



L-Università ta' Malta  
Faculty of Science

Department  
of Biology

# Biology Abstracts 2020



**UNIVERSITY OF MALTA**

**Department of Biology**

**BIOLOGY ABSTRACTS 2020**

B.Sc. (Hons)

M.Sc.

Ph.D

Edited by

David Dandria

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***ERA was set up in April 2016 to safeguard the environment in order to achieve a sustainable quality of life. This ERA achieves by mainstreaming environmental targets and objectives across Government and society and by taking the leading role in advising Government on environmental policy-making at the national level, as well as in the context of international environmental negotiations. Evidence-based policy covering all environmental topics is developed by ERA and is backed by a robust data gathering structure. The Authority also draws up plans, provides a licensing regime, monitors activities having an environmental impact and integrates environmental considerations within the development control process.***

***ERA is instrumental in the formulation and implementation of legislation aimed at designating protected areas harbouring important habitats and species, as well as legally protecting many species of flora and fauna. The Flora, Fauna and Natural Habitats Protection Regulations, 2006 (SL 549.44) are an important legal instrument in this respect. These Regulations, together with the development of the National Biodiversity Strategy and Action Plan (NBSAP), are significant contributions to the conservation of Malta's biodiversity.***

***This booklet has been partly funded by the Environment and Resources Authority (ERA). Any views expressed are those of the authors and are not to be considered as the views of ERA.***

Cover design by Gabriel Izzo (Communications Office, University of Malta) featuring the Egyptian St. John's Wort (*Hypericum aegyptiacum*).

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## B. Sc. (Hons)

### ASPECTS OF ECOLOGY OF *CLADOCORA CAESPITOSA*

Hannah Abela

Supervisors: Joseph A. Borg and Patrick J. Schembri

(Plate 4 p.16)

*Cladocora caespitosa* is a temperate, zooxanthellate scleractinian coral, which is endemic to the Mediterranean Sea and adjacent Atlantic areas. This coral occurs on hard substrata in a wide range of illumination conditions, from well-lit to dim light, and at depths ranging from shallow waters, down to around 40m. Locally, the abundance and state of health of this legally protected species appears to be declining in pristine waters; however, this aspect has not been investigated previously.

The main aim of the present investigation was to determine the occurrence and abundance of *C. caespitosa* colonies in local pristine waters, and to assess their state of health. During the present study, *C. caespitosa* was found to be more abundant in well-lit areas and colony size did not appear to bear any relationship to the depth at which the colonies were found. The present results indicated that more than half (56.8%) of the total polyps assessed were pigmented, however, the remaining polyps (43.2%) appeared to be adversely affected in some way. Most of the latter were mainly dead polyps, while bleached and partially necrotic polyps occurred in fewer numbers. The state of health of the polyps varied between localities, apparently as a result of differences in edaphic factors.

This study also assessed algal growth on the colonies of *C. caespitosa*. A significant relationship between the number of dead polyps and algal biomass was found. It is unclear whether the polyps died as a consequence of being overgrown by the algae or if their demise was caused by other factors, and algal growth occurred subsequently.

Data from the present study can be used as a baseline for further studies on the state of the local *C. caespitosa* population.

## RECOGNITION OF VISUAL STIMULI BY THE WARTY CRAB *ERIPHIA VERRUCOSA*

Edward Attard Selvagi

Supervisors: Julian Evans, Ian Thornton

The aim of the present study is to determine the visual capabilities of the crab *Eriphia verrucosa* by exposing it to footage simulating a looming stimulus which varies in approach speed, colour, sharpness, and shape. Crabs were harvested from a local shore and allowed to acclimatise for 24 hours in the ambient laboratory conditions. Individuals were then shown footage and the nature of their response and the time taken for it to be initiated were recorded.

Behavioural responses were categorised into three main types: a retreat from the stimulus, a squat and a deimatic display. Initial tests indicated that the animals' sex or presence of injuries did not influence their behaviour; this allowed the data to be pooled when analysing the responses to different stimuli.

The crabs showed no statistically significant variation in reaction nature or time as stimulus colour varied. This could be due to *Eriphia verrucosa* being unable to differentiate between the colours chosen in this experiment (black, red, green and blue). However, it is also possible that the crabs can see the different colours but react in the same manner to them. The results showed that there was a significant association between response type and stimulus speed and shape. It was seen that with faster and more complex, or 'crab-like', stimuli, the crabs were more likely to show an aggressive response. This suggests that the animals do obtain information from the velocity, profile and appearance of the stimulus.

The results also showed a significant association between response time with stimulus shape, speed, and sharpness. Post hoc analysis showed that the faster, sharper, and more complex a stimulus was, the faster they would respond. This variation in reaction speed would suggest that the animals alter their behaviour based on the amount of information they can resolve from the stimulus. Statistical analysis also showed that mean response time varied according to the type of behavioural response. This showed that the responses each took a different average time to occur suggesting that the decision-making process to get to those responses may be different.



The ability for *Eriphia verrucosa* to distinguish stimuli based on their clarity (sharpness), profile and speed is essential when inhabiting a shallow water environment. The capabilities of their vision are still not fully understood however there is scope to perform follow-up experiments. By showing a greater variety of stimuli and harvesting crabs from different locations, a more thorough understanding of their visual capabilities can be developed.

## **EFFECTIVENESS OF DRONE IMAGERY IN MAPPING SHALLOW-WATER BENTHIC ASSEMBLAGES**

Andrea Francesca Bellia

Supervisor: Julian Evans

The present study aimed to assess the efficiency of using drones to map shallow-water benthic assemblages, compared with that of manual survey techniques. It also aimed to develop an 'optimum protocol' to characterize shallow-water benthic assemblages. The efficiency of mapping was assessed in terms of its comparative accuracy and cost-effectiveness.

The study was carried out in six coastal sites in Malta, characterised by varying benthic complexity and fragmentation. The bays were imaged using a DJI Mavic 2 Pro drone, at altitudes between 100-300m above mean sea level. Aerial images obtained were subsequently processed using k-means clustering to distinguish between different benthic assemblages, based on their colour profiles. The results of the automated drone surveys were then compared with those of the direct manual surveys to assess their relative efficiencies in terms of area mapped per unit effort.

Results obtained indicated that using a drone to map benthic cover greatly reduced the time and manpower requirements relative to direct surveys. This method also generated biotope maps with greater coverage, accuracy, and efficiency relative to manual methods. Nonetheless, while a general procedure was followed, an optimum protocol could not be produced, as each study site was treated according to its own specific characteristics.

## PRELIMINARY STUDIES ON *COLAPHELLUS PALAESTINUS* ACHARD IN THE MALTESE ISLANDS

Luke Camilleri

Supervisors: Joseph A. Borg and David Mifsud

*Colaphellus palaestinus* Achard is an alien invasive species belonging to the Chrysomelidae family, which feeds on plants from the Brassicaceae family. The main objectives of this preliminary study were to establish the distribution of this species in Malta and Gozo, determine the host plants this species is associated with, and assess the damage done to the host plant.

Field surveys lasted from 17th October to 23rd November 2019. Observations were maintained occasionally up to 15th December 2019, after which date no more adults were recorded from the field. Sweep netting was the main technique used to sample the insect. Photographs were taken to document the areas sampled and the damage incurred by the host plants.

An area of 100m<sup>2</sup> from each of 62 sectors was sampled in Malta and from each of 19 sectors in Gozo. Each sector had an area of 2.5km<sup>2</sup>. In Malta, *C. palaestinus* individuals were found in 59 sectors, whilst in Gozo *C. palaestinus* was only found in 3 sectors that were located close to Mgarr Harbour. Larval individuals were found in 16 sectors in Malta, concentrated in the northern and western regions, whilst in Gozo no larval individuals were recorded. In Malta, a total of 3130 adult individuals and 311 larval individuals were recorded throughout the study, whilst in Gozo only 5 adult individuals were encountered.

The main host plants were *Brassica rapa* subsp. *sylvestris*, *Diplotaxis erucoides* and *Diplotaxis tenuifolia*. Two occurrences of *C. palaestinus* were also recorded from *Sinapis* sp. Furthermore, a few individuals of the insect were also recorded on *Matthiola incana* subsp. *incana* outside the main sampling programme.

