITERRANEAN MARINE FLORA AND FAUNA**

The present day flora and fauna of the Mediterranean is composed of four elements. A large number of Mediterranean species (ca. 62%) are also found in the East Atlantic. Biogeographically therefore, the Atlantic and the Mediterranean form one unit known as the **Atlanto-Mediterranean province**. A number of species (ca. 29%) are **endemic** to the Mediterranean, that is, they are only found in this sea; a few (ca. 13%) are cosmopolitan, that is, they are found in most of the world’s oceans, while a small number (ca. 5%) are Indo-Pacific. This mix of species is a result of the evolutionary history of the Mediterranean.

As already described above, some 65 My BP the Mediterranean (or what was to become the Mediterranean) was part of a stretch of ocean called the Tethys Sea which connected the Atlantic to the Indo-Pacific. The Tethys Sea was populated by a rich subtropical biota of Indo-Pacific affinity. When the Palaeomediterranean became totally cut off from both the Atlantic and the Red Sea about 6 My BP, climatic changes caused the Palaeomediterranean to partially or totally dry up resulting in the mass destruction of nearly all the original marine biota, an event known as the **Messinian Salinity Crisis** or **Messinian Salinity Event**. Subsequently, further tectonic movements re-established a connection with the Atlantic at the Strait of Gibraltar around 5 MY BP and the Mediterranean was repopulated by Atlantic species, this being the reason why the bulk of the present day Mediterranean species are also found in the East Atlantic.

In very recent times (1869), a link with the Red Sea was re-established via the Suez Canal and this has resulted in a number of species migrating from the Red Sea into the eastern Mediterranean, a process which has been called **Lessepsian migration** after the French diplomat Ferdinand de Lesseps who was largely responsible for the development of the Suez Canal. The present day Indo-Pacific element of the Mediterranean is mainly represented by these recent immigrants; however, a few species may be relicts from the days of the Tethys Sea, for example, the Neptune Grass *Posidonia oceanica* [Alka].

**SPECIES DISTRIBUTION PATTERNS**

The Mediterranean is divided into two great basins, the West Basin and the East Basin, separated by a shallow sill (the **Siculo-Tunisian Sill** with maximum depth ca. 430m) running from Sicily to Tunisia. While close to this sill, the Maltese Islands do not actually lie on it but are geographically part of the East Basin. There is a pronounced difference between the two Mediterranean basins in floral and fauna diversity: the number of species is much lower in the eastern than in the western Mediterranean, the most impoverished region being the south-eastern corner - the Levant or Levantine Sea. This is probably because the warmer and more saline eastern Mediterranean is inhospitable to the majority of Atlantic-derived species. Species abundance also falls from west to
east. Many West Basin species reach their easternmost limit of distribution in the Central Mediterranean, for example, the mussel *Mytilus galloprovincialis* [Masklu] and the precious coral *Corallium rubrum* [Qroll Ahmar]. There are also some differences in species numbers and abundance from north to south: the northern waters of the Mediterranean generally being richer than southern waters, again probably an effect of increasing temperature as one proceeds south. On the other hand, some species common in the eastern Mediterranean are very rare or not found at all in the West Basin, while others are found only along the southern shores. Examples of the former include the starfish *Hacelia attenuata* [Stilla Hamra Lixxa] and the long-spined sea-urchin *Centrostephanus longispinus* [Rizza tax-Xewk Twil], while an example of the latter is the stony coral *Astroides calycularis* [Qroll tad-Dell]. For species such as these then, the Central Mediterranean is again on the border of their distribution. Some of the recent Lessespsian immigrants from the Red Sea, finding the warm saline and underpopulated Levant Sea a suitable environment, have established themselves there and are slowly spreading westwards. One example is the sea-grass *Halophila stipulacea* which entered the Levant Sea soon after the Suez Canal was opened (it was first recorded from the Mediterranean in 1885) and has since progressively colonised the East Basin and has reached the Maltese Islands in 1971. Their position makes the Maltese Islands particularly interesting from a biogeographical point of view since they are close to the region where the several different Mediterranean sub-regions meet. Our knowledge of the Maltese marine fauna and flora is still poor; however, the few studies that have been made have shown that our marine life includes components from both the western and eastern regions as well as some North African forms, as expected. There is only one marine endemic species known: the Maltese Top-shell *Gibbula nivosa* [Gibbula ta’ Malta] a species known only from a few inshore localities.

### ‘TROPICALIZATION’ OF THE MEDITERRANEAN

Over the past couple of decades, changes in species occurrence and distribution patterns in the Mediterranean Sea have occurred at unprecedented rates. New alien\(^7\) thermophilic\(^8\) species have entered and established themselves in the Mediterranean from the Indo-Pacific (via the Suez Canal) and the subtropical Atlantic (via the Strait of Gibraltar); already established non-indigenous species that were limited to particularly warm corners of the Mediterranean have expanded their range, and indigenous thermophilic species have done likewise. On the other hand, less thermophilic species have contracted their range to now occur in the coldest parts of the sea. This process has been called the ‘tropicalization’ of the Mediterranean and has been linked to an observed warming of Mediterranean surface waters, but remains poorly understood.

The ‘tropicalization’ process has resulted in new species appearing in Maltese waters, including Lessespsian immigrants previously limited to the Levantine Sea as well as new immigrants from the Atlantic and the Red Sea.

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\(^7\) alien = not native = non-indigenous = exotic = not naturally occurring in the Mediterranean Sea.

\(^8\) thermophilic = species adapted to live in warm waters, usually taken as a winter temperature of not less than 15°C.
MEDITERRANEAN MARINE COMMUNITIES

From the biological point of view, the Mediterranean marine environment may be divided into several zones depending on the relative contributions of three key physical factors: light, wetness and pressure. The uppermost region is the Supralittoral zone, which is permanently exposed to the air except for occasional wetting by sea spray and the highest waves. Next comes the Mediolittoral zone, which is regularly exposed and submerged, mainly due to wave action. This zone corresponds to the intertidal zone of tidal shores, however, in most of the Mediterranean, the tidal range is small (average of 0.06m in the Maltese Islands) and changes in sea level brought about by wave action and atmospheric conditions are far more important. The next zone, the Infralittoral, extends from the lower limit of the Mediolittoral, which is never (or hardly ever) exposed, down to the lowest limit where sea-grasses and photophilic algae can live. The lower limit of this zone depends largely on light penetration, but on average lies at ca. 40m depth. Next comes the Circalittoral, which extends from the lower limit of the Infralittoral down to the maximum depth at which plant life of any sort can live, in practice about 200m. All these zones collectively make up the phytal or littoral system. Below this is the aphytal or deep water system, which is poorly known in the Mediterranean. The great attraction of this system of classification of the marine environment is that the various zones are not delimited solely by bathymetry but also by a consideration of the biota.

Within each zone of the phytal system, a number of subdivisions exist characterised by differences in ecological factors such as water agitation, salinity fluctuations, temperature, and the nature of the substratum. Of these, perhaps the most important is the last, giving for each zone two distinct assemblages of biotic communities: soft substratum communities and hard substratum communities.

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8 photophilic algae = those that require a substantial amount of light for photosynthesis.
TOPIC 1 – ADLITTORAL HABITATS

Lecture: ADLITTORAL MACROPHYTIC VEGETATION ON ROCKY SHORES OF THE MALTESE ISLANDS

Lecturer: Dr Sandro Lanfranco

Introduction
Gently sloping rocky shores outcropping on Lower Coralline Limestone represent one of the most characteristic coastal habitats of the Maltese Islands. Such environments are generally characterised by a karstified substratum colonised by a sparse, low-diversity aerohaline assemblage dominated by halophytic perennials.

Abiotic constraints
The principal abiotic constraints in these environments include the following:

1. Exposure to wind: geomorphologic relief on rocky shores is generally of low amplitude and much of the surface area is therefore not sheltered from wind. Consequences of exposure include increased rates of evapotranspiration, increased fallout of saline aerosol, increased rates of soil erosion and possible mechanical damage to vegetation.

2. Exposure to wave action: the direct effects of wave action are most evident in an elongated zone (henceforth referred to as the wave-zone) running roughly parallel to the shoreline. The breadth of the wave-zone (perpendicular to the shoreline) is a function of prevailing wind direction and wind speed, local topography and slope. The wave-zone is generally characterised by complete absence or very low density of macrophyte populations as well as comparative absence of loose sediment as a direct consequence of the mechanical influence of wave action, particularly during storm episodes. The intensity of wave action is also proportional to the extent of fallout of saline aerosol throughout the entire rocky shore habitat.

3. High soil salinity: proximity to the shore promotes fallout of saline aerosols derived from seawater into soil.

4. Shallow soil and absence of confluent soil cover: topsoil is largely absent as a result of erosion by wave action and generally confined to restricted accumulations of sediment in sheltered depressions. The confluence and depth of soil generally increases with increasing distance from the wave-zone. Shallow, discontinuous soils do not promote extensive vegetation cover due to restriction of suitable sites for anchorage of vegetation, reduced nutrient volume, reduced water content (and consequently, elevated salinity) and restricted volume of seed-banks.

Plant communities
The climax communities colonising rocky shores are generally consistent with a maritime steppe/garrigue dominated, in terms of biomass and coverage, by low halophytic shrubs. Much of the perennial biomass of such communities is contributed by a core framework of species including the following:
(1) Golden Samphire (*Inula crithmoides*)
(2) Sea Fennel (*Crithmum maritimum*)
(3) Shrubby Glasswort (*Arthrocnemum macrostachyum*)
(4) Sea Lavenders (*Limonium* spp.)

The relative abundance of these core species is obviously variable across sites and within sites although, in many cases, *I. crithmoides* has been observed to contribute more biomass than the other core species. Several other species, some of which may be relatively site-specific, also occur on rocky shores. These include Maltese Salt-Tree (*Darniella melitensis*) and Maltese Sea-Chamomile (*Anthemis urvilleana*), both of which are endemic to the Maltese Islands.

A variable suite of opportunistic species is generally superimposed on this perennial framework. The spatial and temporal infiltration of such species into the background community is dependent on the frequency and intensity of disturbance events. A high-amplitude disturbance event or a sustained low-amplitude disturbance may remove existing plants from the community releasing free habitat-space that would generally be exploited by opportunistic colonisers.

**Survival strategies**

The presence of a particular species in a rocky shore community is dependent on its physiological tolerance to the prevalent abiotic constraints and on its ability to harvest and retain resources in the presence of competing species.

The principal physiological constraint in vegetated zones close to the shoreline is osmotic stress. Species that colonise this zone are therefore K-selected halophytic specialists that are structurally or physiologically adapted towards the tolerance or reduction of such stresses. Such adaptations include succulent tissues (as in *Inula crithmoides*), very small leaves (as in *Arthrocnemum macrostachyum*) or restricting leaves to a basal rosette (as in *Limonium* spp.).

Further away from the shoreline, where soils are less shallow, more confluent and less saline, the physiological effects of osmotic stress would be less pronounced and species characterised by more generalised adaptations would compete for resources with the halophytic perennials.

**Zonation of vegetation**

The distribution of macrophytes along an axis perpendicular to the shoreline defines a number of general zones:

- (1) Wave-zone: generally characterised by complete absence of macrophytes.
- (2) *Arthrocnemum* zone: a narrow zone that may be situated 30m or 40m from the shoreline. Characterised by individual Shrubby Glasswort (*Arthrocnemum macrostachyum*).
(3) Crithmum-Limonium zone: the presence of these two species is generally taken to define a phytosociological association termed “Crithmo-Limonietum”. This zone is dominated (although not usually exclusively colonised) by Sea Fennel (Crithmum maritimum) and Sea Lavender (Limonium spp.

(4) Inula crithmoides zone: this is, in many rocky shores, the broadest halophyte zone and is indicated by higher abundance of Golden Samphire (Inula crithmoides).

(5) Generalist zone: usually situated well up away from the shoreline, where the effects of wave action are negligible and where accumulations of soil are more confluent. This zone is colonised by a wide variety of species, including several annuals, many of which may not be specifically adapted towards survival in saline environments. This is the broadest vegetation zone in the rocky shore environment and is also the one with the highest diversity of plants and the highest turnover of species.

It should be pointed out that the zonation referred to here is a broad, generalised model that may not be applicable to every such environment. In many cases, differences in slope or exposure would compress the zonation of vegetation with consequent merging of two or more zones.
INTRODUCTION

The distribution of plants across a landscape is seldom random and is generally dependent on the presence of other plants of the same or of different species, on environmental discontinuities and on the availability of resources. The habitat available to a plant may therefore be perceived as a bank of resources with each individual plant utilising a certain minimal amount of resources for its basic survival. Acquisition of larger quantities of the available resource would promote growth, flowering and subsequent reproductive success. Large, competitive plants would generally gain access to more of the resource whilst smaller or less competitive plants would require (or gain access to) fewer resources. Various morphological or physiological adaptations of plants (such as tolerance to saline soils) permit selective access to resources that may be underutilised by other species. As such, different parts of a habitat may be colonised by different species depending on their particular adaptations and consequent resource requirements. Patterns of species distribution would therefore be expected to follow resource gradients. The present investigation will deal with patterns of spatial distribution of plants on a rocky shore.

Plant community analysis

The results of field surveys are generally recorded in an occurrence matrix on which further analysis would subsequently be carried out. An occurrence matrix would usually comprise sample data in its rows and species data across its columns. Species data may be coded as abundance (counts of individuals or area of coverage) or in a presence/absence format. Abundance data is obviously preferable, since it conveys more information about the community under study. Furthermore, an abundance matrix may be converted into a presence/absence format but the reverse process is impossible. An example of an occurrence matrix is given below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glebionis coronarium</th>
<th>Oxalis pes-caprae</th>
<th>Erica multiflora</th>
<th>Teucrium fruticans</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>19</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>102</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
The same matrix may be coded in a presence/absence format as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>Glebionis coronarium</em></th>
<th><em>Oxalis pes-caprae</em></th>
<th><em>Erica multiflora</em></th>
<th><em>Teucrium fruticans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**OBJECTIVES**

The broad objectives of this field session are as follows:

1. Familiarisation with plant species present in the area of study
2. Analysis of species distribution in the adlittoral zone
3. Analysis of species diversity in the adlittoral zone
4. Analysis of population structure of Golden Samphire (*Inula crithmoides*)

**MATERIAL AND METHODS**

**Preparatory material**

Students are required to download the following freeware programs:

1. PAST (PAlaentological STatistics) from [http://folk.uio.no/ohammer/past/download.html](http://folk.uio.no/ohammer/past/download.html)


**Task 1: Identification of species**

1. Conduct a brief familiarisation survey in order to acquaint yourself with the most characteristic plant species present in the community under investigation.
2. Look for structure and arrangement of leaves, colour and structure of floral structures and general growth habit, all of which can be diagnostic in identification. You are being provided with a number of photographs of the most characteristic plants in the habitat being investigated as an aid towards *in situ* identification.

**Task 2: Distribution of species**

1. Use the table of random numbers at the end of this document to select a random point in the area of study. Record this point as S1.
2. Position the frame quadrat on the ground and note the species present within the boundary of the quadrat. Take note of the number of individuals belonging to each species.

3. Select another random point and note it as S2. Record the species present within the frame quadrat at S2 and the abundance of each.

4. Repeat this procedure until you have sampled 25 points across the area of study.

5. Record the data as an abundance matrix.

6. Enter the data as an abundance matrix in PAST and save this file as **matrix1.dat**

7. Use the *Edit>Transform* command in PAST to prepare a transposed version of the abundance matrix and save this file as **matrix2.dat**

**Task 3: Population structure of *Inula crithmoides***

1. This task shall be carried out in parts of the habitat in which Golden Samphire (*Inula crithmoides*) is present.

2. Select a point close to the edges of this area as your *reference origin*. Place a marker to identify your reference origin. This could be a large stone, a pile of stones, a pole, etc.

