

"A comparative analysis of sessile macrofloral and macrofaunal communities from two neighbouring shipwrecks within the Ċirkewwa Marine Park, with an inferential analysis of the potential factors contributing to the observed differences"

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For a better world

No blue - No green

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Abstract

This study provides a comparative analysis of benthic organisms on two scuttled shipwrecks, the MV Rozi and the P29, within the Ċirkewwa Marine Park in Malta. These wrecks function as tourist attractions and artificial reefs and offer insights into the ecological benefits of such structures in marine conservation and tourism. The study used scuba-diving-based surveys and hydrochemical data loggers to assess biodiversity metrics, including species richness, abundance and environmental parameters over a four-month period. Statistical analyses revealed significant differences in species composition on the two vessels, which are influenced by vessel-specific characteristics and environmental factors. The P29 exhibited higher species richness, whereas the MV Rozi showed higher biodiversity. Seasonal variations were modest, with the most notable differences occurring when comparing May with August. The findings underscore the ecological role of shipwrecks as artificial reefs and provide guidance for future scuttling practices to improve marine biodiversity while trying to balance conservation and recreational interests.

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List of Abbreviations

SCUBA Self-Contained Underwater Breathing Apparatus	3
MP Marine Park	11
AR Artificial reefs	8
AW Atlantic Water	6
LIW Levantine Intermediate Water	6
ROI regions of interest	20
NMDS Non-metric Multidimensional Scaling	21
PCA Principal Component Analysis	21
SIMPER Similarity Percentages	21
MDS Multidimensional Scaling	30
PCO Principal Coordinates Ordination	32
PERMANOVA Permutational multivariate analysis of variance	24

Introduction

The purpose of this study is to conduct a comparative analysis of benthic communities from two neighbouring shipwrecks within the Ċirkewwa Marine Park, which is located in the North-West of Malta (35°59'23.78" N and 14°19'38.86" E).

The marine park — which is located within the precincts of the Mellieha Local Council — is a site of significant ecological and recreational value. This study will focus on the shipwrecks of the MV Rozi, scuttled in 1992 and the P29, scuttled in 2007, two of the most popular dive sites within Malta. Both wrecks are situated at similar depths and in comparable environmental conditions, providing an optimal setting for a comparative analysis.

As part of the Marine Park's (hereafter referred to as 'MP') long-term vision, the goal is to ensure that by 2050, all natural habitats and marine flora and fauna within its boundaries are protected and thriving. The MP is envisioned as a site for safe, sustainable and environmentally conscious marine-based activities, with full legal protection. Further it is aiming to be an important resource for education and research while maintaining its high visual quality for conservation and public enjoyment (Cutajar, 2022).

Artificial reefs (hereby 'AR') have been created and used by humans for about 3,000 years (Jensen et al., 2000). These structures are defined as submerged artefacts on the seabed that are intentionally placed and constructed to simulate natural reefs and their function as a protection zone, regeneration and habitat area for underwater organisms (International Maritime Organization, 2009). Early examples for AR in the Mediterranean are rocks left over from tuna fishing, Japan started constructing AR during the 17th century and the concept spread around the world reaching Europe in the mid-20th century. In Europe alone over 252 AR have been created in the last century, with the majority of these within the Mediterranean Sea (Fabi et al., 2011).

Today, artificial reefs are not only essential for fisheries management but also for nature conservation, habitat restoration, and recreational activities across Europe. Their construction and placement are carefully regulated under international guidelines to ensure environmental safety and sustainability, reflecting a broader commitment to in-

egrated coastal zone management.

1.1 | Shipwrecks as artificial reefs

Shipwrecks often function as artificial reefs, and if scuttled are intentionally placed to serve as attractions for divers and as habitats for marine life (Silvestre, 1989), as well as providing substrates for colonisation of benthic organisms (Bohnsack & Sutherland, 1985). Benefits for these AR can be of ecological or economic and social nature. Besides the obvious reasons such as habitat creation, diving attraction and fishing ground creation, these vessels have many more positive things to add to the environment.

As steel-hulled vessels are considered to last way over 60 years in the water, they contribute not only to the underwater community, but are serving a more economical way of reusing them instead of scrapping them (International Maritime Organization, 2009). Due to the huge vertical profile of the bigger vessels, they do not only serve benthic and sessile organisms, but also pelagic and demersal fish communities. With the many complex structures built on a ship, local marine life can find increased shelter opportunities and niches in which to settle. Furthermore, not so well known vessels or vessels less frequently visited by divers can serve as immensely important refuge for various reef (fish) species (International Maritime Organization, 2009).

While anecdotal evidence from the diving community in Malta suggests differences in biodiversity between the two wrecks, there is limited scientific data to support or refute this hypothesis.

Given the increasing number of shipwrecks being scuttled around Malta, understanding their ecological impacts is essential for sustainable marine resource management. This study aims to fill the current knowledge gap by providing detailed comparative data on the biodiversity of two well-known wrecks in the Ċirkewwa Marine Park. The findings might have implications for the future scuttling practices and the management of artificial reefs in the region (Leeworthy et al., 2006).