The recorded damage was restricted to the leaves, with larvae causing the most damage. Many adults were also noted to congregate around the inflorescences of the first 3 plant host species mentioned above, with damage also being noted on the flower petals although it was not extensive. *B. rapa*

subsp. *sylvestris* and *D. erucooides* were the two plant species that were affected most with regard to leaf damage.

Rearing, which was undergone in an attempt to understand the life cycle of *C. palaestinus*, was unsuccessful for all 9 trials undertaken. The insect individuals died after a few days, and many adult individuals were noted to have burrowed into the soil present at the bottom of the container in which they were being kept.

Further studies should be undertaken, given the dearth of information on the local ecology and impacts of *C. palaestinus*. Preferably, such studies would be undertaken over several years, and include further assessment of its occurrence and distribution in the Maltese Islands, its life cycle, feeding habits and economic impacts.

## **EFFECT OF MULTIPLE TREATMENT REGIMES OF CYTOTOXIC EXTRACTS AND DRUGS ON TUMOUR CELL SPHEROIDS**

Martina Ciantar

Supervisor: Joseph Buhagiar

(Plate 7 p. 18)

Multicellular tumour spheroids (MTS) are produced by the hanging drop method to mimic the features of solid tumours due to their layered assemblage and organisation. Cell spheroids consist of 3 regions; the outer zone known as the proliferating zone, the quiescent viable cell zone and the necrotic core. When tumour cells are cultured as MTS, they develop a resistance to various chemotherapeutic drugs, known as multicellular resistance (MCR). This phenomenon is also observed in in vivo tumours. For this reason, there has been an increase in interest to reduce MCR by using multiple drug treatment singly or in combination.

The aim of this study was to investigate the effect of a 0h, 12h and 24h multiple treatment regime of Paclitaxel (D) and *Laurus nobilis* leaf essential oil (EO) either separately or in combination on MCF-7 human breast cancer cell spheroids, and to determine if it is possible to overcome this resistance by disaggregating the spheroids or induce apoptosis. Every 12 hours, the spheroid diameter was measured and microphotographed up to 36hours.

Resazurin viability assay was used to calculate percentage transformation of resazurin to resorufin and these values were compared to the values with the percentage reduction in spheroid diameter. Four combinations were tested namely: EO-EO-EO (COMB 1), D-D-D (COMB 2), D-EO-D (COMB 3) and EO-D-EO (COMB 4).

When comparing the mean average percentage reduction in diameter of the four combinations, the highest percentage reduction was observed in Combination 2 (13.54%), followed by Combination 1 (12.19%) and Combination 3 (9.78%). Combination 4 actually produced an increase in diameter (1.35%). Values for the conversion of resazurin to resorufin does not correlate well with the values for decrease in diameter for Combination 1-4. The main limitation has been the 36hour interval allowed for ending the experiments. A longer exposure time would have increased the disaggregation of spheroids and the loosed cells surrounding them , which indicate cell death by apoptosis.

## **DEEP-WATER FISH DISTRIBUTION AND HABITAT ASSOCIATION IN THE MALTESE FMZ**

Yasmin Clark

Supervisor: Joseph A. Borg

(Plate 10, p. 19)

Knowledge on deep-sea habitats inside the Maltese Fisheries Management Zone (FMZ) is scarce and mainly limited to information obtained through trawl surveys. The main aim of this project is to identify the distribution of fish assemblages and their respective habitat associations in the FMZ, at depth ranges of 200 m – 1000 m. The effect of habitat complexity and depth on fish composition is investigated.

The data was collected using a Remotely Operated Vehicle (ROV) as part of the 'LIFE BaHAR for N2K' project in 2015. The habitat complexity, microhabitat types and depth ranges within which fish species were found was recorded and analyzed.

The Shannon-Weiner Diversity Index and Margalef Species Richness was highest in low complexity habitats. Depth had an overall effect on the fish assemblages exhibited across the different ranges. Whilst the Shannon-

Weiner Diversity Index did not decrease drastically with depth, the fish species which were observed changed when moving from 200 m – 1000 m.

The present study suggests that the researched area acts a biodiversity hotspot, indicating an urgent need for further research into the deep-sea environment around the Maltese Islands.

*(N.B. The data used in the present work belongs to the LIFE BaHAR for N2K project. This data was collected through marine research surveys authorised by the Environment and Resources Authority, the Continental Shelf Department, and the Superintendence of Cultural Heritage. The Project (LIFE12 NAT/MT/000845) is 50% co-financed by the EU LIFE+ Funding Programme and implemented by the Environment and Resources Authority (ERA), the University of Malta, Fundacion Oceana, the Ministry for the Environment, Sustainable Development and Climate Change (MESDC), and the Department for Fisheries and Aquaculture within MESDC. All data are copyrighted and cannot be used or quoted without written permission from the Environment and Resources Authority. © LIFE BaHAR for N2K, 2019. All rights reserved.)*

## **EXTRACTION OF HIGH VALUE COMPOUNDS FROM FISH WASTE**

Neil Cutajar

Supervisors: Joseph Buhagiar and Alexia Massa Gallucci (Plate 9. P.19)

Worldwide fish production has continued to increase and reached 171 million tonnes as of 2016. Concurrently, large volumes of fish waste are generated, with an estimated 35% of fish mass, being discarded, usually with negative environmental effects. This also represents a huge economic loss since a wide range of chemicals, including proteins (enzymes, collagen), protein hydrolysates, polyunsaturated fatty acids (PUFAs), as well as bioactive peptides and hydroxyapatite could be recovered from these discards. The aim of this study was to investigate the potential of extraction and quantification of high value extracts from fish waste.

An estimated 5900 metric tons of waste are generated by the local Atlantic bluefin tuna (*Thunnus thynnus*) fattening industry. Protocols for the retrieval of high value fractions from the discards of the local fisheries industry were reviewed. Discarded tuna heads were harvested for skin, cheek muscle and subocular fat from a local tuna farm operator; gilthead seabream heads were obtained for comparison. Different fish tissues were treated for lipid retrieval. Enzymatic collagen extraction was also carried out on tuna skin and tuna bone tissue. Yields obtained from oil and collagen extraction indicated that

there are considerable amounts of these valuable fractions in tuna waste: sub-ocular Tuna fat gave the highest yields recorded whereas gilthead seabream waste gave considerably lower lipid yields. ATR-FTIR spectra obtained for tuna oil samples were characteristic of high EPA and DHA omega 3 fatty acids. The most effective method to extract tuna oil samples proved to be enzymatic (pepsin), closely followed by warm extraction. Mixed tuna tissue, which most closely represents approximate composition of tuna heads, gave a 19.8% lipid yield. This means that from the lipid containing tuna waste generated yearly, upwards of 700 metric tonnes of fish oil may potentially be retrieved. Collagen extracted from tuna skin gave an approximate yield of 10.9%, this is still substantial when considering the €41,465/MT value for marine collagen.

If such valuable products are properly retrieved using state-of the art methods which prevent the breakdown of important components, they may prove to be the basis a lucrative local industry.

## **PREPARATION OF COLLAGEN SCAFFOLDS FROM DIFFERENT SKIN SOURCES FOR FIBROBLAST SEEDING**

Gianni Delia

Supervisor: Joseph Buhagiar

The repair of damaged tissues in the body can be accelerated by replacing or regenerating such tissues. Regeneration can be achieved by using biological structures such as 3D porous scaffolds which are seeded with cells and maintained under the necessary environmental conditions including growth factors.

Tissue scaffolds can be generated from a variety of materials, one being type I collagen which is the most prominent structural protein found in the majority of hard and soft tissues in animals. The aim of the project was to investigate the potential of different animal skins to yield free Type I collagen and a decellularized collagen matrix. Specific objectives were to find the optimal source of animal tissue from which Type I collagen can be extracted, and to

optimize the experimental conditions to maximize the quality and quantity of collagen that can be extracted from the selected animal skin source.