3. Mark a line 3m in length and starting from the reference origin. This line could be marked out using string or using a tape measure.

4. Mark a second line, equal in length to the first and perpendicular to it, also starting from the reference origin. These two lines will subsequently represent the *x* and *y* axes of a two-dimensional plane with their zero points at the reference origin.

5. Record the position (in metres) of each individual Golden Samphire plant as (*x*,*y*) coordinates relative to the reference origin. You may either do this by measuring the perpendicular distance between the plant and each axis or by measuring the perpendicular distance to one of the axes and the distance, *w*, to the reference origin (Figure 1). The second option will probably generate fewer errors of measurement since one of the points (the reference origin) is always fixed. In the latter case, the position along the axis that has not been utilised for direct measurement may be calculated as follows:

   If *x* and *w* have been measured:
   
   \[ y = \sqrt{w^2 - x^2} \]

   If *y* and *w* have been measured:
   
   \[ x = \sqrt{w^2 - y^2} \]

6. Measure and record the maximum height (in metres) of each plant. Call this value *h*.

7. Measure and record the approximate coverage of each plant. Do this by recording two measurements: the maximum breadth (in metres) of the plant and the longest axis (in metres) perpendicular to the maximum breadth. Call these values *c₁* and *c₂* respectively. This is illustrated in Figure 2.

8. Record your field data in the following format (some examples are given):
<table>
<thead>
<tr>
<th>Plant number</th>
<th>x (m)</th>
<th>y (m)</th>
<th>c₁ (m)</th>
<th>c₂ (m)</th>
<th>h (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.96</td>
<td>3.10</td>
<td>1.02</td>
<td>0.84</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>7.95</td>
<td>13.73</td>
<td>0.48</td>
<td>0.67</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**TREATMENT OF DATA**

**Task 1: Identification of species**

Compile a visual catalogue (photographic/graphic) of six plants encountered in the area of study. Each plant image should be accompanied by BRIEF notes (not more than 100 words) on its structure and adaptations. Please use appropriate botanical terms rather than sweeping general terms. Use of correct scientific nomenclature (i.e. correct binomials, correctly spelt) is imperative.

**Task 2: Distribution of species in the adlittoral zone**

**Detection of zonation of vegetation**

1. Open the file `matrix1.dat` using PAST.
2. Highlight all the data by clicking the cell in the top left-hand corner of the data entry screen.
3. Look for relationships between your samples by carrying out a Cluster analysis using the `Multivar>Cluster Analysis` command.
4. Look for relationships between your samples by carrying out a Principal Components Analysis using the `Multivar>Principal components` command. Choose the “View Scatter” option and tick the “Row Labels” check-box when the results screen appears.

**Task 3: Population structure of Inula crithmoides**

**Part 1: Basic data**

1. Enter your field data into a data analysis program of your choice in the following format (some examples are given):

<table>
<thead>
<tr>
<th>Plant number</th>
<th>x (m)</th>
<th>y (m)</th>
<th>c₁ (m)</th>
<th>c₂ (m)</th>
<th>Coverage (m²)</th>
<th>h (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.96</td>
<td>3.10</td>
<td>1.02</td>
<td>0.84</td>
<td>2.69</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>7.95</td>
<td>13.73</td>
<td>0.48</td>
<td>0.67</td>
<td>1.01</td>
<td>0.18</td>
</tr>
</tbody>
</table>

2. The value for Coverage in the table above is calculated as follows:

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9 Various statistical calculations may be carried out online at [http://statpages.org/](http://statpages.org/)

---
This assumes that the area covered by the plant (described by \(c_1\) and \(c_2\)) approximates an ellipse (Figure 2) and the formula given above therefore calculates the area of this ellipse.

3. Draw scatter diagrams showing the following relationships:
   (a) \(c_1\) with \(c_2\)
   (b) \(c_1\) with \(h\)
   (c) \(c_2\) with \(h\)
   (d) Coverage with \(h\)

4. Use Pearson’s Product-Moment Correlation Coefficient (available in most basic software packages) to calculate the correlation between the pairs of variables listed above.

5. Data from a previous study indicated that individual Golden Samphire plants may be classified into one of four height categories as follows:
   (a) Height Class 1: 14cm or less;
   (b) Height Class 2: between 15cm and 45cm;
   (c) Height Class 3: between 46cm and 65cm;
   (d) Height Class 4: taller than 65cm.

Using your data, construct a histogram showing the abundance of Golden Samphire individuals in each Height Class.

**Part 2: Patterns of spatial distribution**

1. Are the individual plants making up the population distributed at random? This hypothesis may be tested by calculating the T-square Index, a method that detects departures from randomness for individual plants within a sample area based upon measurements of nearest-neighbour distances or point-to-plant distances.

2. Rearrange your field data in the following format (some examples are given):

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>number</th>
<th>edge</th>
<th>species</th>
<th>radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.96</td>
<td>3.10</td>
<td>1</td>
<td>e</td>
<td>i</td>
<td>0.45</td>
</tr>
<tr>
<td>7.95</td>
<td>13.73</td>
<td>2</td>
<td>e</td>
<td>i</td>
<td>0.38</td>
</tr>
</tbody>
</table>

3. The values of “edge” and “species” are dummy variables in the context of the present investigation and may be entered as “e” and “i” for every plant. The value for “radius” is calculated as \((c_1/2)\). Save the data file in Comma-Separated Variable (CSV) format. Name this data file “distribution.csv”.

4. Construct a scatter diagram showing the distribution of plants in the population under study (an example from a previous study is given in Figure 3).

5. Print out the scatter diagram referred to above and select ten random points within the area of study bounded by the axes of the scatter diagram. Label these points \(R_0\) through \(R_9\). The coordinates representing these points may be obtained from a random number generator.
6. Start with $R_0$ and measure the distance, $j$, from $R_0$ to the nearest plant, $P$. Measure the distance $k$ from $P$ to the nearest neighbouring plant, $Q$ beyond the plane line at $P$ perpendicular to $R_0P$. This is clarified in Figure 4.

7. The distances $j$ and $k$ do not actually need to be measured manually but may be derived from the coordinates of $R_0$, $P$ and $Q$.

8. Let $(x_0, y_0)$ represent the coordinates of $R_0$, $(x_1, y_1)$ represent the coordinates of $P$ and $(x_2, y_2)$ represent the coordinates of $Q$. Distances $j$ and $k$ may be calculated as follows:

$$j = \sqrt{(x_1 - x_0)^2 + (y_1 - y_0)^2}$$

$$k = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

9. Repeat steps 6-8 for $R_1$ through $R_9$.

10. These values would then be used to calculate the value of the T-square $C$, an index of spatial pattern:

$$C = \frac{\sum [j^2 / (j^2 + k^2)]}{N}$$

Where $N$ is the number of random points taken (in this case, 10).

11. Values of $C$ of approximately 0.5 indicate random patterns, significantly less than 0.5 indicate uniform patterns, and significantly greater than 0.5 indicate clumped distributions. The significance of a departure of $C$ from 0.5 may be determined by calculating the test statistic $z$ as follows:

$$z = \frac{C - 0.5}{\sqrt{1/(12N)}}$$

Where $N$ is the number of random points taken (in this case, 10).

At $p=0.05$, $z=1.96$, so calculated test values exceeding this critical value indicate a significant departure of $C$ from 0.5.

Part 3: Representing spatial distribution using Voronoï tessellations

1. The neighbourhood of individual mapped plants can be studied by analysing the distribution of space among individual plants. One way of doing this is by dividing the mapped area into Voronoï tessellations or polygons. Each polygon describes the region of the mapped area closer to a plant than any other plant and may therefore be used to represent the resource area exploited by individual plants. More information about Voronoï diagrams may be obtained from http://www.beloit.edu/~biology/zdravko/voronoi.html

2. Use the data file “distribution.csv” as your input file and run the program VPPlants with Euclidean metric and Voronoï diagram. The output should produce a diagram similar to the example shown in Figure 5.

3. Press F12 to save the results file as “results.csv”. The variable DelArea in the output file represents the area of habitat occupied by each plant. Note that this does not represent coverage but indicates the area of influence of each plant.
4. Draw scatter diagrams showing the following relationships (the large polygons along the edges of the Voronoi diagram should be omitted from any analysis):
   a. \( c_1 \) with DelArea
   b. \( h \) with DelArea
   c. \( c_2 \) with DelArea
   d. Coverage with DelArea

5. Use Pearson’s Product-Moment Correlation Coefficient to calculate the correlation between the pairs of variables listed above.

DISCUSSION
1. Use your results to give a brief description of the community you have sampled. Identify any zonation of vegetation and correlate this with environmental factors (distance from shore, slope.)
2. Comment on the distribution of plant height in the \textit{Inula crithmoides} population in the area you sampled. Are the Height Classes used in Task 3 justified or should they be revised?
3. Is plant height a good predictor of the plant’s area of coverage?
4. Comment on the pattern of distribution of the \textit{Inula crithmoides} population in the sampled area. Is it random, clumped or uniform? Suggest reasons for the observed patterns of spatial distribution.
5. Is the area of influence of each individual plant correlated with the plant’s physical dimensions? Do small plants influence smaller areas than larger plants? If not, suggest any factors or processes that may be affecting the observed patterns of distribution.
# ASSESSMENT

Your report shall be assessed according to the following criteria:

<table>
<thead>
<tr>
<th>Organisation &amp; presentation</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>This refers to logical flow of ideas, accuracy and efficacy of graphical presentation and appropriate presentation of results. It does not refer to the ability to apply fancy fonts or novelty borders round pictures. Students should be aware that all graphs and histograms should be presented as 2-D images rather than as eye-catching 3-D diagrams with a false third dimension.</td>
<td></td>
</tr>
</tbody>
</table>

**Assessment criteria:**

- **20%:** Logical flow of ideas, graphics relevant and useful. Very few or no misspellings or grammatical errors;
- **15%:** Work that is well-structured; minor inconsistencies or incongruence in flow of ideas. Graphics mostly relevant and useful. Sporadic misspellings or grammatical errors;
- **10%:** Structure discernible. Graphics mostly relevant and useful. Frequent misspellings or grammatical errors;
- **5%:** Some self-contained structure present in document. Graphics may be repetitive or irrelevant. Frequent misspellings or grammatical errors;
- **0%:** No discernible structure; flow of ideas seems to follow little pattern. Frequent misspellings or grammatical errors.

<table>
<thead>
<tr>
<th>Accurate use of botanical nomenclature</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Students should ensure that botanical names are up-to-date, spelt correctly and formatted correctly.</td>
<td></td>
</tr>
</tbody>
</table>

**Assessment criteria:**

- **10%:** no errors in use of terminology and scientific names;
- **8%:** sporadic point errors in spelling or formatting of technical or scientific terms;
- **6%:** frequent point errors in spelling or formatting of technical or scientific terms;
- **4%:** use of inappropriate terminology; major errors in spelling and formatting of scientific names;
- **2%:** use of incorrect terminology and incorrect scientific names;
- **0%:** no usage of terminology and scientific names.

<table>
<thead>
<tr>
<th>Accuracy in calculations and precision of results</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>It is expected that calculations would be carried out using computer software. As such, the accuracy of results is dependent on accuracy in data entry. Students should ensure that any results that do not seem to make sense, that are illogical or that defy the laws of physics are double checked. Results of calculations should be</td>
<td></td>
</tr>
</tbody>
</table>
expressed to a suitable number of decimal places (usually two); avoid long and meaningless mantissas that have been copied and pasted straight from your spreadsheet program.

**Assessment criteria:**
- 10%: No apparent numerical errors; calculations logical; appropriate precision of numbers;
- 5%: No apparent numerical errors; calculations logical; precision of numbers not always appropriate;
- 0%: Several numerical errors; calculations may be incorrectly applied; precision of numbers seldom appropriate,

**Discussion of results and conclusions**
Use your skill as a scientist, as a biologist, to make sense of your observations and calculations. Your interpretation does not have to match mine but they do need to be defensible. Ensure that any predictions that you make are testable.

**Assessment criteria:**
- 30%: Content comprehensive, accurate and updated. All relevant points treated. Evidence of critical appraisal of method and results;
- 25%: Content wide-ranging, accurate and updated. Most relevant points treated. Evidence of critical appraisal of method and results;
- 20%: Content incomplete but accurate and updated. Many relevant points treated. Little evidence of critical appraisal of method and results;
- 15%: Content incomplete with some inaccuracies. Many relevant points omitted; Little evidence of critical appraisal of method and results;
- 10%: Content sketchy; several inaccuracies noted. Many relevant points omitted. Little evidence of critical appraisal of method and results;
- 0%: Content does not treat all material covered. Most relevant points omitted. No evidence of critical appraisal of method and results.

**Evidence of further reading**
This practical exercise should be viewed in its wider context and it is expected that your output is not merely a regurgitation of the material presented to you. Further reading will be credited as follows:

**Assessment criteria:**
- 20%: All material derived from primary sources or specialist secondary sources; comprehensive coverage of subject matter;
- 15%: Most material derived from primary sources or specialist secondary sources;
comprehensive coverage of subject matter;

| 10%: Most material is secondary; uncritical use of standard textbooks. May include uncritical use of unverified internet sources that may or may not be accurate; |
| 5%: Most material is secondary; extensive use of textbooks and unverified internet sources. Evidence of extensive cut-and-paste from Internet sources; |
| 0%: No evidence whatsoever of any further reading. |

**Referencing**

This is essential. Any statements of fact that you make need to be substantiated with a reference. Use the APA style sheet. Refer to instructions concerning its use at [http://home.um.edu.mt/biology/12_links.htm](http://home.um.edu.mt/biology/12_links.htm). Remember that web pages are also to be referenced.

**Assessment criteria:**

| 10%: Statements of fact substantiated with a reference; consistent use of a recognised style sheet. Correct formatting of citations and reference list; |
| 6%: Most statements of fact substantiated with a reference; use of an ad hoc style sheet. Inconsistent formatting of citations and reference list; |
| 2%: Little reference to sources; style sheet inconsistent. Inconsistent formatting of citations and reference list; |
| 0%: No reference to sources |

**A CAUTIONARY NOTE ABOUT USING MICROSOFT EXCEL**

Whilst Microsoft Excel is excellent for data entry and manipulation and for the calculation of simpler summary statistics, its suitability for more complex statistical calculations is not universally accepted. Please refer to the following documents in this regard:

- [http://home.um.edu.mt/biology/12_links.htm](http://home.um.edu.mt/biology/12_links.htm)
- [http://www.practicalstats.com/xlsstats/excelstats.html](http://www.practicalstats.com/xlsstats/excelstats.html)
- [http://www.mis.coventry.ac.uk/~nhunt/pottel.pdf](http://www.mis.coventry.ac.uk/~nhunt/pottel.pdf)
Figure 1: Determination of the position of an individual plant
Figure 2: Determination of the approximate coverage of a plant. The area covered by the plant is assumed to approximate an ellipse; $c_1$ and $c_2$ are the major and semi-major axes of this ellipse.
Figure 3: Scatter diagram showing distribution of Golden Samphire. The values on the x and y axes represent distances (in metres) from the reference origin.
Figure 4: Method for identifying points in calculation of T-square Index. \( R_0 \) represents a random point, \( P \) is the plant nearest the random point and \( Q \) is the nearest plant beyond the perpendicular at \( P \) to \( R_0P \).
Figure 5: Voronoï diagram showing area utilised by individual plants. The large, open-ended polygons at the margin of the diagram should be omitted from further analysis.
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TOPIC 2 – ROCKY SHORES

Lecture: ECOLOGY AND BIOTA OF ROCKY SHORES AND OTHER HARD SUBSTRATUM SHORE TYPES.