1.2 | Aims

This study aligns with the MPs vision by contributing valuable research on the biodiversity associated with artificial reefs within the park and aims to scientifically evaluate the differences in benthic biodiversity between two shipwrecks, the MV Rozi and P29, located in the Ċirkewwa Marine Park. By assessing these differences, the research seeks to contribute to a broader understanding of artificial reefs and their ecological impact

in Maltese waters. This objective will be pursued through the specific goals outlined in Section 1.3.

The study seeks to understand how vessel-specific factors, and oceanographic data contribute to observed differences in biodiversity of sessile biota.

1.3 | Objectives

1.3.1 | Biodiversity assessment

To systematically collect and analyse biodiversity data on both shipwrecks with the help of Self-Contained Underwater Breathing Apparatus (SCUBA) diving, at varying points throughout the summer, focusing on the current state of the benthic assemblages colonising both wrecks.

1.3.2 | Comparative analysis

To compare the biodiversity data from the MV Rozi and the P29 to determine if there is an existing difference in terms of species richness, abundance and overall coverage.

1.3.3 | Vessel-specific and oceanographic factors

To investigate how vessel-specific factors (such as structure and distance to the reef) and oceanographic data (such as temperature, salinity, pH and dissolved oxygen) influence biodiversity assemblage differences between the P29 and MV Rozi shipwreck. This includes collecting and analysing data from both sites and assessing how these factors correlate with observed biodiversity variations.

Literature review

2.1 | Main macrofauna and macroflora of the Mediterranean

The Mediterranean Sea is one of the world's richest regions in terms of animal and plant diversity (Cuttelod et al., 2008). It is home to a wide range of macrofauna including species of fish, crustaceans, molluscs, echinoderms and others organisms (Coll et al., 2010). In 2010, Coll et al. estimated the biodiversity for the Mediterranean Sea to approximately 17,000 marine species. A significant portion of the diversity is still unexplored, particularly for areas that are harder to reach such as deep-sea areas, or small organisms such as microbes.

Biodiversity within the Mediterranean Sea decreases from the north-west to south-eastern regions, with diversity hotspots being the western Mediterranean, the Adriatic and the Aegean Sea. The main fish species found in the Mediterranean include groupers (*Epinephelus* spp.), sea breams (Sparidae), mullets (Mugilidae), moray eels (Muraenidae), damselfish (*Chromis chromis*) and wrasses (Labridae). Among molluscs, the most common species are octopus (*Octopus vulgaris*), cuttlefish (*Sepia officinalis*) and squid (*Loligo vulgaris*).

Next to animals, a big part of our oceans is inhabited by plants, or macroflora. In the Mediterranean Sea, one can find a variety of species including seagrasses, algae, and others. The most popular and well-known endemic seagrass of the Mediterranean is *Posidonia oceanica* or neptune seagrass, which is a keystone species that forms massive and dense underwater meadows. Another common species of seagrass, which is smaller but thinner than *P. oceanica*, is *Cymodocea nodosa*. The 1,124 known macroalgae species can be classified into green algae (Chlorophyta) with about 190 known species, brown algae (Phaeophyceae) with an estimate of 277 known species and red algae (Rhodophyta) including approximately 657 known species (Bartolo et al., 2020). The Mediterranean Sea hosts a diverse range of sponges and other benthic organisms,

contributing significantly to the ecosystem's complexity and health. Specific numbers for sponges and other benthic organisms can vary, but they are an essential part of the marine biodiversity in the region.

Whilst the Mediterranean is home to many species, most found throughout different worldwide oceans, it is also home to many endemic species (Cuttelod et al., 2008). Endemic species are organisms that are only found in a specific environment or area, in this case the Mediterranean Sea. Of the 17,000 known marine species about 25-30% are endemic (Coll et al., 2010; United Nations Environment Programme, 2021). The IUCN Red List (Cuttelod et al., 2008) outlines many of these, the most common ones within the Mediterranean Sea being:

- *Posidonia oceanica* - the neptune grass
- *Pinna nobilis* - the noble pen shell
- *Monachus monachus* - the Mediterranean monk seal
- *Caretta caretta* - the loggerhead sea turtle
- *Scyllarides latus* - the Mediterranean slipper lobster
- *Coris julis* - the Mediterranean rainbow wrasse
- *Serranus cabrilla* - the comber
- *Lithophaga lithophaga* - the date mussel

Many of these endemic species are threatened by so called alien species. These non-indigenous species are primarily introduced to the Mediterranean Sea via ship traffic since the opening of the interoceanic maritime routes about 50 years ago. The Suez Canal is the main pathway for these species (Galil, 2000). An inevitable threat that biologists throughout the Mediterranean are awaiting, is that of the invasive lionfish (*Pterois miles*), a generalist predator that is rapidly expanding its range from the East to the Central Mediterranean Sea, including Malta, Cyprus and Tunisia (Ulman et al., 2021).