The extraction was performed using acid hydrolysis, enzyme hydrolysis, and a combination of both. Type I collagen was extracted from the skin and tendons of porcine and chicken feet using varying concentrations of 0.3 to 0.7mol/L acetic acid, hydrolysis times (24-72 hours), and concentrations of 0.1% or 0.2% pepsin to find out which combination maximized yield. Overall, the porcine trotters were found to provide a better collagen yield than chicken feet. With acetic acid only, porcine tissues yielded best when using 0.5mol/L acetic acid for 72 hours. Chicken feet showed optimal extraction with 0.7mol/L acetic acid for 24 hours. Incorporating pepsin, the best yield from both animal tissues was achieved using 0.7mol/L acetic acid and 0.2% pepsin for 48 hours of hydrolysis.

Additionally, porcine skin was decellularized using Triton X-100 and trypsin, monitoring the process at various reaction periods. This was done to further understand the efficiency at which a collagen scaffold can be produced by decellularization, compared to extracting collagen. The use of 200 $\mu$ l Triton X-100 with 5ml 2.5M trisodium citrate and 1ml 0.25% trypsin on porcine dermis indicated the possibility of successful denaturation, but this could not be quantified.

Both extraction of type I collagen and dermal decellularization showed promise for further applications in scaffold engineering.

## **DIETARY PREFERENCES OF SELECTED SHALLOW-WATER HERBIVORES**

Dylan Farrugia

Supervisor: Julian Evans

*Sarpa salpa* and *Sparisoma cretense* are the two main herbivorous fish native to the Mediterranean Sea. The present study aimed to investigate the dietary preferences of these two species, determine any temporal trends in their feeding behaviour, and assess whether a dietary niche overlap exists between them. This was done through field observations carried out at Anchor Bay, Ghar Lapsi, Jerma, Xghajra and Zonqor over the summer of 2019, and

stomach content analysis procedures on specimens collected from these sites.

During the on-field procedures, cover estimates of phytobenthic species, fish density counts and grazing rate per dietary species at different times of day (morning, afternoon and evening) were done at each site for each fish species. From the data recorded, Ivlev's Electivity Index was calculated for each phytobenthic species. Stomach content analysis was carried out on specimens of *S. salpa* (n = 48) and *S. cretense* (n = 4). Descriptive parameters and the percentage gravimetric composition of each phytobenthic species in the stomach were measured for each specimen.

From the data recorded, the foraging ratios for each phytobenthic species were determined. Analysis of data shows that these two species have comparable diets. This is largely supported by statistical tests and Schoener's Index results. However, grazing rates on different dietary species were statistically different, corroborating the fact that they are functionally different herbivores. For example, turf algae *Cladophora* sp. were mainly consumed by *S. salpa*. Coralline alga *Jania rubens* was consumed ubiquitously by both species while encrusting algae *Lithophyllum incrustans* and *Hildenbrandia rubra* were never fed upon by either species. A degree of diet-niche partitioning was noted. *S. salpa* selected *Halopteris scoparia* and *Dictyota dichotoma* when in sites where *S. cretense* populations were negligible or not recorded but consumed these algae at a much lower rate in sites where both herbivores were abundant.

The statistical tests carried out on the grazing rates show some support for the 'Optimal Foraging Theory' and the related 'Diel Feeding Hypothesis'. Nevertheless, more research is required to assertively state if the nutritional content of phytobenthic species affects herbivorous fish feeding behaviour. Stomach content analysis for *S. salpa* specimens corroborated field observations. Statistically no difference in dietary preferences was noted when comparing foraging ratios by fish maturity stage. However, males appear to prefer *D. polypodioides*/*D. dichotoma* and *Posidonia oceanica*.





*Photo Daniel Mangion*

**Plate 1. *Narcissus tazetta* in full flower** (See abstract by Daniel Mangion on p. 36)



*Photo Gabriel Pace*

**Plate 2. Two *Euphorbia* congeners, namely *E. dendroides*, the widespread species (left), and *E. melitensis*, the endemic species (right).** (see abstract by Gabriel Pace on p. 26)



*Photo © LIFE BaHAR for N2K, 2019. All rights reserved.*

**Plate 3.** Screenshot of maerl bed showing rhodolith accumulations and associated species located south-east of Malta at a depth of 72.3 metres. (see abstract by Luke Tabone on p. 38)



*Photo Hannah Abela*

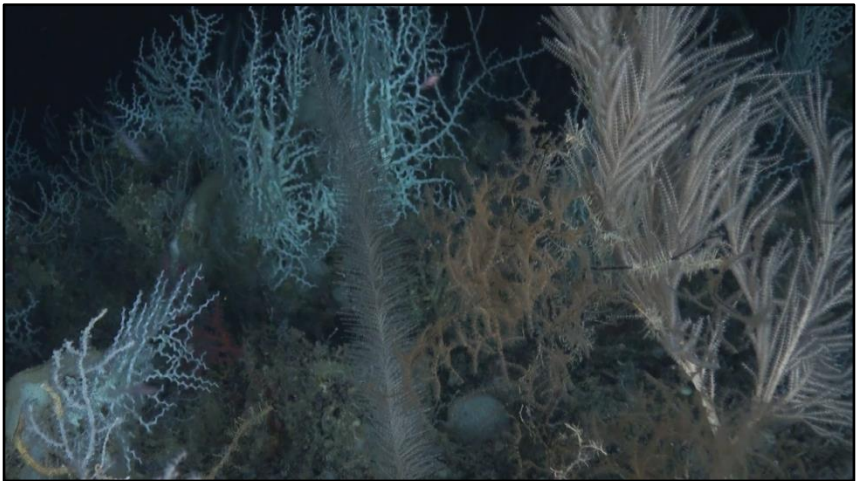
**Plate 4.** A colony of the coral *Cladocora caespitosa* photographed in Maltese waters. (see abstract by Hannah Abela on p. 5)





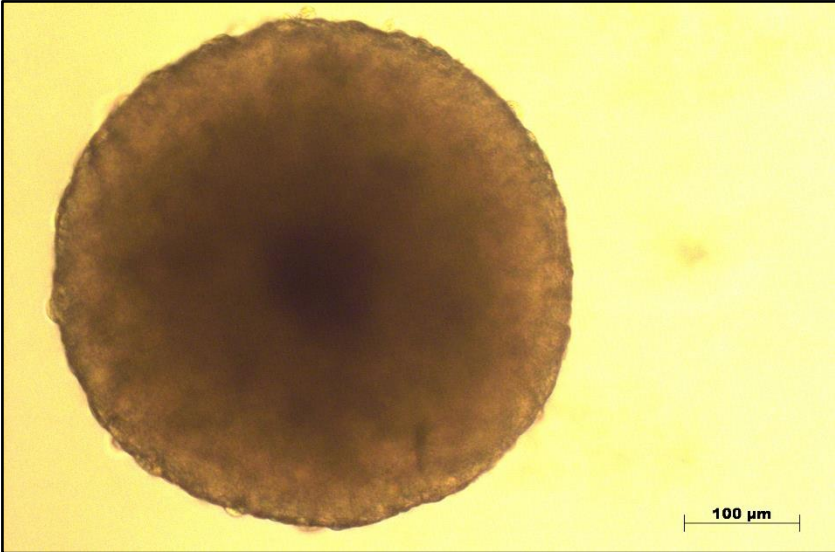
*Photo Jurgen Gatt*

**Plate 5. *Brachypodium retusum* grassland, I/o Majjistral Nature and History Park (see abstract by Jurgen Gatt on p. 34)**



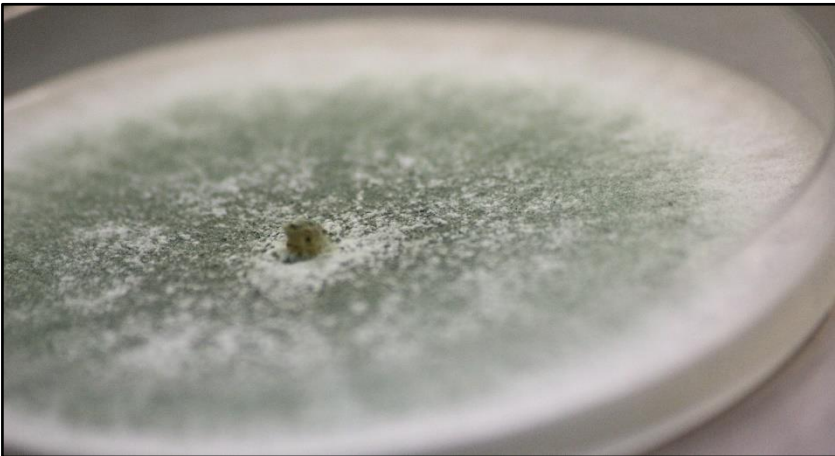
*Photo © LIFE BaHAR for N2K, 2019. All rights reserved.*