Lecturers: Dr Joseph A Borg & Prof Patrick J Schembri

THE SHORE

The shore is the transitional area between land and sea. The shore takes the form of a band, the width of which varies from place to place and is determined by the interaction of marine and terrestrial coastal processes, both natural and those due to human activities. There is a great diversity of views as to what constitutes the shore, both between and within disciplines. Thus ecologists, geomorphologists and economists all have different ideas of the limits of the shore. Ecologically, the shore can be regarded as that zone in which the marine environment grades into the terrestrial environment. Natural processes that affect the shore may be physical, chemical or biological in nature and include: coastal currents and sediment flows, which lead to coastal erosion or accretion; storm and wave conditions, which alter coastal profiles; sedimentation processes, which affect water and sediment quality; ecological succession, which leads to changes in habitat types and biodiversity; and energy and material cycles, which affect biological production.

ABIOTIC AND BIOTIC FACTORS

Several physico-chemical and biological factors influence the occurrence and composition of biotic assemblages on rocky shores. These include exposure to wave action, the nature of the substratum and its topography, the influence of sunlight and shade, climatic conditions, biological interactions, salinity and the concentration of nutrient salts, and tidal cycles. On rocky shores, water movement associated with tides, waves and spray results in environmental conditions that are neither fully terrestrial, nor fully marine. As a result, the biotic assemblages found on rocky shores do not survive full immersion or full emersion, but conditions of wetness between these two extremes. The pattern of zonation on rocky shores results from the gradient of environmental factors that characterizes this habitat. However, two factors in particular play an important role: (i) competition for resources, and (ii) the restricted potential of a given species to perform optimally under different environmental conditions. Other factors may also be important in particular circumstances, for example, predation by terrestrial and marine consumers.

Overall, rocky shores constitute a very harsh environment in which the biota is exposed to extremes of conditions. As a result, the flora and fauna associated with this habitat type have evolved morphological, physiological and behavioural adaptations, which enable them to survive there.
ECOLOGICAL ZONES

Ecologically, Mediterranean shores may be divided into several zones depending on the relative contributions of two key physical factors: light and wetness. The first region of the shore is the Adlittoral – that part of the terrestrial environment that is under some maritime influence from wind-blown salt and spray. The next region is the Supralittoral – that zone on the shore which is permanently exposed to the air except for occasional wetting by sea spray and the highest waves. Next comes the Mediolittoral – that zone which is regularly exposed and submerged, mainly due to wave action. This zone corresponds to the intertidal zone of tidal shores, however, in most of the Mediterranean, the tidal range is very small (in the Maltese Islands, this is not more than 25cm at the extreme) and changes in sea level brought about by wave action and atmospheric conditions are far more important.

What follows is a brief description of the typical biotic communities that are found on the rocky shores of the Maltese Islands.

THE SUPRALITTORAL ZONE

This zone is characterised by organisms that require some wetting with seawater but not immersion. On Maltese rocky shores the main species are various maritime lichens, mainly species of *Verrucaria* (HAZIZ TAX-XATT) which are adapted to live in this extreme environment, microscopic algae which live on or in the calcareous rock and give it a characteristic blackish-brown colour, two species of periwinkle, of which the commonest on Maltese shores is *Melarhaphe (= Littorina) neritoides*, [ZIBGET IL-BLAT] and the isopod *Ligia italic*, [DUD TAS-SAJD].

THE MEDIOLITTORAL ZONE

In this zone live organisms that tolerate more or less regular immersion in seawater but not continuous submersion. On rocky shores, the upper reaches of this zone are characterised by barnacles [KOCCLI] of which there are three species: *Euraphia depressa* [KOCCLA CATTA] occurs in the highest levels and *Chthamalus stellatus* [KOCCLA KOMUNI] further down; the third species *Chthamalus montagui* occurs at intermediate levels on some shores. At the lower limit of the barnacle zone occurs the limpet *Patella ulyssoponensis* [MHARA TAL-FURAN], and further down another limpet (*Patella caerulea* [MHARA KAHLA] and the chiton *Lepidochitona corrugata* [HANZIR IL-BAHAR TAX-XATT]). On some shores, a third species of limpet occurs: *Patella rustica* [MHARA TAS-SAMMA]. Close to sea-level the shore is dominated by the attached snail *Dendropoma petraeum* [FARRETT TAX-XATT] in exposed parts while another attached snail, *Vermulus triquetrus* occurs in sheltered microhabitats. On most shores, the shells of *Dendropoma* are embedded in encrustations of the calcareous alga *Neogoniolithon notansii* to form characteristic platforms known as ‘trottoirs’. The vagile top-shells *Osilinus (=Monodonta) turbinatus* and *Osilinus (=Monodonta) articulatus* [BEBBUX TAL-MAZZA] occur throughout the mediolittoral during calm weather but seek the shelter of pits and holes in the lower reaches of the shore during rough seas. In the midmediolittoral, macroalgae (large algae) become evident. On most shores there is a fringe of red algae (mainly *Polysiphonia setularioides*) followed by fringes of
Polysiphonia opaca, Ceramium ciliatum and Laurencia papillosa, with encrusting coralline algae becoming dominant in the lowermost reaches of the mediolittoral.

PRACTICAL 2: Rocky shores

Lecturers: Prof Patrick J Schembri and Dr Joseph A Borg
Demonstrators: Ms Sarah Debono & Mr Edwin Zammit

INTRODUCTION

The Maltese Islands are composed of carbonate rock, mainly limestones, and are tilted towards the NE, producing a submerged and generally lowland northern coastline and an emerged and almost exclusively cliff-dominated southern coastline. Rocky shores are the most widespread coastal habitat in the Maltese Islands constituting ca. 90.5% of the 272km coastline of the islands, as opposed to the 2.4% constituted by 'soft sediment' shores. Some 7% of the coastline is built-up, some 60% is cliffs or boulder shores and the remainder is gently sloping lowland shore.

Globally, on a large spatial scale, the biota of rocky shores is determined by such factors as exposure to wave action, the nature of the substratum and its topography, influence of sunlight and shade, climatic conditions, biological interactions, salinity and the concentration of nutrient salts, and tidal cycles. However, differences at much smaller scales are also possible if some of these factors vary significantly between different shores, or between sections of the same shore.

On the microtidal Maltese shores (maximum tidal range = 0.25m), differences between the biotic assemblages of different lowlands are primarily due to differences in exposure to wave action, substratum geology and topography.

OBJECTIVES

This practical is intended to introduce you to the typical biota of lowland Maltese rocky shores and to their zonation on such shores. During this practical you will be required to:

- Examine and identify the characteristic biota of a lowland Maltese rocky shore.
- Qualitatively examine differences between the biota of flat stretches of rocky shore and those that occur in gullies, crevices and rock pools on the same shore.
- Construct a shore profile.
- Quantitatively sample the biota along a belt transect laid on the shore in order to study zonation patterns.
- Study the microtopography of different sections of shore.
- Calculate an exposure index for the shore.
- Analyse your results to (1) compile a species list for the stretch of shore you have studied; (2) construct graphs of species abundance against position along the belt transect you have sampled; (3) relate any observed patterns in the distribution of biota to position on the shore, slope and microtopography; and (4) compare your
results with those of other groups, relating any observed differences to differences in the physical characteristics of the stretches of shore studied.

MATERIALS

Items marked with an asterisk (*) must be supplied by course participants themselves.

Notebook and pencil and preferably a clipboard inside a large plastic bag (it tends to be wet on the shore!) (*); long forceps (*); penknife with strong blade (*).

Hammer and chisel; collecting buckets; specimen tubes; plastic bags.

Three metre rulers and one spirit level (or 2 rulers and one long spirit level), one measuring tape or graduated line per group.

One 0.5m X 0.05m quadrat and sighting frame per group.

Plastic profiler (to be borrowed as necessary); fine-point dividers (*); calculator (*); graph paper (*).

Identification guides (especially the keys to Maltese rocky shore biota provided on the Department of Biology’s website).

FIELD PROCEDURES

The class will be divided into groups. Each group will select a stretch of rocky shore and will carry out all the following procedures in their study area. Do not overlap with groups working an adjacent area. It is suggested that within each group, the tasks are shared amongst the group members.

Compilation of a species list

‘Walk’ the stretch of shore that you have chosen as your study area and note any gross morphological features such as flat stretches of bedrock, ledges, crevices, gullies, rockpools, etc. Make a rough sketch map of your study area (remember to include a North-pointer and scale). Make sure that your shore has a flat area that extends from sea-level to the upper shore as you will need this to lay your belt transect on (see below).

Search the shore [NOTE: You need to get very close to the rock to spot most species – this is a ‘hands and knees’ job and involves you getting wet! Wear a mask to help you search at the air/water interface]. Only pick up one or two of each species you find [NOTE: Not more! Be conservation minded – 30 students picking indiscriminately will significantly impact the biota], noting where on the shore they come from and especially microhabitat (for example, flat bedrock, under ledge, in crevices, from shallow rockpool etc.). For attached organisms use a hammer and chisel to chip off a small piece of rock with the specimen. Do not collect vermetids – these are protected species!

Place these specimens in a bucket in seawater and transport to the laboratory for identification [NOTE: Do not leave the bucket in the sun and change the water frequently].
You will also need these reference specimens to help you identify species in your belt transects.

**Shore profile**

Carry out shore profiling along the same section of shore where you lay your belt transect (see below).

Place a metre ruler vertically at ‘biological zero’ (mean sea level)\(^{10}\) and a second ruler also vertically some distance up the shore; arrange a third metre ruler horizontally on the surface between them as shown in Fig.1 (or the spirit level itself if you are using a long spirit level). Place a spirit level on the horizontal ruler and adjust the ruler until the spirit level indicates that it is perfectly horizontal. At this point take a reading on the first vertical ruler (marked A in Fig.1); this is equal the height ‘\(y\)’ of the second ruler (marked B in Fig.1) above biological zero. Repeat this procedure by ‘walking’ the two rulers up the shore. A plot of height above mean sea level against horizontal distance from mean sea level give a faithful representation of the shore profile (Fig.2). Use this profile to calculate the general slope and local variations in slope. Correlate the results of your belt transect studies with topographic variations along the shore as revealed by the profile.

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\(^{10}\) ‘Biological zero’ is taken as the point where the first stands of the alga *Cystoseira* spp. occur. This is a reasonable approximation of ‘mean sea level’ for ecological work. Biological zero is ca 23.5 cm ± 1 cm above chart datum (Mallia & Schembri, 1996).
Fig. 2. Graph of height above biological zero against horizontal distance along the shore from biological zero.

Belt transect
Select a 2m wide strip of shore that is relatively flat and free of large crevices, gullies, ledges and rock pools. Lay a measuring tape (or graduated line) from some distance below ‘biological zero’ to the upper shore beyond the supralittoral\textsuperscript{11}. This ‘shore-normal’ line transect (i.e. perpendicular to the shore) will form the midpoint of your belt transect.

Estimate the abundance of biota on the shore starting from biological zero and working your way upshore using a 0.5m X 0.05m quadrat laid along the line as shown in Figs. 3 and 4. This shape of quadrat is necessitated by the very compressed zonation on Maltese shores and was found to be a good compromise between sampling an adequate area, detecting changes in abundance over small vertical distances along the shore, and the size of the biota to be sampled. In the lower parts of the shore, where most change occurs, lay your quadrats contiguously. However, once you enter the ‘Littorina zone’, variation with distance becomes much less and here you can sample at appropriate intervals.

For each quadrat, count the number of individuals of each faunal species within the quadrat. Make sure that you distinguish the different species, if not taxonomically, then morphologically — you can put names to the species later in the laboratory once you identify the species from the ‘general collecting’. If you are in doubt, collect one individual for later identification in the laboratory. In particular, the three species of patellid limpets (\textit{Patella caerulea}, \textit{Patella rustica} and \textit{Patella ulyssiponensis}), the two species of the topshell \textit{Osilinus} (\textit{Osilinus turbinatus} and \textit{Osilinus articulatus}), and the three species of shore barnacles (\textit{Euraphia depressa}, \textit{Chthamalus stellatus} and \textit{Chthamalus montagui}) present special problems. It is possible to distinguish the two \textit{Osilinus} and the three species of \textit{Patella} from the shell alone, so learn how to do this before you start the quadrat counts (see Annex 1, an identification guide for barnacles, \textit{Inula crithmoides}).

\textsuperscript{11} On lowland Maltese rocky shores, the supralittoral/adlittoral boundary is conveniently taken as the point of first occurrence of the terrestrial maritime shrub \textit{Inula crithmoides}. 
limpets and top shells). It is also possible to distinguish *Euraphia* from *Chthamalus*, however it is difficult to distinguish between the two species of *Chthamalus*, especially in the case of juveniles. It is therefore permissible to record these as ‘*Chthamalus* spp.’ in the transect counts.

![Diagram of belt transect positioning on the shore and sampling using 0.5m X 0.05m quadrats.](image)

**Fig. 3.** Positioning of belt transect on the shore and sampling using 0.5m X 0.05m quadrats.

![Quantification of abundance of shore biota using a 0.5m X 0.05m quadrat. The tape measure acts as the midline of the belt transect.](image)

**Fig. 4.** Quantification of abundance of shore biota using a 0.5m X 0.05m quadrat. The tape measure acts as the midline of the belt transect.

For algae and colonial animals, if any (e.g. sponges, ascidians), do not count individuals but estimate cover (the percentage area of quadrat covered by these organisms). To help you estimate cover, use the gridded sighting frame and count the number of grid
squares occupied by each species. Note that species may overlap so estimate cover for each species separately. Unless you are sure what the species is, take a small piece of each for later identification in the laboratory.

**Microtopography**

The microtopography of different zones along the transect can be quantified and recorded as an 'index of roughness' by means of a profiler (Fig. 5). This device was originally developed for use by carpet fitters in order to accurately trace the shape of awkward corners. However, it has proved to be very useful for this type of work. Make a minimum of five traces of the microtopographic profile, distributed along the length of your transect with at least three in the mediolittoral zone and the other two in the supralittoral (you may make more traces if you wish). Do this by placing the profiler on the rock as shown in Fig.5 and then tracing the outline on paper. An 'index of roughness' can be determined by calculating the ratio between the actual profile line and the 100% line, that is, the straight line which joins the upper and lower points of the profile. You will be shown how to this in the laboratory.

![Fig. 5 Method of using a 'profiler' to make a template of the microtopographic profile of the rock on the shore.](image)

**LABORATORY SESSION 2: ROCKY SHORES**

**Species identification**

Use the manuals, keys, books and reference material to identify the species you have collected, including voucher specimens from the belt transect, to the lowest possible taxon.

**Exposure index**

Locate the exact position of your field station on the Admiralty charts provided and use these to calculate the Thomas Exposure Index for your site (see Annex 2). You will be shown how to do this as a class demonstration.

‘Index of roughness’
Use the traces of the microtopographic profile made in the field to compute an index of roughness by calculating the ratio between the actual profile line and the 100% line, that is, the straight line which joins the upper and lower points of the profile (Fig.6). Measure the lengths of the profile line by ‘stepping’ a pair of dividers along the line to be measured, using a separation of the dividers’ points of not larger than 4mm. Repeat the measurement at least once and take the average of the results as the length of the profile line. Measure the length of the 100% line using an accurate ruler. The length of the profile line divided by that of the 100% line gives the ‘index of roughness’; a value of 1.0 is indicative of a flat surface while the higher the index the more topographically complex (‘rougher’) is the surface of the rock.

Fig. 6. Measurements taken for the computation of the ‘index of roughness’. Line ‘a’ is the 100% line and line ‘b’ is the profile line.