2.2 | Hydrodynamic conditions within the Malta-Sicily channel

Malta is an archipelago consisting of three main islands positioned in the central Mediterranean Sea. It is located within the so-called Malta-Sicily Channel a crucial area of in-

teraction between the eastern and western Mediterranean basins. The Maltese islands lie about 93 km south of Sicily and about 300 km north of Libya, making them a crucial link between the water exchange between these two basins, which differ in terms of temperature, salinity and thermohaline properties (see Figure 2.1).

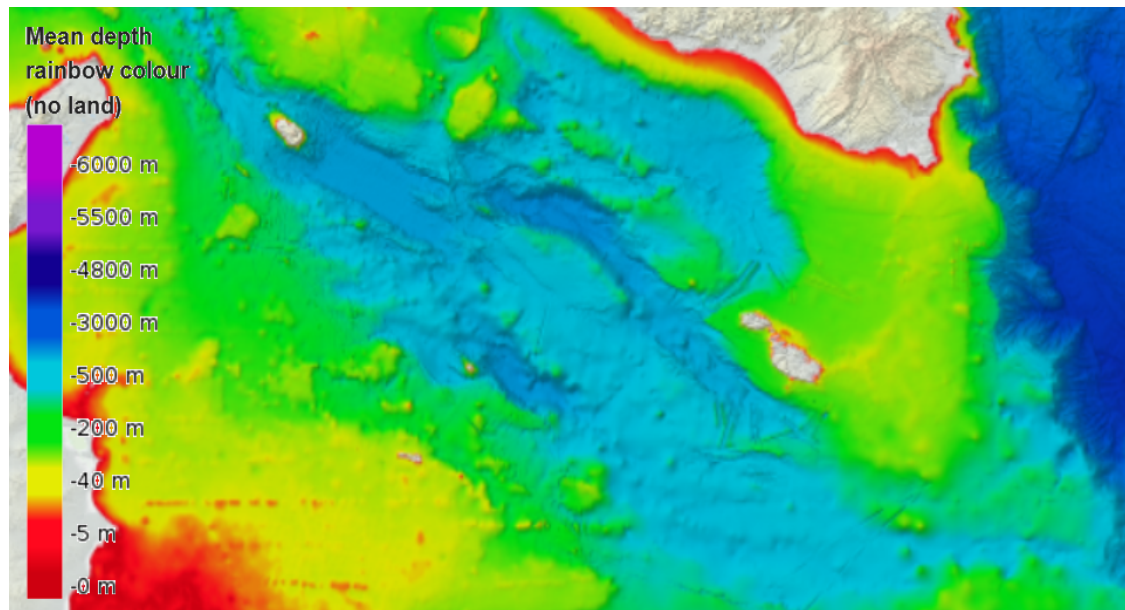


Figure 2.1: A topographic map of the Malta-Sicily Channel with bathymetric data derived from the European Marine Observation and Data Network (<http://www.emodnet.eu/>).

This Malta-Sicily Channel serves as a conduit where water masses and thermohaline properties (density differences driven by temperature and salinity) exchange between these basins, creating a unique hydrodynamic environment (Suarez et al., 2019).

The surface water moves eastwards, carrying less saline water coming from the Atlantic Ocean (AW), while the saltier Levantine Intermediate Water (LIW) flows westwards in intermediate waters. This exchange of saltier and less salty water is essential for maintaining the Mediterranean's salt balance and affects the broader thermohaline circulation patterns within the Mediterranean Basin (Menna et al., 2019).

In the Malta-Sicily Channel, mesoscale oceanic features—such as eddies, gyres, and fronts—are largely influenced by regional wind patterns and the complex bathymetry (underwater topography) of the strait. Mesoscale features refer to oceanic processes and structures that operate on scales ranging from tens to hundreds of kilometres and over time-frames from days to months. These features impact the movement and mixing of water masses, nutrient transport, and biological activity within the region.

A prominent mesoscale feature in the Channel is the anticyclonic Malta-Sicily Gyre.

This gyre is shaped by the interaction between wind-driven currents and the bathymetric profile of the strait, resulting in a complex circulation pattern that further enhances the exchange between the Eastern and Western Mediterranean waters (Menna et al., 2019; Suarez et al., 2019).

Whereas the Mediterranean Sea is well known for its low productivity as an oligotrophic Ocean, the hydrodynamic conditions in the channel impact and influence the nutrient availabilities nevertheless. This is mainly due to upwelling events and mesoscale circulation. Upwelling — which is the rise of deeper, nutrient richer waters — supports the primary production by providing essential nutrients to phytoplankton (Mann & Lazier, 2005) and therefore the biological diversity of the region. Within the Malta-Sicily Channel, these events occur primarily along the southern coast of Sicily (Placenti et al., 2022).