**Plate 6. A mixed assemblage of habitat-forming cnidarians on hard substrata. (see abstract by Maria Attard on p. 33)**



*Photo Martina Cianciar*

**Plate 7.** A spheroid on exposure to 25 ppm *Laurus nobilis* essential oil after 36hrs. The inner dark area is the necrotic core. (see abstract by Martina Cianciar on p. 9)



*Photo Marco Iannaccone*

**Plate 8.** Cultured *Trichoderma harzianum* Rifai, an ascomycete fungus found in Maltese soils. (see abstract by Marco Iannaccone on p. 35)



*Photo Neil Cutajar*

**Plate 9.** Fish discards, such as these bluefin tuna heads can be a valuable resource. (see abstract by Neil Cutajar on p. 11)



*Photo © LIFE BaFAR for N2K, 2019. All rights reserved.*

**Plate 10.** An electric ray (*Torpedo* sp.) photographed by a Remotely Operated Vehicle in deep waters off Malta. (see abstract by Yasmin Clark on p. 10)

## **THE ENVIRONMENTAL IMPACT ASSESSMENT PROCESS AS APPLIED IN MALTA TO MAJOR COASTAL ENGINEERING WORKS: AN EVALUATION**

Loriana Ghita

Supervisors: Victor Axiak and Alex Camilleri

The scope of an Environmental Impact Assessment (EIA) is to ensure that when public or private projects are proposed, and prior to planning consent is decided upon, any potential environmental impacts are duly taken into consideration and the relevant authorities can take an informed decision based on appropriate investigations. This study aims to determine the effectiveness of the EIA process in Malta, as applied to a named example of a major coastal engineering work.

The evaluation attempts to use a local coastal engineering development from 2001, namely a proposed beach nourishment project at St George's Bay, San Giljan. After the execution of the works, the stability of the nourished beach as well as any environmental degradation have been monitored for five years by carrying out an Ecological Monitoring Plan. Several surveys were conducted and the scientific information about the shore and sub littoral system was collected by using adequate tools and methodologies.

The overall monitoring results and conclusions showed that the EIA ecological impacts were correctly predicted and the mitigation measures with the respective recommendations were accurately identified and implemented. While analysing the project retrospectively, improvement opportunities can be observed, thus a number of recommendations regarding methodology, study accuracy and objectivity have been proposed.

## **BIOEROSION RATES ON SHORE AND SHALLOW WATER ROCKY BOTTOMS ALONG MALTESE COASTS**

Alistair Grima

Supervisor: Julian Evans

Biological factors are often overlooked when viewing the morphodynamics of rocky coasts. Bioerosion occurs when, due to the effect of bioeroding

organisms, material is broken down and fractionated. This subsequently alters the habitat in which these organisms live, possibly affecting the entire ecosystem.

The most common bioerosive organisms found on Maltese rocky shores, namely limpets, chitons, top shells and sea urchins, were considered during this study. Limpet home scars were measured to study the effects of rock type (Globigerina Limestone and Coralline Limestone) and exposure on limpet scar volumes. The interaction of rock type and exposure was deemed as not statistically significant. The same could be said regarding the individual effect of both rock type and exposure. Correlation between the rock type and exposure of an area and the population density of bioeroding organisms present was also examined using transect data collected from 16 sites. The areas examined showed no correlation between the rock type and exposure and the resultant population density of the shore for any of the species.

Quantitative measures of the bioerosion rate of the different species were taken and related to the exposure and rock type of the sites sampled. This was done by fasting the collected organisms for 24 hours and collecting the excreted faecal matter. These faecal pellets were then tested via a back-titration to gather estimates of CaCO<sub>3</sub> in the faeces.

Results obtained showed that there was significant influence of exposure or rock type on some species and not others. *Arbacia lixula*'s percentage CaCO<sub>3</sub> in faecal pellets was affected by both exposure and rock type. Rock type also influenced the percentage CaCO<sub>3</sub> in faecal pellets of the species *Paracentrotus lividus*, *Patella caerulea* and *Lepidochitona caprearum*. Further statistical tests were done on the mass of CaCO<sub>3</sub> per unit body mass. It was discovered that *Patella rustica* was affected by changes in both exposure and rock type whilst *Paracentrotus lividus* was affected by a difference in rock type. The mass of CaCO<sub>3</sub> per unit body mass indicated that different species have varied bioerosive abilities. *Paracentrotus lividus* had the highest, followed by *Lepidochitona caprearum*, *Patella caerulea*, *Arbacia lixula*, *Patella rustica* and *Phorcus turbinatus* with similar bioerosive abilities. The remaining species, *Patella ulyssiponensis*, *Phorcus articulatus* and *Melarhaphé neritoides* had a much lower mass of CaCO<sub>3</sub> per unit body mass.

Using the bioerosion rate and the population densities extrapolated from this study, the total bioerosive rate of each shore was estimated and compared. When compared statistically the different shore types produced no statistically significant difference between them.

## **THE *BRACHIDONTES PHARAONIS* MUSSEL BED IN BIRŻEBBUĠA, NINE YEARS ON**

Ema Marmarà

Supervisor: Patrick J. Schembri

The Indo-Pacific mytilid bivalve, *Brachidontes pharaonis*, has colonised many rocky shores around the Maltese Islands. As far as is known, it has only managed to successfully establish a mussel bed in Birżebbuġa; elsewhere it is found scattered as single individuals or small groups. In Malta, it is primarily a mediolittoral mussel however it can also colonise the uppermost infralittoral zone as well as extend to the upper reaches of the mediolittoral.

A previous study made by Bonnici in 2010 assessed aspects of the ecology and population biology of the *B. pharaonis* mussel bed at Birżebbuġa by comparing the biotic and abiotic factors at the bed with those of reference sites. The present study, carried out nine years later, was a follow up to this. The key research questions addressed are whether the ecology of the mussel bed has changed over nine years and what may be the underlying reasons why *B. pharaonis* only formed a bed in one area and not at the reference site.

Similar methods to those of the 2010 study were employed in the present study to ensure comparability of the results obtained. Mussels were collected from quadrats along transects on the shore and sex, size, and weight were determined in the laboratory. The denuded areas were then monitored at bimonthly intervals for signs of mussel recruitment. Changes in abundance over a period of eight months at the mussel bed site and two reference sites were monitored through analysis of photoquadrats. The overall abundance of mussels was found to have declined drastically compared to 2010. This could have been due to several factors, most likely physiological since no obvious changes in the physical environment have occurred in the interim period.



Mussel sizes varied greatly between the two studies. In 2010, mussels greater than 20 mm in length were not observed at the mussel bed, but in the present study, these were relatively abundant. From this, it was evident that factors limiting the growth of mussels in 2010, were not operating in recent years. Mussels at the reference site, however, did not grow to these sizes. The sex ratio of mussels at both the mussel bed and the reference site was roughly equal to 1:1 and this did not differ from the 2010 study. Mussel abundance varied seasonally. The number of mussels declined during the autumn and winter months, showing a slight population recovery at the onset of spring. No recruitment was observed over the eight-month period in the denuded transects and these became densely populated by macroalgae.

From the results of the present study, it seems likely that the mussel bed at Birżebbuġa will continue to thrive but the population ecology of the mussels here is dynamic and show marked interannual variations.