DATA ANALYSES

Compile a table giving a **classified list** of the species identified, approximate distance from mean sea level these species were collected at, and the microhabitat they were collected from.

Compile a matrix of species abundance and quadrat number starting from Quadrat 1 at biological zero. Express abundance values as ‘number of individuals per square metre’ and cover as a ‘percent cover’.

Draw the shore profile to scale. Calculate the average slope of your line transect.

Calculate the ‘index of roughness’ for each ‘roughness’ measure you took and mark these values on your shore profile indicating the position where the microtopographic profiles were measured. What is the mean ‘index of roughness’ for the mediolittoral and for the supralittoral?
QUESTIONS

Inspect your table of species and those obtained by two other groups whose position on the shore was not adjacent to yours. Are there any correlations between species and distance from mean sea level and between species and microhabitat?

Take your species-abundance data from the belt transect, and for the more abundant species, plot graphs of abundance against distance from biological zero (classically, ‘kite diagrams’ have been used for this but you can plot ordinary histograms). Overlay the shore profile over the abundance graphs. Inspect the graphs for the species from your transect. Are there any zonation patterns evident? Can you distinguish the bare zone, supralittoral zone, upper mediolittoral zone and lower mediolittoral zone from your data? Is there any correlation between abundance and shore profile for any species?

Compare your abundance-distance graphs with those of two other groups whose position on the shore was not adjacent to yours. Are there any similarities? Are there any differences? Can these differences be accounted for by differences in shore physical parameters such as differences in microtopography, exposure, and slope?

BIBLIOGRAPHY AND REFERENCES


Methodologies and illustrations were adapted from:


BIO3060 - Field Biology

PRACTICAL 2: BIOTA OF ROCKY SHORES

Annex 1 Distinguishing between barnacles, limpets and top-shells of Maltese shores

Far left: Osilinus articulatus
Left: Osilinus turbinatus
BIO3060 - Field Biology

PRACTICAL 2: BIOTA OF ROCKY SHORES

Annex 2 - The Thomas Exposure Index for Maltese shores

INTRODUCTION

Waves are a very important factor on all shores since, directly or indirectly, they affect erosion, deposition, availability of water and oxygen, the supply of dissolved and suspended matter, and the distribution and behaviour of shore biota. However, the extent of wave action on a given shore (referred to as the ‘exposure’) is very difficult to measure directly. For this reason, a number of indirect methods of estimating exposure for the purposes of comparative studies of different shores have been devised. Some of these are based on biological indicators and others on physical characteristics of the shore. One method that has been used to describe exposure on Maltese shores is the physically derived index devised by Thomas (1986), based on the premise that a coastline will be affected by waves in proportion to the angle through which it is open to the water, its aspect relative to the range of wind directions, and the distribution of shallow waters within the fetch.

METHOD

The Thomas Exposure Index (EI) is based on the assumption that the general magnitude of wave action is predictable, given information on wind velocity, duration and direction, provided that such wave action is modified to account for the direction, shape and angle of exposure of the shore, the wind fetch and the distribution of shallow water close-to and off-shore. This method, as adapted for local use, is described below.

Since the amplitude of wave energy bearing on a particular shoreline is intrinsically tied to the wind energy, a transparent wind rose overlay, divided into twelve 30° sectors of the compass rose, is used to determine which of the wind sectors are important in determining wave action on the shore.

![Wind roses and wind rose overlays](image.png)

Fig. 1. Examples of the use of the transparent overlay to determine sectors to be considered (shaded) at two locations, one in Bermuda and one in the Bay of Fundy, Canada. Wind energy roses for the two locations are also shown.
The rose is centred on the precise site on a map, with the centre of sector 1 aligned with true north. From the overlaid wind rose, those wind sectors which are at least 50% unobstructed by the shoreline or by nearshore islands are identified (i.e. the ‘open’ sectors). Further calculations are carried out on these sectors only (see Fig. 1 from Thomas, 1986).

For each of the sectors under consideration, the following parameters are determined:

- Wind Energy ($W$)

This is calculated by using the following formula:

$$\text{Wind Energy (W)} = \left( \frac{\text{% time wind blows in sector}}{100} \right) \times (\text{mean wind speed})^2$$

Wind speed and duration data for the period 1972-1991 obtained from the Meteorological Office are reported below, with the last column ($W_T$) giving the wind energy for each sector.

- Fetch in nautical miles ($F$)

This is the distance in nautical miles from the nearest headland and is taken to be a maximum of 100 nautical miles for open shorelines.

- Extent in nautical miles of water <6m deep adjoining the shoreline ($CS$)
This parameter is calculated from bathymetric charts. Thomas (1986) considered 6m as the critical depth for shallow water that will reduce wave height and so affect exposure. This is because waters greater than 6m depth have little or no effect on most waves impinging on a coast.

**INDEX ADAPTED FOR THE MALTESE ISLANDS**

The original exposure index proposed by Thomas (1986) incorporated an additional factor, $D_S$, which is the extent in nautical miles of water within the fetch but not adjoining the shore and having a depth of less than 6m. However no such conditions occur in Maltese coastal waters and this term can be eliminated from the equation, which therefore becomes:

$$ EI = \sum \log W \times \log (1+F/CS) $$

Values of $EI$ have been calculated for most Maltese shores. The most exposed site in the Maltese Islands is San Dimitri Point in Gozo, with a $EI$ value of 27.58, which can be used as a reference value for other sites; thus the closer a calculated value is to the San Dimitri Point value, the more exposed that shore is by local standards.

**Useful conversion factors:**

- 1 nautical mile = 1.852 nautical miles = 1.1507 miles
- 1 km = 0.5399 nautical miles
- 1 fathom = 6 feet = 1.829m

For a worked example of the calculation of the Thomas Exposure Index, see the schedule for Practical 3 on Sandy Shores.

**REFERENCE**

Bathymetry of the coastal water from Qawra Point to Ras l-Irqiqa on the northeast coast of the island of Malta (from Admiralty charts)
TOPIC 3 – SANDY SHORES

Lecture: ECOLOGY AND BIOTA OF ADLITTORAL HABITATS AND SANDY SHORES.

Lecturer: Dr Alan Deidun

MOBILE SEDIMENT SHORES
Sand and shingle are defined as mobile sediment shores since the particles are non-cohesive due to the high-energy status of such shorelines, making settled life difficult for plants and animals. Two thirds of the world’s shorelines are classified as mobile (Reise, 2001), although such shore types comprise just 2.4% of the total coastline of the Maltese Islands (Mallia et al., 2002).

VERTICAL ZONATION
The vertical zonation scheme most widely used for Mediterranean shorelines is that proposed by Pérès & Picard (1959) who replaced ambiguous terms such as the ‘intertidal’ which does not have the same meaning in different parts of the world. The main ‘zones’ within this scheme are:

Supralittoral – a vertical zone which is very rarely immersed in seawater but is under the effect of sea-mediated factors, such as sea spray. This is often taken to delineate the highest landward extent of the coastal zone.

Mediolittoral – a zone which is alternately exposed and immersed by wave action. It corresponds to the mean sea level or the highest level of wave immersion on microtidal Mediterranean shores.

Infralittoral – a zone that is permanently immersed and is never exposed by wave action. Its lower extent is that level where normal photosynthesis is no longer possible due to the low light intensity.

Circalittoral – a zone extending from the lower infralittoral boundary to that level where there is no light from the surface (roughly ca 200m depth).

Some authors, like Jedrzejczak (2002), identify an additional zone, the adlittoral or backshore zone on which no organic material is deposited and which extends from the highest landward limit of the supralittoral to the foot of sand dunes, where these are present.
GRAIN SIZE CLASSIFICATION OF SEDIMENTS

The single most important physical parameter pertinent to a mobile shore is the grain size of its sediment particles since this correlates with many other physical parameters, such as sediment organic content. Grain size analysis (granulometry) determines particle size by classifying sediments according to the Udden-Wentworth scale, part of which is represented below.

<table>
<thead>
<tr>
<th>Grain size (mm)</th>
<th>Phi (φ)</th>
<th>Sediment description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;256</td>
<td>&lt;-8</td>
<td>Boulder</td>
</tr>
<tr>
<td>64 – 256</td>
<td>-6 to -8</td>
<td>Cobble</td>
</tr>
<tr>
<td>4 – 64</td>
<td>-2 to -6</td>
<td>Pebble</td>
</tr>
<tr>
<td>2 – 4</td>
<td>-1 to -2</td>
<td>Granule</td>
</tr>
<tr>
<td>1.0-2.0</td>
<td>0 to -1</td>
<td>Very coarse sand</td>
</tr>
<tr>
<td>0.50-1.0</td>
<td>1 to 0</td>
<td>Coarse sand</td>
</tr>
<tr>
<td>0.25-0.50</td>
<td>2 to 1</td>
<td>Medium sand</td>
</tr>
<tr>
<td>0.125-0.25</td>
<td>3 to 2</td>
<td>Fine sand</td>
</tr>
<tr>
<td>0.0625-0.125</td>
<td>4 to 3</td>
<td>Very Fine sand</td>
</tr>
<tr>
<td>0.0039-0.0625</td>
<td>8 to 4</td>
<td>Silt</td>
</tr>
<tr>
<td>&lt;0.0039</td>
<td>&gt;8</td>
<td>Clay</td>
</tr>
</tbody>
</table>

Note: A more detailed treatment of grain size analysis will be given in the laboratory session.

OTHER PHYSICAL PARAMETERS OF SANDY ENVIRONMENTS

Besides grain size parameters, it is also useful to measure the following physical parameters that are relevant to life in sandy habitats:

- **Penetrability** – a measure of the ease with which sands can be burrowed into. This is of the utmost importance to burrowing fauna and is measured using a piston with a known cross-sectional area. Fine sands exhibit thixotropy, whereby once pressure is exerted on them, they become more easy to penetrate since their viscosity is lowered.
- **Porosity** – a measure of the volume of void space in a sand. This is usually measured
by taking the percentage water content of the sand. It is inversely proportional to sand median grain size.

- **Permeability** – a measure of the rate of flow or drainage of water through the sand. It is also inversely proportional to sand median grain size.

- **Dissolved oxygen content** – Oxygen plays a vital role in sandy beach ecosystems due to its influence on chemical and biological reactions taking place. In fact, the level of oxygen controls the redox equilibrium maintained by elements, such as iron, sulphur, manganese and nitrogen (Aller, 1980) and so influences the cycling of these nutrients. In some sandy beaches, a sharp boundary occurs at a particular depth in the sand, where the colour of the sand changes abruptly from yellow to black; this boundary is usually marked by a thin grey layer. Above the grey layer, the interstitial water contains sufficient oxygen for the contained fauna to oxidize all the organic waste products of the immense populations of micro-organisms. Below the grey boundary, the black sand is anoxic; free oxygen is totally absent and the fauna must survive through anaerobic processes, such as fermentation. There is a difference in electrical potential between the reduced ions of black sand and the oxidized ions of the upper, yellow layers and this is used to express the oxygen demand, or redox potential, of the black sand. In the total absence of free oxygen, the redox potential drops to –200 millivolts, compared with a value of +400mV at the surface. The grey boundary layer marks the transition from reducing to oxidizing conditions and is termed the **redox potential discontinuity (RPD)**. In coarse, well drained sand, there is no black layer as all organic material is rapidly oxidized. As sand becomes progressively finer, so the black layer moves closer to the surface. The depth of the black sand boundary also varies seasonally. The depth of the boundary is greatest during the winter months, but decreases during the summer as bacterial populations bloom and oxygen demand rises.

- **Estimation of the percentage organic carbon content** is made using the Walkley and Black titration method following wet-oxidation by potassium dichromate, as described in Morgans (1956) and Buchanan (1984). This method gives an estimation of organic carbon excluding carbonates (Morgans, 1956) and is preferred to that of loss of weight on ignition because the latter only gives a rough estimate (Buchanan, 1984). This is inversely proportional to mean grain size (In fact, Dale, 1974, found that sediment with a mean grain diameter of 0.01mm contained 10% organic carbon, while sand with a mean grain diameter of 0.2mm contained only 0.1% carbon) and is important for sustaining infaunal communities. An important source of organic content input on local beaches are the banks of beached seagrass (banquettes) that accumulate.

- **Salinity and pH.**

**EXPOSURE AND SLOPE**

On the basis of exposure to wave action, sandy beaches stretch between two extremes. On **dissipative beaches**, wave action is fierce. At the other extreme, on **reflective beaches**, wave action is relatively mild. Between these two extremes there is a whole range of intermediate states. The **beach slope** is intrinsically tied with its degree of exposure to wave action. Brown & Mclachlan (1990) and Little (2000) state that exposed beaches are characterised by a gentle slope. Also, the effect of exposure to wave action on beach profile changes radically with season. Such seasonal variability in the beach slope also results in seasonal shifting of the position of the **berm**, a relatively horizontal platform which extends to a break in the slope.
Exposure to wave action is a fundamental parameter on sandy beaches since it directly affects sand particle size distribution, which in turn is a sensitive indicator of the ecological conditions pertaining on a particular beach (Little, 2000). In fact, fine sands provide the greatest surface area for the attachment of micro-organisms, such as bacteria and diatoms, and their rich organic content is capable of supporting large macrofaunal populations.

In the literature, however, there are conflicting views about the relation between exposure to wave action and grain size. Little (2000) claims that where exposure to wave action is high, sands are usually fine (less than 200µm), whereas low exposure to wave action results in sediments tending to be coarser, and the beach may even be shingle – composed of pebbles (4-6mm) and cobbles (64-256mm). Brown & Mclachlan (1990), on the other hand, claim that it is only under sheltered conditions (i.e. where exposure to wave action is low) that very fine sand can remain on a beach.

CLASSIFYING SANDY BEACH FAUNA

Two major categories of sand-dwelling animals may be recognized: the **epifauna** and the **infauna**. Whilst the former are mostly non-resident species living on the surface of the sand, the latter live within the sand. According to Hayward (1994), the latter is the larger of the two categories and may be further divided into three major types – the **macrofauna**, the **meiofauna** and the **microfauna**. Macrofaunal are animals large enough to be retained by a 0.5mm sieve; the meiofauna pass through a 0.5mm sieve but are retained by a 0.05mm mesh, while the microfauna are organisms of less than 0.05mm diameter (Hayward, 1994).

Psammophiles are strict sand dwellers (hence, highly stenoecious). Examples from Maltese beaches include *Phaleria acuminata* (Coleoptera: Tenebrionidae), *Labidura riparia* (Insecta: Dermaptera) and *Tylos europaeus* (Crustacea: Isopoda). Psammatophiles are even more habitat specific since they are restricted to the drift line of sandy beaches, feeding of stranded wrack; for example, many fly (Diptera) species. Sandy beaches also harbour littoral species which are not restricted to the sand habitat only, many ubiquitous/euryecious species.

THE DIVERSITY OF SANDY BEACH FAUNA

Despite the fact that at first sight sandy beaches give the impression of being ‘ecological deserts’, they do, in fact, harbour, a rich faunal diversity, dominated by arthropods. These include:

• **Insects**, such as beetles (Order Coleoptera) with many representative families, such as the darkling beetles (Tenebrionidae), the ground beetles (Carabidae), the rove beetles (Staphylinidae), the weevils (Curculionidae) and the small ground beetles (Anthicidae) being the most common. Other insects include earwigs (Order: Dermaptera), bees, wasps and ants (Order: Hymenoptera) and flies (Order: Diptera).
• **Peracarid crustaceans**, such as the beachhoppers (Order: Amphipoda) and the woodlice (Order: Isopoda) but also opossum shrimps or mysids (Order Mysidacea) in the

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53
shallow beach surf zone.