2.3 | Shipwrecks as artificial reefs

In the realm of artificial reefs, shipwrecks have emerged as one of the most effective and intriguing methods for enhancing marine biodiversity and creating new habitats (Jensen, 2002; Leeworthy et al., 2006). These sunken vessels, whether accidentally wrecked or intentionally scuttled, provide complex structures that mimic natural reefs. Their vertical profiles and large surface areas make them ideal substrates for colonisation by marine flora and fauna. Over the past few decades, a number of European countries, such as France, Turkey and Italy (Jensen et al., 2000) have increasingly turned to shipwrecks as a means to support marine life, manage fisheries, and boost diving tourism (Fabi et al., 2011; Jensen et al., 2000).

Shipwrecks as AR play a crucial role in enhancing marine biodiversity by providing substrates for colonisation and habitat for various marine organisms (Bohnsack & Sutherland, 1985; Silvestre, 1989). Understanding their role and benefits for protecting our ocean and its many habitats will be an asset for conservation in the future and an important research topic.

Several factors can influence the biodiversity observed on artificial reefs, including the material of the wreck, the hydrodynamic and hydro chemical conditions, and the duration of submersion (Paxton et al., 2017). The positioning of the wreck, exposure to currents, and light availability are critical in determining the types of species that colonise the wreck (Mallet & Pelletier, 2014). Additionally, the pre-scuttling preparation of the wreck, such as the type of paint used, can impact the initial colonisation stages (Mallia, 2022). The debate on whether artificial reefs merely attract fish or increase fish

production is also relevant, with various studies presenting differing viewpoints (Pickering & Whitmarsh, 1997).

The use of wrecks as AR in Europe has a rich history since the mid-20th century. Many of the existing shipwrecks within the Mediterranean were unintentionally scuttled, a result of maritime accidents or due to war practices. However, over time, the intentional scuttling of vessels that are no longer fit to operate became a strategic practice which aims to enhance marine ecosystems and boost economic activities such as tourism at the same time (Bohnsack & Sutherland, 1985; Fabi et al., 2011).

Many European nations have scuttled shipwrecks as artificial reefs over the last years. The HMS Scylla, scuttled off the coast of Cornwall in 2004, has boosted the local tourism significantly (Firth et al., 2023). Similar observations have been made in the Ligurian Sea off the coast of Italy, where scuttled shipwrecks have enhanced local biodiversity and become major diving attractions – highlighting again the dual importance of shipwrecks as AR (Fabi et al., 2011).

2.3.1 | Environmental guidelines

Nowadays shipwrecks are well established tools in the European marine management. Their role as AR goes way beyond the ecological benefits and has a significant role in the diving tourism industry. Careful selection, preparation and sinking of these vessels is managed to maximise ecological outcomes while minimising environmental risks (Fabi et al., 2011; “OSPAR Convention”, 1992).

Before sinking a huge vessel such as a ship intentionally, considerations must be made to protect the ocean from pollution. The sinking site must be selected considering local and marine environments to avoid harm to sensitive ecosystems, such as seagrass meadows or coral reefs. If ships are scuttled with the idea of creating a diving attraction or an AR they have to be cleaned and made safe for penetration, a process that is called ‘prepping’. Ideally, these newly created habitats should be monitored and managed to improve future projects.

Countless regulations have been created to make sure these ARs do not create more harm than good. These regulations consider the protection of the ocean against pollution and irresponsible dumping at sea. The following conventions have come up with some of these regulations and are especially important in Europe and the Mediterranean Sea: London Convention (“London Convention”, 1972), Barcelona Convention (“Barcelona Convention”, 1976), HELCOM (Annex II) (“Helsinki Convention”, 1992) and the OSPAR Convention (“OSPAR Convention”, 1992).

2.3.2 | Artificial reefs in Malta

Since the 1980s Maltese authorities have been scuttling ships by intention to create diving and tourism attractions (Fabi et al., 2011). The intentional scuttling of ships to create diving attractions and marine habitats is a common practice in Europe (Fabi et al., 2011; Jensen, 2002) and the Maltese islands. The 13 shipwrecks that have been scuttled as a diving attraction are as follows: MV Rozi and P29 at Ċirkewwa Marine Park; P31 at Comino close to the Blue Lagoon; Um El Faroud oil tanker off Żurrieq; Imperial Eagle off Qawra Point; tugboats Tug 2 off Exiles, Sliema, Tug 10, St. Micheal and the P33 patrol boat off Żonqor Point, Marsaskala; MV Xlendi, MV Cominoland, MV Karwela and the newest shipwreck the M/T Hephaestus in ix-Xatt l-Ahmar in Gozo.

In figure 2.2 one can see the scuttling of the P29 patrol boat on the 14th of August 2007 in the Ċirkewwa Marine Park.



Figure 2.2: Scuttling of the P29 patrol boat on the 14th of August 2007 (Adi Associates Environmental Consultants Ltd, 2007).