## DISTRIBUTION AND POPULATION SIZE STRUCTURES OF THE MEGABENTHIC CNIDARIANS *ISIDELLA ELONGATA* AND *FUNICULINA QUADRANGULARIS* IN MALTESE WATERS

Luke Mifsud

Supervisor: Julian Evans

Cold water communities play an important role as benthic habitat formers in the deepsea environments, providing a variety of ecosystem services to many fish and invertebrate communities, including food, shelter and breeding grounds. Information and data about these deep-sea communities, including diversity, abundance and distribution is to the present day very limited. This study analysed two important megabenthic cnidarians occurring in Maltese waters, namely *Funiculina quadrangularis* and *Isidella elongata*, with the aim of bridging knowledge gaps regarding spatial distribution, bathymetric distribution and colony size structures.

These two critically endangered octocorals were analyzed using underwater video footage recorded in years 2015 and 2016 by a remotely operated vehicle within the Malta 25-nautical mile fisheries management zone at depths ranging between 100 and 950m. Results show distinctive preferences with regards to bathymetry and terrain slope, with similar preferences with

respect to sediment type. From all the stations analyzed, *Funiculina quadrangularis* was found to occur in wider bathymetric ranges (135- 930m) with a total of 686 individuals. Whilst the occurrence of *I. elongata* was present between depth ranges of 320-790m, with a total of 629 individuals recorded. The highest density of *F. quadrangularis* was recorded in depths ranging between 100- 150m, with a total of 40 individuals per 1000m<sup>2</sup> and a general trend of a decrease in density with an increasing depth. The highest density of *I. elongata* was present in depths ranging between 600-650m, with a total of 21 individuals per 1000m<sup>2</sup> and a general trend of an increase in density with an increasing depth. A large proportion of *F. quadrangularis* analyzed at the colony level was of intermediate size (class C), with larger size classes being much less common (in terms of mean standard abundance) than smaller size classes. Similarly, a large proportion of *F. quadrangularis* analyzed at the colony level was of intermediate size (class C), however the populations seem to be shifting to one with larger colonies. In addition to this, a change in colony size was indicted with a variation in depth.

Distribution and population size structures are also affected by anthropogenic impacts such as bottom trawling, varying also based on the morphological structure of the species. Ultimately this knowledge can serve as an important contribution for ecological and biological studies, with the main aim being that of safeguarding the species and their ecosystems given that Mediterranean CWC habitats have been recognized and classified as Essential Fish Habitats.

*(N.B. The data used in the present work belongs to the LIFE BaHAR for N2K project. This data was collected through marine research surveys authorised by the Environment and Resources Authority, the Continental Shelf Department, and the Superintendence of Cultural Heritage. The Project (LIFE12 NAT/MT/000845) is 50% co-financed by the EU LIFE+ Funding Programme and implemented by the Environment and Resources Authority (ERA), the University of Malta, Fundacion Oceana, the Ministry for the Environment, Sustainable Development and Climate Change (MESDC), and the Department for Fisheries and Aquaculture within MESDC. All data are copyrighted and cannot be used or quoted without written permission from the Environment and Resources Authority. © LIFE BaHAR for N2K, 2019. All rights reserved.)*

## ASPECTS OF THE ECOLOGY OF *ELATINE GUSSONEI* (SOMMIER) BRULLO & AL.

Stella Nuccio

Supervisor: Sandro Lanfranco

The aim of this study was to investigate the aspects of the ecology of the sub-endemic Maltese Waterwort, *Elatine gussonei* (Sommier) Brullo & al. This study focused on surveying temporary rock pools inhabited by *E. gussonei* and evaluating how this species was influenced by abiotic factors and by the presence of syntopic species.

This was done by sampling and reviewing sixteen rock pools in San Pawl tat-Targa, Naxxar, Malta, over a period of three months, between October and December 2019. The environmental variables recorded include pH, temperature, water surface area and water depth. These were then analysed against the frequency data of *E.gussonei* in the various rock pools, in order to acquire a plausible model for the distribution of this species amongst neighbouring pools.

The results showed that some of the factors studied did in fact have an effect on denoting and predicting the presence or absence of *Elatine gussonei*. On the other hand, when it was attempted to grow the species in the laboratory, in order to test other combinations of environmental conditions with the aim of optimising the species' growth, the seeds failed to germinate, hence the experiments did not have the expected outcome.

Generally, *Elatine gussonei* prefers growing in places with high temperature, alkaline pH and shallow water depth. The presence of different macrophytic communities in temporary rock pools, usually indicates the type of factors characterising the pools. Nevertheless, due to time constraints and unpredicted weather conditions, the results obtained during the period of study might be incomplete.

## **A COMPARISON OF THE BIOLOGY AND ECOLOGY OF *EUPHORBIA DENDROIDES* AND *EUPHORBIA MELITENSIS***

Gabriel Pace

Supervisor: Sandro Lanfranco

(Plate 2 p. 15)

Comparing congeneric pairs of plants in which one is endemic and the other is widespread may shed light on the conditions which favour endemism to take place. In this study, the biology and ecology of *Euphorbia melitensis*, endemic to the Maltese Islands, and *Euphorbia dendroides*, distributed across the Mediterranean, were analysed alongside each other.

Eight sites across Malta and Gozo were sampled to determine whether the presence of endemic species is driven by stressful conditions. In three of the studied habitats, both species exist in sympatry, while in the rest, either one of the two congeners can be found. Nine environmental factors relating to stress were compared in each habitat along with vegetation richness. Readings of leaf length, plant width and height of the two congeners were also measured together with a sample of 300 propagules from four of the eight sites.

Detrended Correspondence Analysis of the environmental parameters indicated that wind exposure is the leading contributor to the distribution of the two plants based on the nine factors tested. This implied that at higher wind exposures, the endemic species is favoured. Other parameters such as altitude above sea level and distance from the sea were found to be much less effective in the two species' distribution. The size of the plants and leaves was found to be smaller for *Euphorbia melitensis* than for *Euphorbia dendroides*, indicating a reduction in size as a measure to combat stress.

These findings may be specific to the genus *Euphorbia* or to the Maltese Islands and further analysis is required to confirm if these traits are shared among endemic species in different territories.

## CHARACTERISATION OF DISCARDS GENERATED BY MALTESE BOTTOM OTTER TRAWL FISHING VESSELS

Matthew Pace

Supervisor: Simeon Deguara

Discarding is regarded as an economically wasteful and ecologically harmful practice and has been recognised as a major issue in trawling ever since its conception. This dissertation aimed to advance the knowledge of the discards generated by Maltese bottom otter trawlers, which are unmonitored under the EU Data Collection Framework (DCF) or the General Fisheries Commission for the Mediterranean (GFCM) Data Collection Reference Framework (DCFR).

The unmonitored discards of a total of 17 hauls were analysed, originating from five fishing trips undergone between March and September of 2018, covering areas towards the Northwest, North and Northeast of the Maltese islands. A total of 101 species were identified, of which 38 were Osteichthyes, 4 Chondrichthyes, 24 molluscs, 9 crustaceans and 26 other invertebrates. The five defining species for shallow-water hauls (120-140m), by number of individuals and in descending order were: *Argentina sphyraena*, *Arnoglossus laterna*, *Lepidotrigla cavillone*, *Serranus hepatus* and *Lepidotrigla dieuzeidei*. Similarly, the defining species for deep-water hauls (330-590m): *Munida rutilanti*, *Pasiphaea sivado*, *Coelorinchus caelorhincus*, *Chlorophthalmus agassizi* and *Nezumia sclerorhynchus*. 18% of the *C. agassizi* analysed were suffering from the generalist cymothoid ectoparasite *Ceratothoa steindachneri*, which could potentially spread to farmed species. Multiple fragments of the critically endangered bamboo deep-water coral *Isidella elongata*, and one count of the giant tun *Tonna galea*, both protected under the Barcelona Convention, were amongst the unmonitored discards, suggesting that some vulnerable species are going unnoticed by DFA observers. This potentially unrecorded aggregation of *I. elongata* highlights the necessity for enhancing local data collection measures. There was no statistically significant difference between shallow and deep-water discard rates, both in terms of weight and individuals ( $P > 0.05$ ) but there was a significant difference in the species richness between the two depth categories ( $P < 0.05$ ).