- **Arachnids**, mainly represented by spiders (Order: Araneae) and false-scorpions (Order: Pseudoscorpiones).
- **Centipedes** (Order: Chilopoda)

Also present are non-arthropod taxa, including:

- **Polychaetes** (Annelida: Polychaeta). These are very abundant on sandy beaches globally but not on local shores.
- **Oligochaetes** (Annelida: Oligochaeta), mainly members of the family Enchytraeidae, which are minute whitish worms.

### BEHAVIOURAL ADAPTATIONS OF PSAMMOPHILES

Due to the harsh environmental conditions on sandy beaches, psammophiles possess a suite of behavioural and morphological adaptations, including the ability to **burrow** in the sand (along with a streamlined body), **nocturnal activity**, the ability to withstand prolonged **immersion** in seawater, and the ability to exploit different food sources (**polyphagy**). Such adaptations are related to maintaining the position on unstable sediment, to minimizing loss of moisture and resisting thermal stress and to the lack of a reliable food source.

On sandy beaches, food materials from a wide area are concentrated within a narrow band (Gunter, 1979). During late winter and spring, a particularly important source of food on Maltese sandy beaches is stranded drifting material mostly seagrass debris that accumulates on beaches to form ‘**banquettes**’.

Food production and the sources of food for animals living on sand beaches are considerably different from those of rocky shores. Since the substratum on sandy beaches is too unstable for colonization by macrophytic plants, the basic food on such beaches is microscopic ‘plants’ – chiefly diatoms, other unicellular algae, and bacteria – and detritus. As a result, as Brown (1983) points out, a general feature of sandy-beach macrofauna is that they usually have alternative feeding methods (hence, are **generalist feeders**).

The major feeding mechanisms of sandy beach organisms are suspension feeding on organic material in the water column and deposit feeding on organic detritus on or within the sand.

### SAMPLING SANDY BEACH FAUNA

**Pitfall traps** are used to collect sand fauna that burrows during the day and only emerges at night. The traps consist of plastic cups inserted into the sand such that their mouth is flush with the sand surface. The cups are arranged one at the centre and the other four in a cross pattern, and the peripheral traps are connected to the central one by thin strips of wood resting on the sand, which serve as walk-ways; the use of such walk-ways greatly enhances sampling efficiency.
**Hand nets** are used to collect sand-burrowing crustaceans, such as mysids and cumaceans, which live in the upper infralittoral zone and which emerge from the sandy bottom at night to swim and feed in the water column.

**Corers** are used to collect infauna. The corer is pushed into the sand and the top 10cm of sand is transferred to a 0.5mm mesh sieve and wet sieved in the laboratory. The same procedure is repeated for the sand fractions between 10cm and 20cm and between 20cm and 30cm below the beach surface, and so on.

**Standard searches** in a pre-defined area and for a given period of time are carried out to collect mobile epifauna, with fauna being collected using a **pooter**. Alternately a variety of specialised trapping techniques may be used.

**POCKET BEACHES OF THE MALTESE ISLANDS**

The sandy beaches of the Maltese Islands are defined as ‘pocket beaches’ since they accumulate at the heads of bays and have a limited physical extent. Beaches are nourished by terrestrial sources (from rock erosion) and by biogenic sources from the sea (from animal skeletal remains). Maltese rocks, being predominantly carbonates result in local beaches being composed of **carbonate sands**, with very little **silica** sand, resulting from the siliceous skeletons of marine organisms. Over 90% of the sand of Maltese beaches is composed of carbonates (Turi et al., 1990). Minerals in the sand may give it a characteristic colour. Thus the reddish sand at Ramla l-Hamra in Gozo results from the erosion products of the Greensand layer, which is rich in iron minerals.

To date, very few studies have been carried out on supralittoral and mediolittoral bare sand communities, with most of the scientific attention being dedicated to sand dune habitats.

Local beaches are under intense anthropogenic pressure, mainly from tourism and recreational activities. This, coupled with the local paucity of this coastal habitat, the faunistic **compartmentalisation** that seems to be characteristic of local beaches, and the ecological importance of insular ecosystems (due to their isolation), highlights the need for conservation of these poorly-understood biocoenoses.

**REFERENCES**


PRACTICAL 3: SANDY SHORES  
Tutors: Dr Alan Deidun, Dr Joseph A Borg, Prof Patrick J Schembri  
Demonstrators: Ms Sarah Debono & Mr Edwin Zammit

OBJECTIVES
The main aim of this practical and the related laboratory session is to acquaint participants with the typical biota of sandy beaches and with the basic sampling techniques used to study them. Organisms restricted to this type of habitat have a unique suite of adaptations and are called psammophiles. In addition, this practical aims to introduce students to the practical procedures involved in characterizing a shore, such as in the calculation of an index of exposure, or in characterizing a beach sediment, such as determination of granulometric parameters and of organic carbon content. Additionally, students will be introduced to common statistical and graphical methods employed in the description of the faunal assemblages of sandy shores.

MATERIALS
Items marked with an asterisk (*) are to be provided by the course participants themselves.

- Levelling device (3 metre rulers & spirit level); 1mm sieve; trowel; auger (to be shared between the different groups); measuring tape; length of nylon string; hand nets (to be shared between groups as necessary); white sorting tray; fine forceps (*); permanent markers (*); plastic bags, rubber bands (for granulometry)(*); wash bottle; 70% alcohol; 2 collecting buckets per group; specimen tubes; calculator (*); notebook and pencil and preferably a clipboard inside a large plastic bag (*).

FIELD PROCEDURES
The class will be divided into groups. Each group will select a stretch of sandy shore and will carry out all the following procedures in their study area. Do not overlap with groups working an adjacent area. It is suggested that within each group, the tasks are shared amongst the group members.

Transects
Before the actual sampling can start, the sampling area must be standardised. One way of doing this is by using the line transect method, which is appropriate in studies where a transition between different habitats and populations is postulated. Take the measuring tape provided and secure it around a stone or any large object at the Mean Sea Level (MSL). There are many ways to define MSL but one basic way is that it is considered to be the average distance between the uppermost and lowermost level reached by waves. The measuring tape is then extended over the sand across the mediolittoral and supralittoral zones to the first vegetated fringes of the beach. Be conscious of the fact that the measuring tape provided is only 30m long and some transects will span over longer distances than this, in which case sample the first 30m before continuing.
with the second 30m and so on. Use the measuring tape laid down for the transects to measure the length of the wet and dry zones in your transect.

**Grain-size analysis and depth of anoxic layer**

The single most important physical parameter pertinent to a mobile shore is the grain size of its constituent sediment, since this correlates with many other physical parameters such as percentage organic content. The size of granules making up the sediment is a very important factor in shaping biotic distributions on beaches. In grain size analysis (granulometry), sediment-specific parameters such as mean grain size are measured so as to characterise the sediment. Starting from the MSL, walk along the transect and collect the upper 50g of sand from a wet zone station and from a dry zone station in a carefully labelled plastic bag for subsequent grain size analysis. Your group may be asked to collect a sand sample from the 10-20cm or the 20-30cm stratum, rather than from the surface (THIS WILL BE DECIDED ON THE DAY BY YOUR TUTORS). **Brush away the upper few of cm of freshly blown sand before collecting the sample.**

Dig a hole in the sand at the same station from where you have collected your wet zone and dry zone samples. Observe the presence (if any) of the black anaerobic sand layer characteristic of fine sands. Record the position of the station and, the black anaerobic layer is present, the depth below the sand’s surface at which it occurs. If not observed, use an auger at a station around the middle of the wet zone of your transect and penetrate the sand surface until this layer is reached (**Note: The layer may be deeper than the length of the auger**).

**Beach slope/profile**

Organisms are rarely distributed homogenously throughout the sand, and slope is an important determining factor influencing biotic distributions on sandy shores. Although much more accurate ways of measuring the angle of slope along the shore exist today, this can be estimated and plotted using the same method that was employed for profiling the rocky shore.

Carry out shore profiling along your belt transect using the 'rulers and spirit level' method, starting at MSL and working your way upshore. **Take care not to trample on the sand surface whose slope you are investigating.** Tabulate your results and then use them to plot a profile of your shore transect; you can calculate the slope of different sections of the beach from this.

**Core sampling**

Corers are used to collect infauna which lives burrowed amongst sand grains. For stations spaced along the shore-normal transect, use the corer and trowel provided to collect ca. 500g of sediment from the top 0-10cm stratum only. Within the wet zone, stations for core sampling should be spaced every 1m, whilst stations within the dry zone should be spaced every 5m. Transfer the sand collected to the 1.0mm sieve provided and wet sieve this in the sea. Any remaining residue on the sieve is washed onto a white
sorting tray and is subsequently collected using the fine forceps into sampling bottles provided and fixed in 70% alcohol. For that station closest to the transition between the wet and dry zone of your transect, separately collect and sieve cores from the upper 0-10cm stratum of sand (as for the other stations) and also for the 10-20cm and 20-30cm strata. Any macrofauna collected is identified and counted in the laboratory. Make a note of the area of the corer used as you will need this to standardise your counts to individuals per unit area.

**Note:** *Do not be surprised if you DO NOT find any biota. Maltese sandy shores have a notoriously low species diversity and abundance.*

**Hand net sampling**

This sampling technique is used to collect sand-burrowing crustaceans, such as mysids and cumaceans, which live in the upper infralittoral zone and which emerge from the sandy bottom at night to swim and feed in the water column. You are provided with a hand net of mouth area 0.097m² and mesh size 0.5mm to sample the shallow coastal water (depth ca. 1m) adjacent to the beach studied. Make a standard 30 sweeps of the net just above the sandy bottom (disturbing the sand as little as possible). Inspect the inside of the net and wash the contents with 70% alcohol into a small bucket. Any macrofauna collected is identified and counted in the laboratory and the results standardised to counts/cubic metre netted.

**Pitfall traps**

**Note:** *Traps will be set up on the shore by Department of Biology staff in the evening before the actual practical, however, any students who would like to participate in this are welcome to do so.*

This sampling technique is used to collect nocturnal, surface-active macrofauna which burrow within the sand during daytime and is considered to be the most efficient collecting technique in terms of recorded individual abundance amongst the three sampling techniques used. In the evening previous to the sandy shore fieldwork session (at dusk – ca 19.30h) pitfall traps will be set up on the beach and will be collected the next morning, during the sandy shore fieldwork session and the collections will be analysed in the afternoon laboratory session. The pitfall trap constellations set up consist of five plastic cups (7.5cm diameter), with one cup being located at the centre and the other four in a cross pattern separated from the central cup by a distance of 1m. Each cup is inserted into the sand such that the rim is flush with the sediment surface. The peripheral traps are connected to the central one by thin strips of wood resting on the sand, which serve as walkways. Each cup is half-filled with a mixture of water and glycerol (ratio ca. 8:2). The times when each constellation was set up and when the traps were emptied are recorded.
LABORATORY SESSION 3: SANDY SHORES

Faunal diversity
Use manuals, keys, books and reference material to identify the species collected. For the hand net and pitfall trap collections, calculate the relative abundance of insects, polychaetes, isopods, amphipods, mysids and tanaeids in your sample.

Species-abundance matrix
Construct a species-abundance matrix using the species counts that you made and standardize your data to individuals/m$^3$ for the core samples, to individuals per unit sampling effort for the hand net data and to individuals/trap/hour for the pitfall trap collections. Pool your results with that of other groups in a single data matrix.

DATA ANALYSES
Plot a cumulative species-sampling effort graph for your transect to gauge whether your sampling effort was satisfactory or not. Calculate the species richness and overall individual abundances for each of the three sampling techniques for your transect. Graphically represent the relative abundance of fauna in your transect using the following categories: Coleoptera, Formicoidea, Dermaptera, Diptera, Isopoda, Amphipoda, Polychaeta; also represent the zonation of the most frequently-encountered species (only) from the core sampling technique in the form of histograms or kite diagrams.

PHYSICAL CHARACTERISATION OF THE HABITAT

Exposure
Exposure is estimated using the Thomas Exposure Index that was used previously in the practical on rocky shores and reference should be made to the schedule for Practical 2 for a full explanation.

Figure 1 shows a map of the Rdum il-Majjiesa to Ras ir-Raheb area, which includes the field site. Figure 2 shows the wind rose superimposed on a point in the middle section of the beach at Ghajn Tuffieha; this station is exposed to wind sectors 10 and 11.

The Thomas Exposure Index value for the station in Figure 1 is calculated as follows:

Fetch (F) = 100 nautical miles (maximum)
CS = 0.303 nautical miles
Relevant wind sectors = 10 and 11

EI for wind sector 10 = log W x log (1+F/CS)
EI = log 13.789 x log (1+100/0.3030)
EI = 1.14 x log 331.03
EI = 2.873
Figure 1: Map showing the Rdum il-Majjiesa to Ras ir-Raheb costal area.
EI for wind sector 11 = \log W \times \log (1+F/CS)
\[ EI = \log 30.263 \times \log (1+100/0.3030) \]
\[ EI = 1.481 \times \log 331.03 \]
\[ EI = 3.732 \]

**Hence, total EI = 2.2873 + 3.732 = 6.605**

![Figure 2: Wind rose superimposed on a point in the middle section of the beach at Ghajn Tuffieha.](image)

Other sections of the same beach might be exposed to other wind sectors and would therefore show a different EI value – for example, the northeast corner of the beach at Ghajn Tuffieha (i.e. at the ‘stairs end’) is exposed to wind sectors 9 and 10, as shown in Figure 3.

Calculate the Thomas Exposure Index value for this section of the beach (Question 8 in the ‘Data presentation’ section of the present practical).
Figure 3: Wind rose, superimposed over a point at the 'stairs end' of the beach at Ghajn Tuffieha.

Grain size parameters
Grain size analysis (granulometry) determines particle size by classifying sediments according to the Udden-Wentworth Scale, part of which is reproduced below.

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<td>Granule</td>
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<td>0 to -1</td>
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</tr>
<tr>
<td>0.0625 - 0.125</td>
<td>4 to 3</td>
<td>Very fine sand</td>
</tr>
<tr>
<td>0.0039 - 0.0625</td>
<td>8 to 4</td>
<td>Silt</td>
</tr>
<tr>
<td>&lt;0.0039</td>
<td>&gt;8</td>
<td>Clay</td>
</tr>
</tbody>
</table>
Place your sediment in a mortar and grind gently with a pestle to loosen any sand aggregates. Remove any seagrass debris, plastic or any other extraneous material. The sediment is now placed in the sieve stack and shaken on the sieve shaker set at medium amplitude for 15-20 minutes. Using the paint brush and clear white tray as background, the contents of each of the sieves are emptied and weighed and a cumulative curve plotted, as shown below.

From the curve, the following parameters are calculated:

- **median particle size** ($M_d\phi$), which corresponds to the 50% mark on the cumulative curve.

- **sorting coefficient** ($\phi I$), given by:

\[
\phi_I = \frac{(\phi_{84} - \phi_{16}) + (\phi_{95} - \phi_{5})}{4 \times 6.6}
\]

Values below $0.5\phi$ indicate good sorting, values between $0.5\phi$ and $1.0\phi$ moderate sorting, and values above $1.0\phi$ poor sorting, with a wide range of particle size evident.
DATA PRESENTATION

Present the following:

1. A list of species recorded by your group and their individual abundance and standardized abundance for each of the three different sampling techniques (coring = inds/m³; pitfall traps = inds/trap/hr; handnets = inds/sweep).
2. A graphical representation (i.e. pie chart) of the relative number of individuals collected by your group using the different sampling techniques.
3. Histogram showing the relative abundance of each major taxon (e.g. Amphipoda, Coleoptera, etc) in your group’s collections.
4. Species-accumulation curve for your group’s coring data (only).
5. Kite diagrams to show the zonation of the two most abundant species collected by coring (only) by your group.
6. A graphical representation of beach slope for your group’s transect.
7. A cumulative curve for each of your group’s samples used for grain-size analysis and calculation of (a) median grain size and (b) sorting coefficient.
8. A reproduction of the bathymetric map and overlaid wind rose used for calculation of wave exposure values (Thomas exposure index).