Next to these scuttled shipwrecks there are some historical wrecks that are frequently used as diving sites such as the HMS Maori in Valletta, outside the Grand Harbour; SS Margit in Kalkara Bay or the Le Polynesian outside the Grand Harbour ((Adi Associates Environmental Consultants Ltd, 2007); (Borg et al., 2008)).

Borg et al. (2008) conducted extensive studies on artificial reefs in Malta, noting that these structures were not originally designed with ecological considerations in mind. Consequently, scientific monitoring of these reefs has been sporadic, with insufficient data on their long-term ecological impacts. Furthermore, studies on the colonisation of shipwrecks by benthic and other marine organisms are limited and often of poor quality. This gap in knowledge necessitates further research to understand the ecological roles of these artificial structures better.

Malta's approach to using shipwrecks as artificial reefs is primarily driven by tourism, contrasting with other Mediterranean countries like Spain or Italy, who focus on enhancing fisheries and restoring degraded marine environments and habitats. This economic driven strategy underscores the need for a balanced approach that also considers the ecological implications of scuttling ships (Fabi et al., 2011). All existing shipwrecks are made of metal, but cleaned thoroughly before scuttling most of the vessels still have a layer of paint, such as the P29 shipwreck in the Ċirkewwa MP (Adi Associates Environmental Consultants Ltd, 2007).

2.4 | Importance of the hydrodynamic environment in shaping marine ecosystems and assemblages

Marine ecosystems and their structure and function are massively influenced by the hydrodynamic environment which encompasses ocean currents, tides, waves, upwelling and downwelling as well as the bathymetry of the seabed. Habitat structures are influenced by hydrodynamic forces. Artificial reefs — including shipwrecks — can influence these habitats and hydrodynamics massively. Marine environments such as seagrass meadows, corals reefs, rocky shores and artificial reefs are massively influenced by wave action and current (Massel, 2012). Parts of the reefs that are exposed to currents or wave movement compared to sheltered structures, are home to organisms that are resilient to these conditions, hosting coral species or seagrasses which anchor into the sediment. These organisms create habitats for other species that need calmer waters or conditions (Graham & Dayton, 2002). The same can be said about shipwrecks, which create complex physical structures to support a wide range of sessile filter feeders to mobile species such as fish (Jensen et al., 2000). The transformation of the environment by shipwrecks is often a positive one, by establishing populations in otherwise inhospitable areas.

Further, the hydrodynamic environment affects species diversity and distribution by setting physical limits on where organisms can settle down. Barnacles and mussels are species adapted to high-energy environments, so these are dominant in wave-exposed and rocky shores, whereas sediment-dwelling infauna thrive in low-energy environments, such as polychaetes (Thorson, 1950). Surface and deep water currents can additionally play an important role in dispersal of larvae, spores and juvenile stages of organisms to move to different areas or new habitats to settle upon (Cowen & Sponaugle, 2009).

Materials & Methods

3.1 | Site description

The Ċirkewwa Marine Park was established in 2021 and is situated in the North-West of the island of Malta (35°59'23.78" N and 14°19'38.86" E). It is one of Malta's most important marine conservation areas, as it is the first managed marine protected area in the Maltese Archipelago. The MP covers several key dive sites such as the shipwrecks of the MV Rozi and the P29 as well as shallower dives along the rocky reef incorporating for example the Right Arch, Swimthroughs and Ben's Arch.

Furthermore, the Marine Park is home to large sandy seabed areas, extensive *Posidonia oceanica* meadows — a seagrass species that is one of the most important protected organisms within the MP — and shallow rocky topography. It is known for its increasing biodiversity, especially since it has been under the management of Nature Trust Malta in cooperation with the Malta Tourism Authority.

3.1.1 | GPS data collection

To accurately record the locations of the shipwrecks of the P29 and the MV Rozi on the map, GPS data was collected on the 15th and 17th of March 2024 with the help of SCUBA diving and a GRAMPER GPS. The GPS logger was taken underwater and sent to the surface on three points on each wreck — one point on the bow, two points on each side of the stern. Further, GPS points along the reef were taken to get a better understanding of the distance between the shipwrecks and the reef as can be seen in Figure 3.2.