## **THE INFLUENCE OF ROCK POOL MORPHOMETRY ON FLORAL SPECIES DIVERSITY**

Erika Puglisevich

Supervisor: Sandro Lanfranco

The aim of this study was to determine if floral species richness of temporary freshwater rock pools is dependent on basin morphometry or rock pool proximity. 59 pools were observed at San Pawl Tat-Tarġa during December 2019 and January 2020. The plant species in each pool were recorded in terms of presence/absence. The morphometric characteristics of the pools that were noted were the maximum water depth, maximum sediment depth and surface area. GPS coordinates for each pool were also noted.

The results showed that surface area and water depth were the only morphometric factors that showed significant correlation with species richness. Spatial proximity did not influence the species richness of the rock pools, implying that at a local scale, basin morphometry has more of an influence than the spatial distance between pools. The most common lifeforms were hemicryptophytes, followed by therophytes and hydrophytes. This may be due to the anomalous wet season that provided shorter hydroperiods than normal causing the ecotone zone of rock pools to be broader due to the shorter than average wet season.

## **ASSESSING POTENTIAL RECOVERY OF SHORE BIOTIC ASSEMBLAGES FOLLOWING CESSATION OF RAW SEWAGE INPUT**

Vanessa-Ann Scerri

Supervisor: Joseph A. Borg

The presence of the raw sewage outfall at Wied Ghammieq resulted in a pollution gradient along the coast on moving away from the discharge point towards Zonqor Point (Marsascala). Such nutrient enrichment impacted the rocky shore biotic assemblages. Following cessation of discharge of raw sewage in 2011, recovery of the biotic assemblages is expected, whereby the impacted site, Wied Ghammieq, would exhibit environmental characteristics

similar to those of unpolluted sites. In 2015, Attard (2016), indicated partial but not complete recovery of the mediolittoral zone biotic assemblages.

The aim of the present study was to establish potential further recovery of rocky shore intertidal assemblages at the same four locations studied by Attard (2016). Apart from the same four locations used in Attard's study, a further location was used in the present study to assess potential nutrient enrichment that may be resulting from the underwater pipeline that extends 1 km out to sea and carries effluent that originates at the Ta' Barkat STP.

Sampling was carried out using a 20 cm x 20 cm quadrat within the Lower Mediolittoral Zone (LMZ) and Upper Mediolittoral Zone (UMZ) at two sites allocated to each of the five study locations. The results of univariate PERMANOVA tests from the present study indicated that values of the four biological attributes under study: total abundance, number of faunal families, Shannon-Wiener and Pielou's Evenness, were significantly different between some locations for biota in the LMZ. Mean values for number of faunal families at Location 1 were slightly lower than at the other locations but no significant differences were indicated. Significant differences were detected for mean total algal biomass and mean number of algal species amongst the study locations. An increase in the number of faunal families and total faunal abundance for UMZ biota was detected on moving away from the former discharge point; the results of statistical analyses indicated a significant difference for the mean number of families between the five study locations. The results of multivariate PERMANOVA showed no significant differences in the four attributes of biota within the LMZ between the five study locations.

Overall, the results of the present study indicated partial recovery in LMZ biotic assemblages and possible complete recovery in UMZ biotic assemblages, which seems to indicate that the sewage outfall may have affected the LMZ biota more than the UMZ biota. Furthermore, localized potential nutrient enrichment may be present at Location 5, vii which may possibly be due to the influence of the effluent originating from the STP at Ta' Barkat.

## **INTRASPECIFIC MORPHOLOGICAL VARIABILITY IN LESS COMMON SPECIES OF THE FAMILY RAJIDAE**

Emma Jayne Spiteri

Supervisors: Leyla Knittweis-Mifsud and Patrick J. Schembri

The rays and skates (family Rajidae) *Dipturus oxyrinchus*, *Raja miraletus*, *Raja radula*, *Leucoraja melitensis* and *Leucoraja circularis* are amongst the less commonly caught species that nonetheless occur regularly as by-catch in Maltese fisheries. All five have similar habitat and dietary preferences, reproductive biology, size and overall morphology. These similarities, along with intraspecific variation in colouration and morphology, greatly increase the chance of misidentification, especially in the field.

The main aim of this study was to assess the degree of morphological variability in these species collected from Maltese Sicilian waters. The key morphological characters used in identification manuals commonly used in the field and in other literature sources were compiled, compared and quantified by analyzing 179 specimens of the 5 species obtained from commercial bottom otter trawl landings between August 2018 and June 2019 and from the 2019 'Mediterranean International Trawl Survey' (MEDITS) carried out annually in the General Fisheries Commission for the Mediterranean (GFCM) Geographic Sub-Area (GSA) 15. For each individual, data on external meristic (e.g. 'number of alar, malar, pre-orbital and post-orbital thorns'), morphometric (e.g. 'total length', 'distance between the eyes' and 'eyeball length') and qualitative characters (including eye spot colouration, colour pattern and shape of the line between tip of the snout to the pectoral fin) was collected.

Additionally, fisheries independent MEDITS survey data for GFCM GSA 15 as well as fisheries dependent data from recently published Scientific, Technical and Economic Committee for Fisheries (STECF) reports and associated data tables were obtained and analysed graphically. Group average hierarchical clustering, non-metric MDS ordination, and canonical analysis of principal coordinates (CAP) grouped the majority of the specimens into distinct species clusters corresponding to the five species, with the exception of a few anomalous outlier individuals. The variability in these outliers was not due to



age differences or sexual dimorphism, but resulted from a number of morphometric characters (e.g. 'length of snout', 'eyeball length', 'pelvic fin measurements') that overlapped in values.

These results suggest that overall there is low risk of misidentification of the species for the majority of the individuals; however, anomalous intermediate specimens do occur. This may have implications for the monitoring and managing of stocks and for conservation management, since observers on-board fishing vessels, at fish markets, or at fishing ports may misidentify such anomalous individuals. Future studies on these and other 'less common species of Rajidae' are necessary so as to establish the degree of intraspecific variation and whether this may affect species identification based on guides and manuals.

## **DEVELOPMENT OF MATRICES FOR 3D SPHEROID CULTURE AND ASSESSMENT OF THEIR METABOLIC ACTIVITY**

Sarah Zammit

Supervisor: Joseph Buhagiar

Major advancements being made for *in vitro* cellular studies on cancer are currently employing 3D spheroid models as these are capable of mimicking the behaviour of *in vivo* tumours and allow a better understanding of the complex mechanisms behind cancer cell progression. The matrix is a useful component as it can also promote biophysical and chemical cues which would resemble the behaviour of the natural extracellular matrix (ECM). The aim of this study was to compare the development of 3D spheroid cultures using different polymers as matrix supports and follow their progress by monitoring their metabolic activity. Specific objectives centred on the development of appropriate encapsulation techniques for 3D spheroid culture. Additionally, the extraction of collagen hydrogel from easily available raw materials was also attempted to reduce the cost. 3D spheroids were generated using the hanging drop technique and they were encapsulated in three different matrices, these being, calcium alginate, collagen and guar gum. The effect of these matrices on 3D spheroid growth and development was then investigated by measuring the diameter over a 7 day period, using 20

spheroids per sample in 2 independent trials. Additionally, the change in cell viability of the 3D spheroids was assessed using the Resazurin reduction assay, using 12 spheroids per sample in 2 independent trials.

From the results obtained, it was shown that the calcium alginate promoted the largest increase in spheroid diameter, which on average resulted in a percentage increase of 40 % from days 1-7. This was followed by collagen with a percentage increase of 30 %. The values for the Resazurin reduction assay correspond with that of the growth curves as an increase in diameter was accompanied by a larger number of viable cells. Although collagen was extracted successfully, it was expected to promote a larger increase in size due to its protein content resembling that of the natural ECM. Additionally, the guar gum was not a successful matrix as it did not promote increase in spheroid size. Thus, for the latter two matrices further work is needed to make them optimal for 3D spheroid culture. However, this is still a step forward in bridging the gap for the development of spheroid encapsulation protocols.

## M. Sc.

### ASPECTS OF THE ECOLOGY OF HABITAT-FORMING CNIDARIA IN MALTESE DEEP WATERS

Maria Attard

Supervisors: Julian Evans & Patrick J. Schembri

(Plate 6 p. 17)

The present study focused on deep-sea cnidarians occurring within the Malta Graben. Four main habitat-forming species – *Madrepora oculata*, *Lophelia pertusa*, *Leiopathes glaberrima* and *Callogorgia verticillata* – were studied in detail with the aim of updating current information on their spatial and bathymetric distribution. The present study also aimed to determine whether bathymetry and bottom-type have an effect on the structure of the cold-water coral assemblages present in the study area.