Note: In case no biota is collected by your group during the fieldwork, reference should be made to the biotic data collected by another group when addressing the graphical representation questions. Consult your tutor prior to doing this, however.

QUESTIONS

• How did you distinguish between the wet and dry zones along your transects?
• How do you explain the existence of an anaerobic sand layer on a beach?
• How would you expect changes in beach profile to affect distribution of biota on a beach? Suggest an alternative method to determine the beach profile besides the method you used.
• Suggest reasons why so few organisms are collected by the core sampling technique
• Why is it important not to disturb the seabed with the hand net during the sweeping movement?
• Suggest a disadvantage of the pitfall trap sampling technique used.
• Do you consider your sampling effort during the coring technique to have been adequate? Explain.

REPORT CHECKLIST

Each report should have:

• An ‘Introduction’ including a clear statement of the aim and objectives of the study.
• A ‘Materials and Methods’ section which makes reference to the BIO 3060 Handbook, but which does not reproduce it verbatim.
• A ‘Results’ section where data, data analyses and tables/figures are presented.
• A ‘Discussion’, which should include the following:
  1. An interpretation of the results (e.g. species composition, classification of
     beach sand according to Wentworth Scale, general considerations about
     macrofaunal diversity on the beach)
  2. Brief answers to the set questions.

**Note:** Reference should be made to published works on sandy beaches (especially
published material on local beaches) when addressing the set questions.

**BIBLIOGRAPHY AND REFERENCES**


TOPIC 4 – THE SUBLITTORAL

Lecture: ECOLOGY AND BIOTA OF THE SUBLITTORAL

Lecturers: Mr Edwin Lanfranco & Dr Joseph A Borg

PHYTOBENTHOS
Marine plants can be broadly divided into those which are benthic (attached to their substrate) and those which are planktonic (microscopic plants which drift passively in the currents). In this account only macroscopic benthic plants are considered. The great majority of marine benthic plants are algae, which are photosynthetic organisms without vascular tissues and with structurally simple reproductive organs. The algae are very diverse and belong to several unrelated evolutionary lines. Those with benthic macroscopic members are the Green Algae (Chlorophyta), which are related to the higher plants; the Brown Algae (Fucophyta = Phaeophyta); the Red Algae (Rhodophyta), and a very few species of Yellow-green Algae (Tribophyta = Xanthophyta). Apart from the true algae, there are many species of Blue-green Algae (Cyanophyta = Cyanobacteria), which are really photosynthetic bacteria, some of which may reach macroscopic proportions, and a few species of Seagrasses, which are really marine flowering plants.

The sea around the Maltese Islands is microtidal, i.e. the effect of tides on the sea level is minimal – in the order of a few centimetres. Sea level is mainly determined by atmospheric pressure, weather conditions and swell. The seashore is divided into a series of zones. That which concerns us here is the upper or photic zone which is that where sufficient light penetrates to allow the growth of photosynthetic plants. The depth of the photic zone varies according to the clarity of the sea which in turn is determined by the amount of suspended particles. The less suspended material present in the water column, the greater is light penetration. In the seas around Malta this zone can be over a hundred metres deep. The photic zone is itself subdivided into four subzones: The Supralittoral, Mediolittoral, Infralittoral and Circalittoral; the latter is beyond the scope of this account.

The Supralittoral is permanently exposed, except during the most violent storms. It is mainly affected by sea-spray and is sometimes referred to as “spray zone” or “splash zone”. On rocky shores this is mainly characterized by microscopic blue-green algae which impart the characteristic coloration to the rocks. Otherwise this zone is best characterised by animal species, particularly the periwinkle (Melarhaphe neritoides), chthamalid barnacles and the sea slater (Ligia italica).

The Mediolittoral is that zone lying between maximum and minimum sea levels and is thus alternately submerged and exposed, the upper mediolittoral being subjected to longer periods of exposure than the lower mediolittoral. Benthic plant communities are only well developed on hard substrates. The organisms living in the mediolittoral must therefore be adapted to withstand periods of exposure. Insofar as plants are concerned, the upper mediolittoral is marked mainly by a variety of microscopic blue-green algae which impart a brownish to blackish coloration to the rock. Macroscopic blue-green algae such as the gelatinous colonies of Rivularia atra and Rivularia mesenterica mark the
lower limits of the upper mediolittoral. On exposed polluted shores the red algae *Bangia fuscopurpurea* and *Porphyra leucosticta* may be dominant. In cleaner waters, red seaweeds such as *Polysiphonia sertularioides* (often growing on limpets) and the deep red encrusting *Hildenbrandia prototypus* are characteristic of the lower fringes of the upper mediolittoral. In some particularly exposed sites, the lower horizon of the upper mediolittoral is marked by a rim (*cornice*) of the coralline red alga *Lithophyllum byssoides* (= *L. lichenoides*).13

The lower mediolittoral is often characterised by a platform (*trottoir*) consisting of the coralline alga *Neogoniolithon brassica-florida* (in the form which is often named *N. notarisii*) in which are usually embedded the sessile vermetid gastropods *Dendropoma petraeum* thus forming what is known as a vermetid platform. Other encrusting corallines are also common in the fringe zone between the lower mediolittoral and upper infralittoral, the most common are *Lithophyllum incrustans* and *Phymatolithon lenormandii*. This fringe is often covered by a belt of the flexible coralline alga *Corallina elongata* (Żerrieghet il-Hnix) which may be associated with such species as the red seaweeds *Polysiphonia opaca*, *Gelidium crinale*, *Ceramium ciliatum* and the green *Valonia utricularis*. There may also be a fringe of the green spongy alga *Cladophoropsis modonensis*. Horizontal surfaces in the lower mediolittoral are often densely covered by the olive-green *Chondrophycus papillosus* (= *Laurencia papillosa*) together with less robust species such as *Dictyota mediterranea*, *Dictyota fasciola*, *Dictyota linearis* and *Padina pavonica* (*Denb il-Pagun*), generally more characteristic of the upper infralittoral. In exposed situations, the transition zone between the lower mediolittoral and upper infralittoral is often dominated by forests of the brown algae belonging to the genus *Cystoseira* particularly *C. amentacea*, *C. compressa* and *C. brachycarpa*. In sheltered situations, particularly where there is some sewage pollution, the dominant plants are green algae of the genera *Ulva* (*Ħass il-Bahar*), *Enteromorpha* and *Cladophora*.

The **Infralittoral** constitutes that part of the seabed which is permanently submerged and where sufficient light is available for photosynthesis by seagrasses and photophilic (i.e. light loving) algae, and extends in the Maltese Islands to a depth of about 40 m. This zone provides a mild environment that supports numerous species.

Several abiotic factors favour the presence of a particular species. Species are usually specific to certain environmental conditions such as light, water movement, chemical composition of the water and substratum. Therefore, species favouring similar environmental factors will tend to be grouped together in assemblages, which form units comparable to plant communities on land, distributed into 3~4 strata (levels) - an arborescent stratum, a bush stratum, a 'turf' layer and an encrusting ground cover.

**Hard substrata**
The most characteristic communities of hard substrates are those dominated by the brown algae *Cystoseira* spp. Densely branching *Cystoseira* species typically form extensive beds, forming an important phytoocoenosis. The main axes and seasonal branches of *Cystoseira* are generally densely covered by epiphytes such as *Jania*.

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12 Coralline algae are red algae which are heavily encrusted in calcium carbonate. Many species form stony concretions of considerable ecological importance
13 This species is endangered and is in fact protected by Maltese legislation.
rubens. A sciaphilic (i.e. shade loving) assemblage made up of a bush layer (e.g. *Corallina etongata*), a 'lawn' layer (= e.g. *Valonia utricularis*) and an encrusting layer (with red algae e.g. *Peyssonnelia* spp.) develops at the base beneath the canopy. *Cystoseira* populations are very sensitive and are replaced by more tolerant photophilic algae if exposed to pollution. Such species are usually the brown algae *Padina pavonica* and *Stypocaulon scoparium* (= *Halopteris scoparia*) (*Felċi tal-Bahar*). These are not themselves pollution indicators but are generally more tolerant than the more sensitive *Cystoseira*. In more shaded places, *Dictyopteris polypodioides* (*Ħabaq il-Bahar*), a brown alga, forms particularly extensive beds on sloping rocky substrata. It is always found in relatively exposed areas in oligotrophic (i.e. with low nutrient levels) waters. The superior limit gives way to photophilic algal communities of the shallow infralittoral (*Cystoseira* associations), whilst its inferior limit usually leads to sciaphilic algal communities of *Peyssonnelia, Flabellia petiolata* (*Mrewħa tal-Bahar*) and *Halimeda tuna*.

**BENTHIC FAUNA**

The occurrence, distribution and composition of benthic fauna on sublittoral hard substrata follows closely that of the phytobenthos. Algal forests serve as a habitat for numerous species of invertebrates; they provide refuge against predation and food, and serve as nursery areas. Several fish species are also found closely associated with algal forests, since the latter serve as feeding grounds and nursery areas for the fishes. Benthic fauna that are smaller than 0.5mm, but still visible under a stereomicroscope are referred to as **meiofauna**, while those having a size of between 0.5mm and 4cms are known as **macrofauna**. Benthic macrofauna larger than 4cms are known as **megafauna**. Furthermore, benthic fauna that are permanently attached to the rocky substrata are known as **sessile**, while those that can walk, crawl or move around on the substratum by other means are known as motile (or **vagile**).

In shallow waters to a depth of around 25m, where there is considerable light penetration, the assemblages of algae and associated benthic macrofauna present on hard substrata are known as the assemblages of **photophilic algae**. In deeper waters to a depth of 100-120m, where light penetration is reduced, the assemblages of algae and associated benthic macrofauna are known as the assemblages of **sciaphilic algae**. The former assemblages are very characteristic of the gently sloping seabed found in shallow waters (0-25m) off the northeastern coast of the Maltese Islands, and at the same depth on the vertical surface of submarine cliffs (drop-offs) that characterise southwestern coastal areas. The sciaphilic assemblages are characteristic of deep (>25m) local offshore areas along the northeastern coast, and at depths greater than 25m on the vertical faces of submarine cliffs along the southwestern coasts.

The benthic macrofauna associated with the assemblages of photophilic algae include sponges (e.g. *Ircinia* spp., *Aplysina aerophobia* and *Chondrilla nucula*), hydroids and bryozoans (both of which are also found growing on the algae and are hence known as **epiphytes**) and many species of molluscs (e.g. the gastropods *Rissoa variabilis* and *Columbella rustica*), polychaetes and crustaceans (namely amphipods, isopods, tanaids and decapods). Several species of benthic megafauna (e.g. the urchin *Paracentrotus lividus* and the seastar *Echinaster sepositus*) are also found associated with these assemblages.

The benthic macrofauna associated with the assemblages of sciaphilic algae include several large sponges (e.g. *Agelas oroides* and *Petrosia ficiformis*), bryozoans, solitary...
tunicates (e.g. *Halocynthia papillosa*), and many species of molluscs, polychaetes and crustaceans (namely amphipods, isopods, tanaids and decapods). Several species of benthic megafauna (e.g. the seastar *Ophidiaster ophidianus*) are also found associated with these assemblage types.

Therefore, the occurrence, distribution and species composition of macrofauna associated with hard substrata vary depending on the amount of light available underwater (which in turn affects the presence of algal species present), and on exposure and depth. However, another important determinant is the quality of water of the locality in which they are found. Assemblages of photophilic algae and sciaphilic algae found in polluted waters (e.g. ports and harbours) are very different from those found in clean waters. The assemblages of polluted waters tend to be dominated by a high abundance of a few species that are tolerant to pollution (and indeed require the high levels of nutrients found in such waters for their survival), such as some bryozoans and ascidians, and filter-feeding polychaetes (particularly serpulids).

The assemblages of hard substrata referred to above may not necessarily be restricted to rocky substrata! Any ‘hard’ substratum, including man-made ones such as concrete and iron structures may be readily colonised by photophilic and sciaphilic assemblages. Therefore, jetties, piers, quays, and artificial reefs and wrecks all qualify as hard substrata, and may be colonised by the assemblage types discussed above.

**Soft substrata**

The term soft substrata is used to describe mobile sediments, and can therefore refer to boulders, cobbles, pebbles, gravel, sand, clay and mud. Soft substrata are easily disturbed by strong wave action, currents and bioturbation (disturbance resulting from foraging, burrowing and digging by fauna). Because of their instability (which, however, varies depending on grain size), such bottom types do not offer a suitable surface to which plants and animals can attach permanently, and are often ‘bare’.

Although the marine flora is dominated by algae, very few are capable of colonising soft, mobile substrata. Such substrata are dominated by the seagrasses. Five species are found in the Mediterranean, of which only three are known for Malta. Of these, the Neptune Grass, *Posidonia oceanica* (*Posidonja, Alka*), an endemic to the Mediterranean, is the largest, most abundant and most widespread, and is found mainly on sandy bottoms between 2m and 40m depth. It is a highly productive plant that produces large volumes of oxygen. It also plays an important role in reducing coastal erosion and in stabilising the seabed by forming *mattes* (rhizomes and roots compacted by sediment). *Posidonia* is very susceptible to pollution, particularly to agents which increase turbidity, and to sedimentation in general. Under adverse conditions, the *Posidonia* meadows start to die off and, in turn, this affects the various organisms which depend on it and also results in increased shore erosion. The two other seagrasses found in Malta are *Cymodocea nodosa* and the Lessepsian immigrant *Halophila stipulacea*. The most prominent algae that can colonise mobile substrata belong to the genus *Caulerpa* of which two species occur around Malta, the native *Caulerpa prolifera* and *Caulerpa racemosa* which is a recently introduced invasive species which may be a

14 Lessepsian immigrants are organisms which have migrated from the Red Sea to the Mediterranean since the opening of the Suez Canal.
Lessepsian immigrant. This latter species has also colonised hard substrates, starting from the lower mediolittoral down to well into the circalittoral.

Because of their instability, bare sandy and muddy bottoms usually support an impoverished benthic epibiota on their surface, but a rich infauna. Where seagrass beds occur on soft substrata, they greatly enhance the species richness and abundance of associated flora and fauna. In the Mediterranean Sea, the endemic seagrass Posidonia oceanica (Neptune grass; alga) forms meadows that have a high structural complexity and support assemblages of plants and animals that have a high species richness and abundance. Over 1,000 plant and animal species have been recorded from Posidonia beds and a single meadow in one locality may support several hundred different species. Posidonia oceanica meadows support a complex ecosystem. This complexity mainly stems from the structural characteristics of the meadows themselves. The various important roles of Posidonia meadows include (i) they provide of food and shelter, and serve as nursery areas for numerous invertebrates and fishes; (ii) they stabilize the bottom sediment; (iii) they protect the coast against erosion by strong wave action; (iv) they oxygenate the water; (v) they serve as an ‘extended substratum’.

Extensive areas with bare soft substrata occur locally at the head and in the central parts of bays and inlets, and at depths greater than 40m, where the underwater conditions are not suitable for supporting seagrasses. Because of the absence of plant cover on bare soft substrata, the associated fauna is severely exposed to predation. Consequently, soft-bodied animals that have no external protective cover seek shelter from predators by residing in burrows in the sediment. The (relatively) few faunal species that crawl on the surface of soft substrata either carry hard shells (e.g. hermit crabs and gastropods), have a thick armoured skin (e.g. echinoderms which also posses spines), or adopt behavioural strategies involving camouflage.