3.1.2 | MV Rozi shipwreck

The MV Rozi (Figure 3.3) was a tugboat that served as a harbour tug and salvage boat working for different countries before it came to Malta. It was scuttled intentionally in

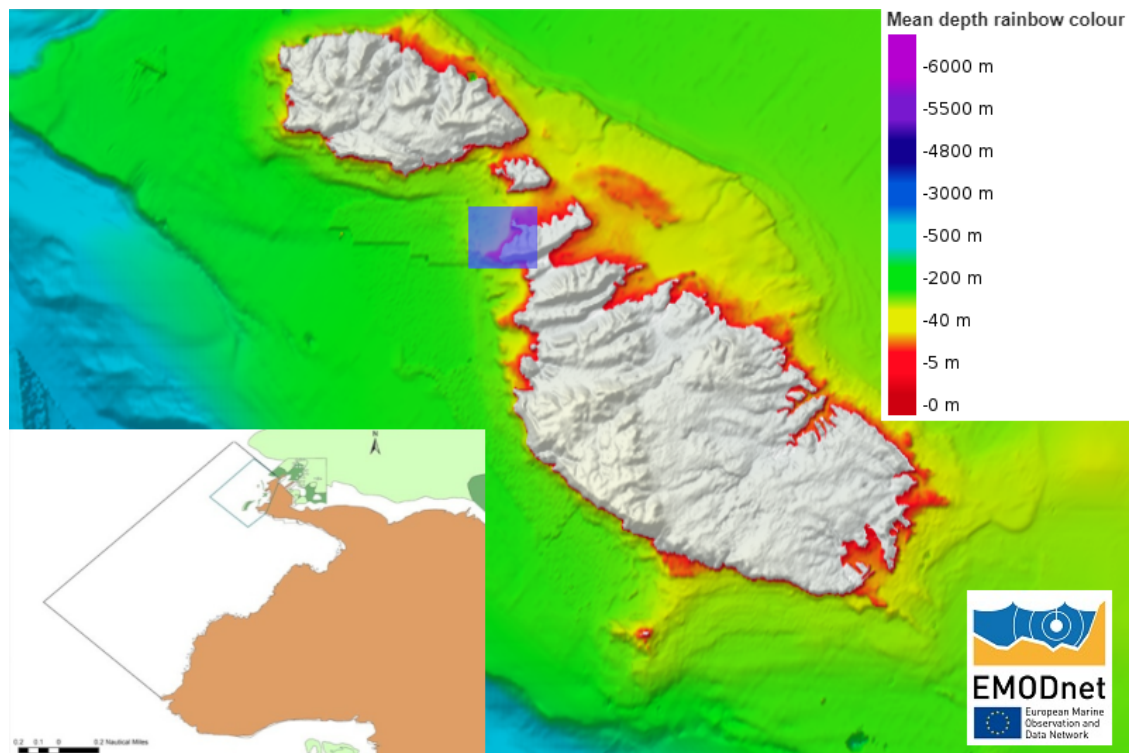


Figure 3.1: Bathymetry map of the Maltese islands, with a scale/legend for depth (Bathymetric Map of the Malta Region [Image], 2024). The blue box marks the Marine Park, the left map shows the Ċirkewwa Marine Park boundaries (Cutajar, 2022).

1992, becoming one of Malta's first example of a vessel scuttled off the coast of north-west Malta so to create an artificial reef and an underwater tourist attraction. The process of scuttling was organised by Captain Morgan Cruises 32 years ago with the intention of providing a tourist attraction for a now no longer existing tourist Submarine Tour operating from Ċirkewwa. Given that the scuttling was organised 32 years ago in the year of 1992, this was a time prior to the creation of the Maltese Planning Authority and therefore no official reports are available for analysis of the pre scuttling processes, if the paint was removed or not, or the scuttling itself.

The 33 metre long and 8.5-metre-wide shipwreck lies on sandy bottom at a depth of about 34 metres, with the shallowest part being around 20 metres. Therefore, the wreck is a well-known and highly frequented dive spot. Usually, the MV Rozi is surrounded by marine life, including various fish species such as damsel fish (*Chromis chromis*), banded bream (*Diplodus annularis*) and scorpion fish (*Scorpaena scrofa*).