Data were collected by analysing video imagery recorded during 84 remotely operated vehicle (ROV) dives along the southeastern end of the Malta Graben as part of the “LIFE BaHAR for N2K” project. The data extracted from the video footage included the location (spatial coordinates) and depth of each cnidarian individual, together with the habitat type where it was found. The size of colonies of *M. oculata* and *C. verticillata* was also determined. These data were used to plot the spatial distribution of the four habitat-forming species, and their bathymetric range. Multivariate analyses (cluster analysis, nMDS, SIMPER and CAP) were carried out on the species-sample data matrix to assess variation in cnidarian assemblage structure in relation to habitat type and depth.

All four habitat-formers were found in high densities in the southeastern end of the Malta Graben, but abundances of *L. pertusa* were notably lower than those of the other three species. *M. oculata* was recorded at depths between 250-1050m, *L. pertusa* was found between 400-1050m, *C. verticillata* was found between 350-600m and again between 750-1050m and *L. glaberrima* was found between 200-500m and again between 600-850m.

The multivariate analyses revealed a significant difference between hard and soft substrata, and also differences in the assemblages in terms of depth for some bottom-types. SIMPER revealed that *M. oculata*, *L. glaberrima* and *C. verticillata* were the main contributors to these differences. As seen in other studies in the Mediterranean region, *L. glaberrima*, *C. verticillata* and *M. oculata* have similar spatial distributions. Their bathymetric distributions are also similar to those found in other regions in the Mediterranean, except for *C. verticillata* which occurs in deeper waters in the Malta Graben.

The data generated through the present study will inform local management of deep-sea ecosystems.

*(N.B. The data used in the present work belongs to the LIFE BaHAR for N2K project. This data was collected through marine research surveys authorised by the Environment and Resources Authority, the Continental Shelf Department, and the Superintendence of Cultural Heritage. The Project (LIFE12 NAT/MT/000845) is 50% co-financed by the EU LIFE+ Funding Programme and implemented by the Environment and Resources Authority (ERA), the University of Malta, Fundacion Oceana, the Ministry for the Environment, Sustainable Development and Climate Change (MESDC), and the Department for Fisheries and Aquaculture within MESDC. All data are copyrighted and cannot be used or quoted without written permission from the Environment and Resources Authority. © LIFE BaHAR for N2K, 2019. All rights reserved.)*

## **EVALUATION OF THE STATUS AND DISTRIBUTION OF *BRACHYPODIUM RETUSUM* STEPPE IN THE MALTESE ISLANDS**

Jurgen Gatt

Supervisor: Sandro Lanfranco

(Plate 5 p.17)

Out of the 31 recognised habitat types associated with grasslands by the EU, only one was identified in the Maltese Islands during the designation of SACs of International Importance: Pseudo-steppe with grasses and annuals (Thero-Brachpodietea), also identified by the EU as ‘habitat 6220’. Due to the looseness of the habitat’s definition, more subtypes within the same habitat type are considered. The Technical Report drafted in 2018 to assist during the interpretation of habitat 6220 uses a phytosociological approach to distinguish three broad subtypes within this habitat, Lygeo-Stipetalia, Poetalia

bulbosae and *Brachypodietalia distachyi* respectively with their own features, ecological requirements and variability.

Grassland communities have attracted attention in the Mediterranean region and, using phytosociology as a means of classification, authors have described different syntaxa related to this habitat. The only data related to grasslands in the Maltese Islands is that collected in relation to obligations under Article 17 of the Habitats Directive and under the Environment Protection Act (CAP. 549) as transposed from the same directive. Therefore, the aim of this work was to characterise the species composition, extent, and distribution of grasslands in the Maltese Islands for the first time and identify any habitat subtypes that may be present locally.

Statistical analysis carried out for the purpose of this study and the observations done on the field revealed that there are various types of grasslands. There are grasslands dominated by *Brachypodium retusum* and *Hyparrhenia hirta* along with other garrigue and phrygana species, such as *Teucrium fruticans*, *Thymbra capitata*, *Euphorbia melitensis* and *Anthyllis hermanniae*. On the other hand, there are grasslands that are nearly solely composed of one species, like *L. spartum*. Furthermore, there were other grasslands dominated by *Avena* species along with other herbaceous species and grasslands dominated by *Andropogon distachyos* and another by *Brachypodium distachyon*.

## **ISOLATION AND CHARACTERIZATION OF MYCORRHIZAE AND MYCORRHIZA-LIKE ASSOCIATIONS FROM SOIL OF THE MALTESE ISLANDS**

Marco Iannaccone

Supervisor: Joseph Buhagiar

(Plate 8 p.18)

Studies of soil fungal diversity in the Maltese Islands are still considered sporadic and limited. The aim of the present work was to carry out studies to investigate the presence of *Trichoderma* spp. within the Ascomycota, ectomycorrhizae and arbuscular mycorrhizae in the Maltese Islands and eventually to effect their isolation and preservation on different substrates.

Soils samples were initially cultured on Potato Dextrose Agar medium at a temperature of 27°C in the light. After microscopic inspection, fast-growing colonies with phialides and conidiophores which are typical of *Trichoderma* growth, were selected for monoconidial isolation. In total, nine distinct isolates were made using a monoconidial isolation technique, and their growth patterns and rate of growth were measured and photographically recorded.

To help in identification of isolates, micromorphological studies were carried out using different staining techniques for light microscopy. In addition, DNA barcoding was carried out. Seven strains of the *Trichoderma harzianum* complex were identified for different soils and media in the Maltese Islands as well as another two new records of *Trichoderma* species namely *T. virens* and *T. citrinoviride*. These *Trichoderma* isolates were preserved in sterile 99% glycerol, sterile distilled water, Synthetic Nutrient Agar, Potato Dextrose Agar, and spent coffee grounds. Spore and mycelium viability were confirmed after one year for all of them.

The search for ectomycorrhizae was conducted during the rainy season between 2015 and 2019. Ten records were confirmed, of which two new records for the Maltese Islands, namely *Russula ochrosporea* and *Hebeloma quercetorum*, were discovered. For *Hebeloma quercetorum* it was also possible to measure the rate of growth of the basidiocarp.

For the arbuscular mycorrhizae study, plant-traps were set up and their presence was confirmed as vesicles within the roots of *Daucus carota*, *Carya illinoensis* and *Quercus ilex*. As far as is known, this was the first record of arbuscular mycorrhizae detected in Maltese soils.

## **DISTRIBUTION AND MORPHOMETRIC VARIATION IN *NARCISSUS TAZETTA* L.**

Daniel Mangion

Supervisor: Sandro Lanfranco

Plate 1, p. 15

The principal aim of this study was to carry out a detailed morphological examination of the *N. tazetta* L. present throughout Malta and Gozo in order to investigate the disparity between 'early-' (Oct-Nov) and 'late-flowering' individuals of the species (Jan-Mar), as described in previous studies in 2006

and 2010. Additionally, the possibility of association between the morphological features investigated and certain abiotic parameters was explored through correlation statistics. At present, field identification of the aforementioned individuals proceeds through the use of a small number of 'Rules of Thumb' which reflect general morphological characteristics.

A total sample of 149 plants was utilised in this study, with specimens (or sampling units; SUs) being collected from 19 sites (15 on Malta and 4 on Gozo) during three different stages throughout the wet season (Oct-Nov, Dec, and Jan-Mar). For each SU, a number of diacritic quantitative and categorical features were measured without discrimination. Additionally, a number of abiotic parameters were also measured from each study-site.

Based on the contributions of several quantitative variables, SUs collected during the Oct-Nov and Jan-Mar flowering periods seem to pertain to two distinct groups, with discrepancies being observed for several vegetative and floral characteristics. However, a more gradual transition in the morphology is observed for the aforementioned characteristics upon extension of the dataset to include the SUs collected during the Dec period. Nonetheless, the differences observed between flowering period categories were found to be statistically significant, with the diameter of the corona and length of the floret being found to be the most influential variables.