Besides sandy and muddy bottoms, other soft substrata include accumulations of small boulders, pebbles and cobbles. This habitat type supports a rather specialized biota. Small boulders, cobbles and pebbles are not easily disturbed by water movement as in the case of sand, mud and clay. Nevertheless, strong water movement (e.g. wave action and strong currents) will disturb such a habitat type. Consequently, only a thin algal turf is usually present on small boulders and cobbles, while most faunal species live beneath them.

Another interesting habitat type that is classified with soft substrata are maerl beds. ‘Maerl’ is a term used to describe calcareous sediments dominated by unattached coralline algae. These algae may take the form of nodules (rhodoliths) or fragmented thalli. Rhodoliths consist either of free-living calcareous rhodophytes, or else of an inner nucleus, such as stone or shell, encrusted by calcareous rhodophytes. Extensive maerl beds occur locally at depths of around 45-100m. Because of the high structural complexity resulting from layers of rhodoliths that characterizes maerl beds, these habitats support a very high biodiversity and constitute important feeding grounds and nursery areas for many species of invertebrates and fishes.

Assemblage of soft sediments in highly polluted waters (e.g. inside ports and harbours) are characterized by fauna that is tolerant to pollution and turbidity, namely polychaetes and molluscs.
PRACTICAL 4: Shallow infralittoral habitats

Lecturers: Dr Joseph A Borg, Mr Edwin Lanfranco and Prof Patrick J Schembri
Demonstrators: Ms Sarah Debono and Mr Edwin Zammit

INTRODUCTION

The lowland northeastern coastline, together with the cliff-dominated southeastern coastline of the Maltese Islands support extensive benthic biotic assemblages on hard and soft substrata. Hard substrata (mainly bedrock) are typically found along the headlands of local inlets, while extensive areas with soft substrata are found at the head and central parts at depths ranging between 0m and 40 m. In the central parts of local inlets, off their mouth, and off rocky shores, local sandy bottoms support extensive beds of seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*) – the most successful plants to colonise this habitat type. Apart from seagrasses, very few algae (e.g. *Caulerpa racemosa*) are capable of growing on soft substrata and, where present, form very sparse stands or single plants. Several environmental factors including exposure to wave action, water quality, the nature of the substratum, availability of light and biological interactions, influence the occurrence and distribution of such assemblages. Furthermore, the species composition and abundance of the flora and fauna making up these assemblages can also vary from place to place (even over very short distances, e.g. a few metres) for the same assemblage type. Soft sediment bottoms are ubiquitous around the Maltese Islands.

OBJECTIVES

This practical is intended to introduce you to the typical biota of sublittoral hard and soft substrata found in local gently sloping coastal areas. During this practical you will be required to:

- Map the distribution of: (i) assemblages of photophilic algae on hard substrata in shallow waters (0-3m); (ii) seagrass (*Cymodocea nodosa* and *Posidonia oceanica*) meadows; and (iii) bare sand assemblages using snorkelling and transect techniques.
- Collect data on percentage cover of algae and large sessile fauna (e.g. sponges, sea anemones etc) using quadrats (and snorkelling) techniques.
- Collect samples of biota associated with assemblages of photophilic algae on hard substrata in shallow waters (0-3m) using quadrats (and snorkelling) techniques.
- Examine and identify the characteristic biota associated with assemblages of bare soft sediments and those of *Cymodocea nodosa* meadows in shallow waters.
MATERIALS

Items marked with an asterisk (*) must be supplied by course participants themselves.

Diver’s slate and pencil (*); measuring tape; 25cm x 25cm quadrats; plastic bags and permanent markers; buckets to transport specimens and samples; chisel to scrape off epibiota from hard substrata; snorkelling gear (*); stereomicroscopes; identification manuals; sorting trays; forceps and dissecting needles (*); rulers (*).

FIELD PROCEDURES

The class will be divided into groups. Different groups will undertake different procedures: mapping survey, estimation of percentage cover by algae and sessile biota, and collection of samples of biota using quadrats, thereby ensuring rotation of tasks.

Mapping survey
Starting from the shore, swim out at a bearing perpendicular to the shore while unwinding the measure tape (which should be kept taut) until the distance from the shore is 25 m. Measure the distance (from the shore) at which boundaries between different benthic assemblages occur (e.g. between rocky substrata and soft/mobile substrata\(^\text{15}\)). Each group should make one such transect. Adjacent transects should be around 10 m apart. Record all data on a diver’s slate. Draw a rough sketch of the stretch of shore where you have collected the data.

Estimation of percentage cover
A sampling station, located at a depth of around 0.5-1m, will be allocated to each group. Place a 25cm x 25cm quadrat at random on the assemblage of photophilic algae. While holding the quadrat firmly, estimate the % cover of species of algae and/or large sessile fauna (e.g. anemones) enclosed in the quadrat. Repeat the procedure 3 times to obtain 3 replicate estimates for one station. All estimates must be carried out at a depth of around 0.5 -1 m. Record all data on a divers’ slate.

Collection of biota
A sampling station, located at a depth of around 0.5 -1m, will be allocated to each group. Place a 25cm x 25cm quadrat at random on the assemblage of photophilic algae. While holding the quadrat firmly, scrape off all the enclosed biota using a chisel, and transfer to a mesh net. Transfer the biota from the mesh to a labelled plastic bag, making sure that the sides of the mesh net have been washed well and examined for any small invertebrates retained on the sides. On completing quantitative sampling, carry out ‘random collection’ of biota to ensure that you also become familiarised with species not collected in the quadrats.

\(^{15}\) Cobbles, pebbles, gravel, sand etc..
Collection of core samples from bare sand and seagrass (SCUBA divers only)
SCUBA divers will collect cores (using a 10 cm metal corer) from adjoining bare sand and seagrass (*Cymodocea nodosa*) habitat. Three cores will be collected from each of the bare sand and seagrass bed habitats.

LABORATORY SESSION 4: SHALLOW INFRALITTORAL HABITATS

Sorting and identification of biota

Transfer the sample material collected (quadrats on hard substrata, sediment cores and seagrass) to a sorting tray and cover with tap water to a depth of around 1-2 cms. Distribute the material evenly by holding the tray and swaying it gently sideways. In the case of samples collected on rock, examine the algae and fragments of rock for the presence of living plants and animals, using forceps. Remove any plants present and transfer onto clean sheets of paper, making sure that the algae are spread out evenly on the paper. In the case of soft sediment samples, distribute the latter evenly by holding the tray and swaying it gently sideways. Using forceps, move small portions (around 1g) of the sediment towards you, starting at the edge of the sediment layer closest to you. Examine carefully for the presence of living plants and animals, distinguishing from dead material (e.g. empty shells of molluscs, spines of echinoderms etc, which should not be taken out). In the case of seagrass samples, remove the seagrass from the sample and wash the vegetation thoroughly over a sieve to retain any biota that wash out. Add the biota retained on the sieve to the sediment from the same sample. In all three cases (samples taken on rock, sediment cores and seagrass samples), any living material encountered should be taken out gently using the forceps and transferred to a labelled specimen container filled with 70% ethanol.

Use the manuals, keys and books to identify the plant and animal species you have collected to the lowest taxon possible. For each of the three different samples, make a list of the recorded species and tabulate the total number of individuals recorded for each species.

DATA ANALYSIS

Pool the data collected by the different groups during the mapping survey and draw a map showing the distribution of biotic assemblages in the study area. Use different hatching/shading to indicate different assemblages of algae and different habitat types.

Estimate the mean percentage cover of plants and/or sessile animals for each set of three quadrats and draw graphs of percentage cover (for the three most abundant species) vs station. You need to pool the data collected by the different groups.

For each group of three stations, estimate the mean total number of faunal species, the mean total faunal abundance, and the mean total abundance of the three most abundant animal species collected from the quadrats. Draw graphs of these attributes vs stations (i.e., station 1-3, 4-6, 7-9 etc).
Estimate the mean total number of faunal species and the mean total faunal abundance recorded from the core samples for: (i) bare soft substratum and (ii) seagrass meadows, and draw graphs to compare the two.

QUESTIONS

Comment on the distribution of different benthic assemblages recorded from the mapping survey. Which factors may be responsible for the observed distribution? What would you suggest to increase the accuracy of the map produced?

Are there differences in percentage cover of different plants and/or sessile animals between the different stations? Interpret the results obtained, giving possible explanations for your findings.

Are there any differences in the total number of species and abundance of fauna between the different groups of stations? Interpret the results obtained, giving possible explanations for your findings.

Are there any differences in the total number of species and abundance of fauna between the bare sand and seagrass habitats? Interpret the results obtained, giving possible explanations for your findings.

What difficulties were encountered during fieldwork?

BIBLIOGRAPHY


INTRODUCTION

These notes are not meant to be an exhaustive account of the relevant subject matter, but should serve only as an outline of the main concepts and issues, which will be covered during the lecture. The lecture itself is aimed at being a primer to the understanding of the basic physico-chemical properties of seawater and how these support marine life with particular reference to plankton and nekton.

Oceanography is a highly practical science and within the context of the present study-unit, the main thrust of the lecture will be more towards the practical aspects of the relevant concepts, especially with respect to measurements at sea. This will prepare students to the various methodologies and techniques for the monitoring of seawater parameters that they will be introduced during fieldwork.

Physical Properties of Seawater

Approximately, 96.5% by weight of seawater is liquid water, and the lecture will outline the basic physical notions of water as a medium for life-supporting systems and as a solvent.

Seawater temperature is one of the most important factors affecting life at sea. The temperature profile of the water column is related to insolation (rate of solar energy falling on the sea surface).

Topics to be covered include:
- Isotherms;
- Thermocline;
- Seasonal changes in thermocline;
- Measurements of surface and subsurface seawater temperatures.

Seawater salinity, although strictly speaking a chemical property of seawater, greatly determines its physical properties such as density. A halocline is a sharp salinity gradient in the water column and often represents the boundary between different water masses.
Density of seawater (g/cm³) depends on temperature, salinity and pressure. Water density controls the vertical structure of the water column. Density increases with a drop in temperature and increase in salinity, and may be measured from temperature and salinity data from tables or graphs.

A pycnocline is a sharp density gradient in the water column and again represents the boundary between different water masses: the surface layer, the pycnocline layer, and the deep layer.

Suspended Solids in the water column greatly influence marine life. Water transparency or turbidity is in turn related to both physico-chemical changes in seawater (e.g. precipitation of evaporates) as well as primary productivity.

Water turbidity (or transparency) may be measured by the classical Secchi disk, as well as by the use of an in situ transmissometer and a nephelometer.

The index most frequently used for this water quality parameter is the Secchi disk depth. In fact the EC Directive (76/160/EEC) for bathing waters sets threshold limits for this parameter. However, past experience has repeatedly shown that this parameter is not suitable for application in the local oligotrophic and clear inshore coastal waters. This is because the waters at the various stations being monitored are sufficiently clear and the waters relatively shallow, so that the bottom is found to be visible most of the time.

Water turbidity may also be monitored through nephelometry (as measured in nephelometric turbidity units, NTU). A nephelometer measures the amount of light scattered by suspended solids. This index is particularly sensitive to a greater size range of suspended solids at low concentrations.

A more reliable index of water transparency is the beam attenuation coefficient as monitored by a transmissometer. For a monochromatic beam travelling through a water column containing suspended particles, light loss is either by absorption into other forms of energy, or by scatter outside of the collimated beam. The amount of light loss depends upon the length of water column and the coefficients of light absorption and light scatter by the particles suspended in the water.

The transmissometer to be used in the field survey measures the attenuation at 660nm of a beam of light with an optical path of 25cm. Its calibration involves the measuring of its voltage output at 100% transmission, at 91.3% transmission (pure distilled water), and then at 0% transmission (blocked beam). The water transparency is then measured in terms of a beam attenuation coefficient.

Other physical properties of seawater which greatly influence life at sea (to be briefly reviewed during the lecture), include:

- light transmission;
- sound transmission; and
- formation of sea ice.
CHEMICAL PROPERTIES OF SEAWATER

Salinity *(as a rough and inaccurate definition)* is the total mass of solutes or salts, expressed in grams per kilogram of seawater. Concentrations of solutes in seawater are usually measured in parts per thousands (ppt) or parts per million (ppm). Other units of measurements (e.g. PSU) will be discussed during the lecture.

Sodium and chloride ions are the main solutes in seawater (together comprising more than 85.65% of all solutes in seawater).

The major constituents (forming up to 99.99% of solutes) of seawater will be reviewed. These vary little over time at most places and are described as conservative properties of the sea.

Although salinity may vary with time and place, the relative proportions of the major constituents are constant. This fact is often referred to as the principle of constant proportion (or constant composition). It provides a very useful method for measuring salinity from chlorinity (i.e. content of chloride and other halogens) of seawater.

The lecture will review the various methods to measure salinity in the lab and at sea.

Other topics to be covered include:
- Factors regulating salinity of seawater;
- Steady-state equilibrium: balance between sources and sinks of various constituents at sea;
- Residence times of different constituents.

Nutrients, primarily: nitrogen (nitrates), phosphorus (phosphates) and silicon are found in much smaller amounts (ppm). Because of their biological uptake and release, their concentrations vary greatly with time and place and are therefore described as non-conservative properties of seawater.

The lecture will briefly review:
- The main factors regulating the concentration of nutrients at sea;
- Vertical nutrient profiles in water column;
- Nutrients and primary productivity;
- Nitrogen: Phosphorus ratio;
- Oligotrophy and the Mediterranean;
- Methods (and units) of measuring nutrient levels.

Gases in seawater include: nitrogen (47.5%), oxygen (varies), carbon dioxide (varies), and hydrogen, argon, neon and helium, which together generally make up 1.4% of gases in surface seawater. The levels of oxygen and carbon dioxide vary greatly in space and time mainly due to biological activities.

The lecture will briefly review:
- The main factors regulating the concentration of gases in seawater;
- Hyperoxic, hypoxic and anoxic conditions;
- Vertical oxygen profile in the water column;
- Carbon dioxide and primary productivity;
• Carbon species and the carbon dioxide system in the marine environment;
• Methods (and units) of measuring oxygen levels at sea.

Other general topics which will be covered include:
• Seawater sampling at surface and subsurface levels;
• Determining sampling depths
• Analytical procedures
• Trace elements and organic constituents
• The sea-surface microlayer: a special habitat.

PRIMARY PRODUCTIVITY

At sea, primary productivity is limited by the availability of solar energy and critical nutrients, particularly nitrates, phosphates, and possibly silica (macronutrients).

Micronutrients are indispensable to plant life but are required at much lower levels. These include iron, copper, manganese, zinc boron and cobalt, among others.

Net primary productivity (the amount of carbon fixed through photosynthesis that exceeds the respiratory demands of the plant and goes into growth) occurs in the water column down to the compensation depth, below which there is no net primary productivity.

Primary productivity may be measured through a number of methods (which will be briefly reviewed during the lecture). One index of primary productivity is a measure of the level of chlorophyll in seawater. An in situ spectrofluorimeter will be used during the field survey to measure chlorophyll as levels at different depths in the water column. The advantages and limitations of this index will be discussed.

Other topics to be discussed include:
• Depth profile of primary productivity;
• The photic / euehotic zone;
• Upwelling and turbulence;
• Patterns of productivity at the global and Mediterranean level;

PLANKTON AND NEKTON

Plankton consists of organisms that drift or swim weakly and hence are powerless to counteract sea currents. Many plankton however go through vertical migrations through the water column both on a seasonal as well as diurnal basis.

Plankton is classically divided into phytoplankton (mainly photosynthetic autotrophs) and zooplankton (chemosynthetic heterotrophs).