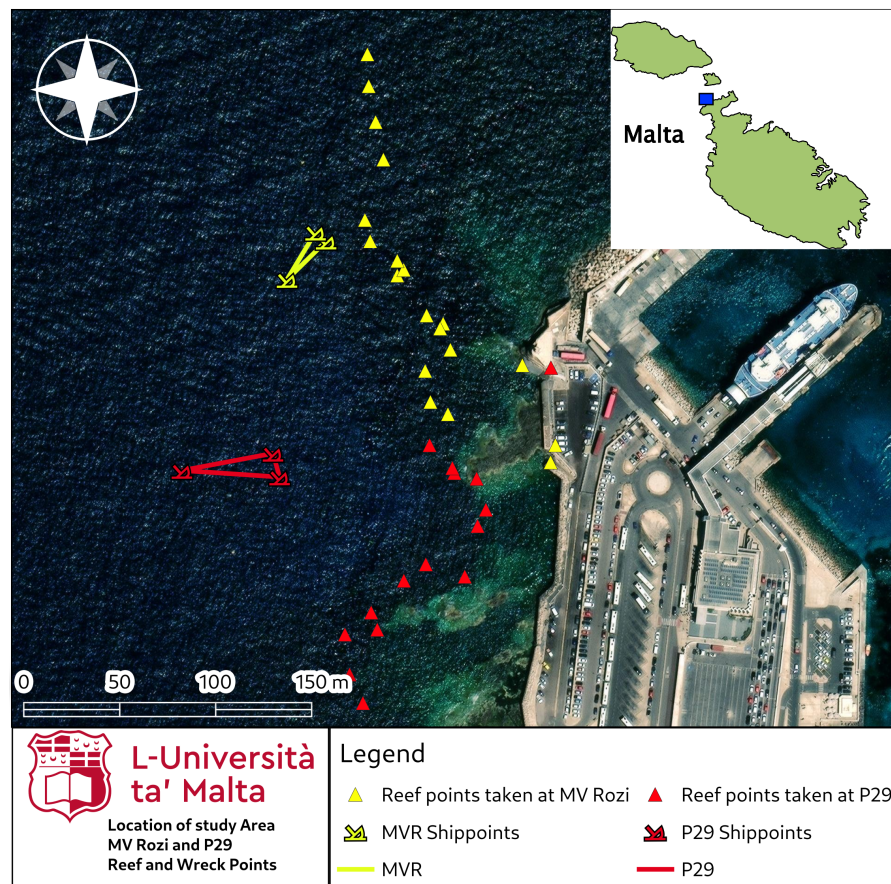


Figure 3.2: GPS data points in relation to both shipwrecks and the reef in the Ċirkewwa Marine Park. Yellow symbols represent the MV Rozi shipwreck, red ones the P29 shipwreck.

3.1.3 | P29 shipwreck

The P29 was a Kondor I-class patrol boat built by the Peenewerft shipyard. Originally used to patrol the riverbanks between East and West Germany, it also served as a minesweeper for the East German Navy under the name Boltenhagen. In 1992, the vessel became part of the Maritime Squadron of the Armed Forces of Malta. The former patrol boat is 51.98 metres long, 7.12 metres wide and has a draught of 2.3 metres. After being decommissioned, the P29 was scuttled as a dive site in Ċirkewwa, Malta, on August 14th, 2007 (see Figure 2.2). In Figure 3.4 a picture of the shipwreck as it looks nowadays after being in the water for 17 years.

The primary mission of the P29 was to secure the Maltese coast against smuggling and conduct border control operations. Upon being sunk, the 52-metre-long vessel,



Figure 3.3: The 33 metre long MV Rozi shipwreck in the year 2024. Port and bow view.

now lying at a maximum depth of around 37 metres with its mast at about 21 metres, has become a popular diving attraction and an artificial reef. Divers can explore the wreck at depths ranging from 24 to 35 metres.



Figure 3.4: The 52 metre long P29 shipwreck in the year 2024. Port and bow view.

The scuttling of the P29, like the MV Rozi, aimed to create an artificial reef and enhance marine biodiversity and the dive sites attraction. The wreck lies on a sandy bottom, providing a habitat for a variety of fish species and larger predators such as the greater amberjack (*Seriola dumerili*) and the common dentex (*Dentex dentex*). These species are more frequently encountered on the P29 than on the MV Rozi (personal observation).

Despite the intentional scuttling of these wrecks 32 and 17 years ago to improve ecological diversity and health of the area, there has been limited research on their impact since deployment. Continued studies could provide valuable insights into the effectiveness of artificial reefs in promoting marine biodiversity and their long-term ecological benefits.

3.2 | Sampling periods

The data was collected on the shipwrecks MV Rozi and P29 over the May-August 2024 period, with one survey being conducted during the beginning of each month. For this, ten monitoring SCUBA dives were conducted to a maximum depth of 34 metres. Additionally, four more dives were done to place and remove the data loggers (fully described in section 3.3 Hydro chemical data). These loggers have been fixed on a concrete block and placed approx. 20 metres west of the two wrecks, at a depth of 39.9 metres. The equipment used during each monitoring dive is listed below.

- 15l steel cylinders filled with 30% EANX
- 50x50 cm PQ
- Buoyancy control device (BCD)
- Depth gauge
- Diving computer
- Fins
- GoPro 12
- Pressure gauge
- Weights
- Lights and strobes
- Mask
- Olympus Tough TG 4
- Regulators
- Surface Marker Buoy
- Transect notes
- Lift bag

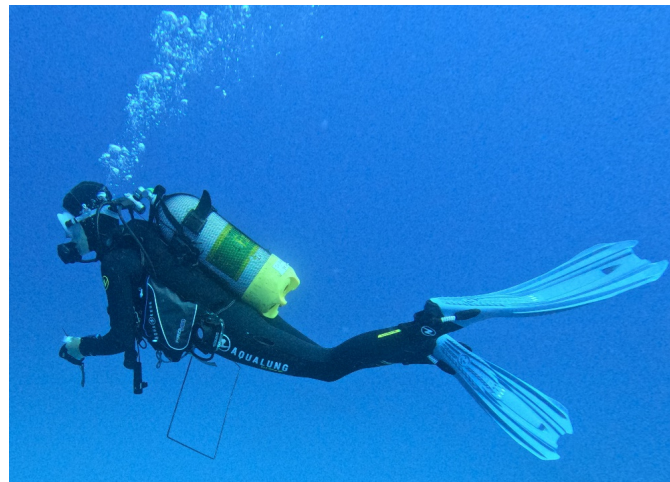


Figure 3.5: Research diver with most of the equipment visible.