A greater degree of heterogeneity was found to be attributed to SUs collected during Oct-Nov, further highlighting the possibility of morphological discrepancy between the Oct-Nov and Jan-Mar data. A number of promising associations between certain key abiotic parameters and a number of quantitative morphological features were also revealed from the correlation analysis, with soil depth being found to be particularly influential. The variable floret length was also found to show the largest number of statistically significant correlations to the abiotic environment.

Collectively, the population of *N. tazetta* L. s.l. found throughout Malta and Gozo was found to be most akin to description of *N. tazetta* subsp. *tazetta*. However, no such rank could be attributed to the SU groups comprising each flowering period category. Nonetheless, there is some evidence for the classification of the latter as ecotypes.

## CHARACTERISATION OF RHODOLITH BEDS IN CIRCALITTORAL WATERS OFF THE SOUTHEASTERN COAST OF THE MALTESE ISLANDS

Luke Tabone

Supervisors: Julian Evans & Leyla Knittweis-Mifsud

(Plate 3, p. 16)

ROV surveys were carried out at 56 stations off the southeastern coast of Malta as part of the LIFE BaHAR for N2K project, primarily at depths of 50-100m, with a view to characterise the rhodolith bed present. Mean percentage cover of live rhodoliths ranged between 0% and 73.6%. High density-patches were found close to the coast and towards the northeast of the study area close to the Fisheries Management Zone boundary, with patches where rhodoliths were absent in between. Conversely, most of the dives in the south, southeast and northwest of the area revealed low rhodolith densities (< 10%) or none at all.

The majority of rhodoliths recorded (96.8%) were considered to be alive, with those having finger-like branches being the commonest morphotype in the overall area. Rhodoliths were mostly present at depths of 60-95m, with peak live rhodolith densities recorded at 78.5m. The shallowest records, with relatively high densities of rhodoliths, were at a depth of 53.7m, whilst the deepest record was at 106.9m albeit at very low densities.

In terms of associated biota, a total of 169 different species and 18,911 individuals of megabiota were identified throughout the entire ROV dives; Actinopterygii and Echinoderms were the most abundant taxonomic groups. *Stylocidaris affinis*, *Centrostephanus longispinus*, and *Antedon mediterranea* were the species found to be most commonly associated with rhodoliths. In addition, *Flabellia petiolata* was observed to grow profusely in areas where rhodoliths were present. No direct correlation between rhodolith density and species richness or abundance was observed.



With respect to anthropogenic impacts, a total of 1,047 anchored vessels were recorded in the area over a one-year period, with the majority anchored where rhodoliths were present. An aquaculture zone was also overlapping with a dense patch of rhodoliths. The majority of the rhodoliths were recorded within one large active trawling zone, and trawl furrows were observed in areas with rhodoliths. Since the present study indicates that large parts of the rhodolith bed falls under the definition of 'maerl', and trawling on this habitat is illegal, locations of the trawl zones need to be revised once more.

Besides its ecological interest, the present characterisation of the rhodolith accumulations and maerl beds located off the southeastern coast of Malta will also serve to inform better management of this habitat.

*(N.B. The data used in the present work belongs to the LIFE BaHAR for N2K project. This data was collected through marine research surveys authorised by the Environment and Resources Authority, the Continental Shelf Department, and the Superintendence of Cultural Heritage. The Project (LIFE12 NAT/MT/000845) is 50% co-financed by the EU LIFE+ Funding Programme and implemented by the Environment and Resources Authority (ERA), the University of Malta, Fundacion Oceana, the Ministry for the Environment, Sustainable Development and Climate Change (MESDC), and the Department for Fisheries and Aquaculture within MESDC. All data are copyrighted and cannot be used or quoted without written permission from the Environment and Resources Authority. © LIFE BaHAR for N2K, 2019. All rights reserved.)*

## Ph D

### RECREATIONAL SPORT SHORE-FISHING OF SELECTED SPECIES OF SPARIDAE IN THE MALTESE ISLANDS: IMPLICATIONS FOR CONSERVATION

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Recreational fishing is very popular in the Maltese Islands, however studies to scientifically investigate this activity have been lacking prior to this research. This study is the first long-term data collection to quantify catches of shore sport fishing and document characteristics, trends, catch composition and impacts on fish populations at the national level. A pilot study on hobby shore angling was also carried out to serve as a guideline for a future larger scale national study. 2,589 roving-access creel surveys which involved directly interviewing each angler on site and investigating his/her catch, were conducted during 132 shore sport-fishing events. An additional 159 catches from hobby fishers were documented using the same methodology which is also applicable to shore fishing taking place in the Mediterranean and elsewhere.

90 species belonging to 29 families were documented with the most common being the Sparidae and Labridae. To this effect, the study focused on 5 Sparidae species: *Diplodus annularis*, *D. vulgaris*, *D. sargus*, *Oblada melanura* and *Lithognathus mormyrus* to assess to what extent their populations are being fished and which sizes are targeted during sport fishing competitions. The most frequently caught study species by sport fishers was *D. annularis* (10.08%) while *D. sargus* was the most targeted species by hobby fishers (14.61%). Catch per unit effort (CPUE) was higher for sport fishers with hobby fishers targeting larger fish. Catch-and-release practices were implemented by 69% of the anglers during sport fishing competitions, however the overall mean mortality rate stood at 35.80% (SD±39.46) indicating that more effort is

required to improve survival of fish. Only 24% of hobby fishers used keep nets. The use of keep nets was observed to be an effective method to reduce fish mortality. Since parameters of growth and weight for the species studied were not available for the Maltese Islands, specimens of the aforementioned species were collected during these activities, together with additional sampling with fishermen, spear fishing competitions, visits to the fish market and 2 fish vendors. They were studied to investigate the length-weight relationship, age structure, reproductive mode, spawning period and diet of the coastal population of these species. A total 3773 fish were examined (*D. annularis*, N=1661, *D. vulgaris* N=1204, *D. sargus* N=346, *O. melanura* N=367, *L. mormyrus* N=195). The length weight relationship was estimated using linear regression where when considering the whole population, a positive allometric growth was observed in *D. annularis*, *D. sargus* and *O. melanura* while growth in *D. vulgaris* and *L. mormyrus* was isometric. Sagittal otoliths were used to estimate the age of the specimens sampled. New age records for *D. annularis*, *D. vulgaris*, *O. melanura* and *L. mormyrus* were established at 17+, 16+, 16+ and 13+ years respectively. The asymptotic lengths ( $L_{\infty}$ ) calculated using the von Bertalanffy equation were: =21.38cm; 26.71cm; 30.86cm; 32.4cm; 35.4cm for *D. annularis*, *D. vulgaris* and *D. sargus* respectively. Fish at age 0+ were the most frequently targeted during competitions.

Reproductive activity in the investigated fish species varied with the three *Diplodus* species showing temporal succession in their spawning period. Histological studies of reproductive tissue were carried out where all species were observed to be hermaphrodites with *D. annularis*, *D. vulgaris* *D. sargus* and *L. mormyrus* displaying non-functional hermaphroditism with partial protandry, while *O. melanura* is a non-functional hermaphrodite species with possible partial protogyny. Sexual inversion was documented for the first time in *D. annularis* and *D. vulgaris* while it was observed in numerous specimens unlike previous work in *D. sargus*. The estimated lengths at first maturity for *O. melanura* and *L. mormyrus* were found to be smaller than the minimum landing sizes permitted by legislation.

All five species were observed to be omnivorous with Crustacea, Teleostei, Polychaeta, Mollusca and algae found in the stomachs of *D. annularis*, *D. vulgaris*, *D. sargus* and *O. melanura*, and Mollusca, Polychaeta, Teleostei and algae in *L. mormyrus*. An overlap between the diets of *D. vulgaris* and *D. sargus* was documented. Findings indicate that recreational fisheries need to

be taken into account when considering conservation measures for national, regional and global fisheries management. The use of keepnets during competitions and respect of the minimum legal sizes among recreational fishers are recommended together with other measures to make this type of fishery more sustainable. Collaboration between managers, anglers and scientists are necessary for successful management of recreational fishing.