The lecture will focus on a few of the sampling techniques for plankton (use of plankton nets, settling and counting techniques) rather than on the classification and systematics of this group.
**Nekton** includes the active swimmers such as fishes, squids, seals and mammals.

The **benthos** includes organisms that are attached to or move on or beneath the sea bottom. The fieldwork will include sampling for offshore plankton using plankton nets as well as remote sampling for benthos using a grab sampler and an anchor dredge.

The characteristics of Mediterranean marine life (including plankton and nekton) reflect the main forcing factors of the abiotic environment, such as nutrient deficiency, deep water temperature, and low tidal amplitudes. Therefore, knowledge of the physical constraints is fundamental to an understanding of the pelagic ecosystem.

The general trend in annual offshore Mediterranean plankton production is based on strong phytoplankton blooms in spring and to a lesser extent in autumn. This trend is associated with maximum variability in temperature and salinity gradients. Plankton levels are at a minimum in summer and winter when water stratification is much more stable.

Primary productivity in offshore Mediterranean areas which are not under the direct influence of major rivers or urban land-based activities, is low. Several factors contribute to this and some will be reviewed during the lecture.

Within their annual evolution, interactions between plankton and nekton groups and their response to abiotic changes are specific to different Mediterranean sub-regions, though long-term observations to confirm such evolution are only available from some areas.

Several attempts have been made in the past to classify the **trophic state (scale) of the sea**, and these will be briefly reviewed during the lecture.

The Mediterranean is one of the most oligotrophic seas in the world and most of its biological productivity takes place in the euphotic zone. Although the development of nutrient / phytoplankton concentration scales seems to be a relatively simple approach, there are numerous difficulties mainly due to seasonal fluctuations.

The development of nutrient / phytoplankton concentration scales has been a difficult task for marine scientists because of the seasonal fluctuations of nutrient and phytoplankton concentrations, phytoplankton patchiness and small-scale eutrophication phenomena. However, there are some attempts reported in the literature, mostly based on probabilistic methods.

Total phosphorus, total nitrogen, chlorophyll a, and Secchi depths were used as the variables expressing eutrophication and trophic scales. Data sets were used from the Adriatic Sea and the geometric mean was calculated. Critical values of phosphate, nitrate, nitrite and ammonia were proposed by some authors, based on probabilistic procedures.

Similar work used phosphate, nitrate, ammonia and phytoplankton cell number values to propose a classification scheme for coastal waters, using the following categories: oligotrophic, lower mesotrophic, upper mesotrophic and eutrophic water types. The procedure was based on spatial analysis. Critical values of ecological indices for characterizing eutrophication and oligotrophy have also been published.
The role of N and P as limiting factors of the primary production and biomass in the Mediterranean Sea is still controversial. Laboratory experiments performed with natural planktonic communities from the NW Mediterranean coast suggest that some biochemical indicators can be used to identify N versus P deficiency. In particular, high N:P ratios were associated to high protein:DNA and chlorophyll:DNA ratios; the opposite trends were observed under low N:P ratios. In addition, low chlorophyll:protein ratios characterized N limited communities.

PRACTICAL 5: Water chemistry and identification of plankton and benthic fauna (collected by remote grab and dredge)

Lecturers: Prof Victor Axiak, Dr Joseph A Borg and Prof Patrick J Schembri
Demonstrators: Ms Ruth Guillaumier, Ms Sarah Debono and Mr Edwin Zammit

INTRODUCTION

Remote sampling techniques are usually employed by biologists when in situ collection of samples and data is not possible, for example, when studying offshore and deep sea pelagic and benthic biotic assemblages, or in extreme climatic conditions. Benthic fauna can be collected using remote sampling gear and techniques. Such gear includes grabs, corers, dredges, trawls and underwater TV cameras (sometimes mounted on a special underwater ‘vehicle’, in which case the equipment is known as Remotely Operated Vehicle – ROV). The biota of soft substrates can be collected remotely using grabs, dredges and trawls, while pelagic biota is sampled using towed nets. For example, plankton is sampled using plankton nets. Such equipment is usually bulky and/or heavy to deploy. Consequently, biologists usually use the services of a marine research vessel and trained crew to assist them in deployment of remote sampling gear. Marine research vessels usually have a length of 50’ or more, have a very sturdy construction and are equipped with winches and derricks to enable deployment of remote sampling gear.

To collect samples of seawater remotely, biologists use water samplers and collection bottles. A popular water collection bottle is the ‘Van Dohrn’ sampler. This sampler is suspended from a line into the water, and can be taken down to any depth.

The Department of Biology at the University of Malta does not have its own research vessel. However, over the past ten years or so, local biologists have used the services of Maltese fishing boats and other commercial vessels, whose crews have also been trained to deploy remote sampling gear. Some local fishing vessels, particularly those used in fishing for Blue Fin Tuna and boats that service them, are well suited for the purpose, since they are large, sea worthy and equipped with winches and derricks that can be used to deploy heavy sampling gear.
OBJECTIVES

This practical will aim to introduce the participant to the following:

- Identification of flora and fauna collected by remote sampling techniques (grab and dredge).
- Demonstration on the use and calibration of the following instruments:
  - Temperature meter
  - Salinity Meter
  - Spectrofluorimeter for the measurement of chlorophyll a
  - Transmissometer for the measurement of beam attenuation coefficient
  - Oxygen meter
- Determination of dissolved nitrates (as given below).
- Determination of dissolved phosphates (as given below).
- Phytoplankton and zooplankton analysis using various methods such as:
  - Settling (Utermohl) technique followed by use of inverted microscope;
  - Use of counting chambers;
- Identification of specimens.

MATERIALS

Items marked with an asterisk (*) must be supplied by course participants themselves.

Life jackets, notebook and pencil (*); Van Veen grab and Kevlar line; Van Dohrn water sampler; sieve (1mm); naturalist’s dredge and wire rope; buckets and lids; specimen bottles; sorting trays; dissecting needles (*); forceps (*); magnifying lens; inverted microscope; stereomicroscope; temperature meter; salinity Meter; spectrofluorimeter; transmissometer; oxygen meter; apparatus and reagents for estimating phosphates and nitrates.

FIELD PROCEDURES

Remote collection of samples of benthic biota and water will take place on board a large vessel equipped with a hydraulic crane and winch. Participants will be required to observe the whole sample-collection procedures, without doing any work themselves. However, they will be required to take detailed notes throughout the field session. Participants who suffer from sea sickness are to ensure that they take appropriate medication (e.g. sea sickness tablets) prior to the field session. All participants are required to wear buoyancy aids throughout the trip and are to take the necessary safety precautions, as instructed by the academic and technical staff.

Details of the meeting point will be given to participants on the day preceding fieldwork. The location of the offshore field session will depend on weather conditions. In extreme weather conditions, the trip may be cancelled.

The use of some of the following equipment at seas will be demonstrated: temperature meter; salinity Meter; spectrofluorimeter for the measurement of chlorophyll a; transmissometer for the measurement of beam attenuation coefficient; oxygen meter.
A suitable site having a water depth of around 50m will be located using the vessel’s Global Positioning System (GPS) and Depth Sounder. At the selected site, a Van Veen grab (having a sampling area of 0.1m$^2$) will be lowered from the vessel to enable collection of a quantitative sample (circa 20L) of sediment for studies of benthic macrofauna. Once the grab has closed on the seabed, it is taken up and the entire contents sieved through a 1mm sieve. The material retained on the sieve is transferred to buckets for transportation to the laboratory.

Following grab sampling, a benthic dredge will be deployed from the vessel to collect benthic megafauna. The dredge has a mesh net size of 5mm and will be towed behind the vessel for a period of 15 minutes. The dredge in then taken back on board and the contents transferred to buckets for later examination in the laboratory. Between 1 and 3 dredge samplings will be made.

Finally, a Van Dohrn bottle is lowered to collect samples from the water column for later analysis in the laboratory.

LABORATORY SESSION 5: WATER CHEMISTRY, SORTING AND IDENTIFICATION OF FLORA AND FAUNA COLLECTED BY REMOTE SAMPLING TECHNIQUES, AND ANALYSIS OF PLANKTON SAMPLES

Sorting and identification of flora and fauna collected by remote sampling techniques, analysis of plankton samples, and water chemistry

Transfer around 500g of sediment collected from the grab sample into a sorting tray, and cover with around 1-2cms of tap water. Distribute the sediment evenly by holding the tray and swaying it gently sideways. Using a forceps, move small portions (around 1g) of the sediment towards you, starting at the edge of the sediment layer closest to you. Examine carefully for the presence of living plants and animals, distinguishing from dead material (e.g. empty shells of molluscs, spines of echinoderms etc, which should not be taken out). Any living material encountered should be taken out gently using the forceps and transferred to a labelled specimen container filled with 70% ethanol.

Repeat the procedure for material collected in the dredge samples.

Identify the biota collected in the grab and dredge samples to the lowest taxon possible. Make a list of the recorded species and tabulate the total number of individuals recorded for each species.

**Determination of reactive nitrate**

Range: 0.05 - 45 $\mu$g-at/litre

**Apparatus:**

- Reduction columns
- 100 mL graduated cylinders
- 100 mL conical flasks
Reagents:
1. Concentrated ammonium chloride solution
Dissolve 125g of analytical grade ammonium chloride in 500 mL of distilled water. Store in a glass bottle.

2. Dilute ammonium chloride solution
Dilute 50 mL of solution 1 to 2000mL of distilled water. Store in a glass bottle.

3. Cadmium-copper filings
Stir about 100g of cadmium filings (0.5-2.0mm) with 500mL of 2% w/v of copper sulphate pentahydrate (CuSO4.5H2O) until all blue colour leaves the solution. Rewash with fresh copper sulphate. Wash with distilled water and 3 runs of dilute ammonium chloride. Roll very fine copper turnings to make a small plug and push this into the bottom of a reductor column. Fill the column with dilute ammonium chloride solution. Pour in sufficient cadmium-copper mixture to produce a column about 30-cm in length. Wash the column thoroughly with dilute ammonium chloride solution. Flow rate: 100 mL of solution should take 8 to 12 mm to flow completely through the column. Add a small plug of copper ‘wool’ to the top of the column. When not in use columns must be left with the metal filings completely covered by dilute ammonium chloride solution.

4. Sulphanilamide solution
Dissolve 5 g of sulphanilamide in a mixture of 50mL of concentrated hydrochloric acid (sp.gr. 1.18) and about 300mL of distilled water. Dilute to 500mL with water.

5. N-(1-naphthyl)-ethylenediamine dihydrochloride solution
Dissolve 0.50g of N-(1-naphthyl)-ethylenediamine dihydrochloride in 500mL of distilled water. Store the solution in a dark bottle.

Sample storage:
In deep-freezer at -20°C.

Procedure:
a) Place 100mL of sample in 100 mL conical flask.
b) Add 2.0mL of concentrated ammonium chloride. Mix
c) Add the sample to the column. Place measuring cylinder under the collecting tube.
d) When 40mL passes through the column, rinse the conical flask with this solution and drain it.
e) Collect a further 50mL of solution and place it in the rinsed conical flask.
f) Add 1.0mL of sulphanilamide solution. Allow reaction to react for 2 to 8 min.
g) Add 1.0mL of N-(1-naphthyl)-ethylenediamine dihydrochloride and mix.
h) Leave for 10 min to 2h.
i) Measure extinction of the solution in a 1 cm cell using 543nm wavelength.

Nitrate concentration ($\mu$g-at N U$^{-1}$) = (corrected extinction x F) - 0.95C
Where C = concentration of nitrite present (negligible for sea water)
F = calibration factor (see calibration part)

Carry out a blank with dilute ammonium chloride.
**Corrected extinction** = absorbance value sample — absorbance value dil.
ammonium chloride

**Calibration**
a) Prepare synthetic sea water as follows:
Dissolve 155g of analytical grade sodium chloride, 50g of analytical grade magnesium sulphate (MgSO$_4$.7H$_2$O) and 0.25g of sodium hydrogen carbonate (NaHCO$_3$.H$_2$O) in 5L distilled water.
b) Prepare standard nitrate solution as follows:
Dissolve 1.02g of analytical grade potassium nitrate (KNO$_3$) in 1L distilled water. Store in a dark bottle.
Dilute 2mL of this solution to 1 L with synthetic seawater. Prepare fresh before use.
c) Add about 110mL of this dilute standard solution and determine extinction as in the method above.
d) Carry out the procedure on a blank consisting of synthetic seawater.

Calculate the factor

$$ F = \frac{20.0}{E_{std} - E_{blank}} $$

F should have a value near to **25** for a 1 cm cell.

**Determination of phosphates**

Range: 0.03 to 5 μg-at/litre

**Apparatus:**
- Screw-capped polyethylene bottles
- 100 mL Conical flasks (washed in 10% HCl)

**Reagents:**
1. **Ammonium molybdate solution**
Dissolve 15g of analytical reagent grade ammonium paramolybdate in 500mL of distilled water. Store in plastic bottle out of direct sunlight.

2. **Sulphuric add solution**
Add 140mL of concentrated (sp.gr. 1.82) analytical reagent quality sulphuric acid to 900mL of distilled water. Allow solution to cool. Store in a glass bottle.

3. **Ascorbic acid solution**
Dissolve 27g of ascorbic acid in 500 mL of distilled water. Store in a plastic bottle in the freezer.

4. **Potassium antimonyl-tartrate solution**
Dissolve 0.34g of potassium antimonyl-tartrate in 250 mL of distilled water. Store in a glass bottle.

5. Mixed Reagent
Mix together:
- 100mL ammonium molybdate
- 250mL sulphuric acid
- 100mL ascorbic acid
- 50mL potassium-tartrate solutions.
This reagent should be prepared fresh before use. The above quantity is suitable for about 50 samples.

Sample storage:
In deep-freezer at -20°C.

Procedure:
a) Warm the samples to room temperature in a water bath.
b) Place 100mL of sample in 100mL conical flask.
c) Add 10mL of mixed reagent and mix.
d) Leave for 5min to 2h.
e) Measure extinction of the solution in a 4 cm cell using 885 nm wavelength.

Phosphate concentration (μg-at P L-1) = (corrected extinction x F)

Calibration
a) Prepare standard phosphate solution as follows:
Dissolve 0.816g of analytical grade potassium dihydrogen phosphate (KH₂PO₄) in 1L de-ionised water. Store in a dark bottle with 1 mL of chloroform.
Dilute 10mL of this solution to 1L with de-ionised water.
Pipette 5mL of dilute standard into a 100mL volumetric flask and make up to 100mL with de-ionised water.
b) Determine extinction of this solution as in the method above.
c) Carry out the procedure on a blank consisting of de-ionised water.

Calculate the factor

\[ F = \frac{3.00}{E_{\text{std}} - E_{\text{blank}}} \]

F should have a value near to 12.5 for a 4 cm cell.

QUESTIONS
Which of the two remote sampling techniques (grab and dredge) can be classified as 'quantitative'? Why? Which factors are expected to reduce the grab's efficiency?
Are there differences in the biological material collected between the two sampling techniques (grab and dredge)?

Do you think that the 1mm sieve mesh size was appropriate for the grab samples? Justify your answer.

The classical way to measure water transparency is by using a Secchi disk. Explain from your own experience during the field survey, the limitations of this method.

Distinguish briefly between the principles of measurements of chlorophyll pigments and of total suspended solids using \textit{in situ} instruments.

List and briefly identify the problems and limitations involved in making quantitative plankton estimations by means of nets.

Assuming that during the marine survey water samples have been taken at different water depths, relate the levels of dissolved nitrates and phosphates, as well as of chlorophyll \textit{a} with different water depths.

**BIBLIOGRAPHY**