3.3 | Hydro chemical data

Three HOBO data loggers were placed underwater on the 8th of May 2024. To make sure, that they will not be moved or otherwise disturbed for each site a concrete block was adjusted accordingly, and the loggers were fixed upon this block (see Figure 3.6).

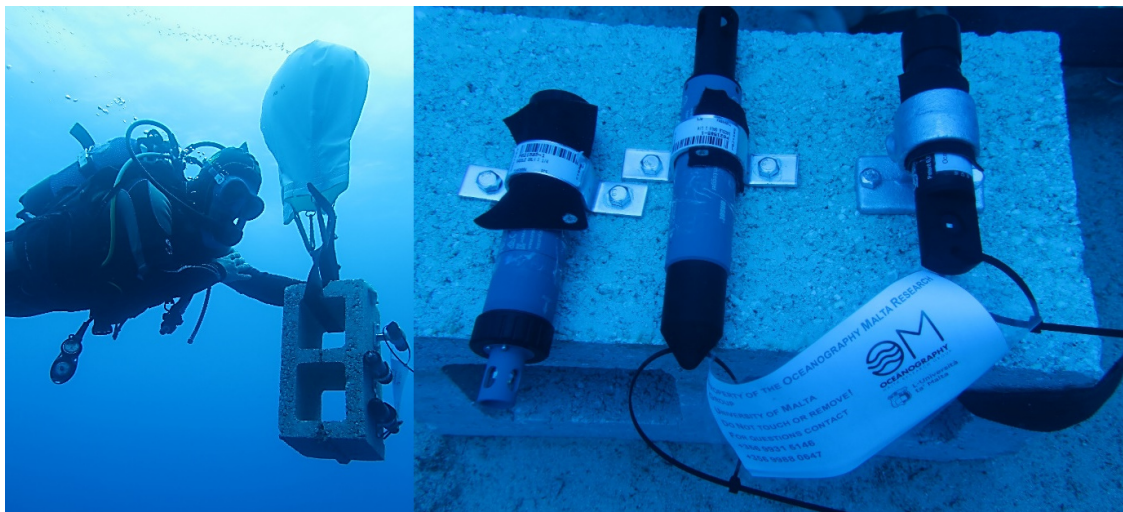


Figure 3.6: Research diver on the way to place the concrete block with the loggers attached on site. To help place them, a 15l lift bag was used. The loggers were placed approx. 20m west of the wrecks at a depth of 39.9m

1. HOBO U24-002-C Saltwater **Conductivity/Salinity** Data Logger. This logger measures salinity, conductivity and temperature in saltwater environments (Onset

Computer Corporation, n.d.-b).

2. HOB0 MX2501 **pH and Temperature** Data Logger. Designed for long – term monitoring (up to six months) of pH and temperature, this Bluetooth-enabled logger can be used in freshwater and saltwater waterbodies (Onset Computer Corporation, n.d.-a).
3. HOB0 U26-001 **Dissolved Oxygen** Data Logger. This logger measures DO concentrations in the water with the help of optical DO sensor technology (Onset Computer Corporation, n.d.-c).

These loggers collect data for pH, dissolved oxygen, temperature, and conductivity — a proxy used to measure salinity hourly. They were retrieved from the water on the 25th of August 2024. The data was analysed with MATLAB, OCTAVE, and R. Time series graphs were generated for each parameter.

3.4 | Sampling techniques

Photographic sampling was conducted through SCUBA diving. The collection of images was conducted on four different days between the beginning of May to the beginning of August:

Table 3.1: Sampling dates of 2024.

Sampling 1	Sampling 2	Sampling 3	Sampling 4
15th May	7th June	10th July	8th August

3.4.1 | Photoquadrates

For this research, a non-invasive sampling technique was chosen. Therefore, pictures were shot underwater with the help of scuba diving and Photoquadrates. Surveys with Photoquadrates (PQ) are a popular method to study benthic organisms; naturally this method has both benefits and limitations (Bravo et al., 2021; Ivkić et al., 2023; Van Rein et al., 2011).

Benefits are

1. that this is a non-invasive method that preserves the natural habitat while allowing repeated analysis;

2. the high resolution, as PQ can capture detailed images that aid in the identification of species;
3. PQ provide quantitative data on species abundance and distribution.

Further, once a method is established PQ surveys can be quicker than traditional in-water identification, especially when dealing with larger and deeper areas.

Limitations come in different forms

1. some organisms are hard to identify from (poor quality) images alone, leading to potential misidentifications;
2. SCUBA diving has depth and significant time restrictions for safety reasons and surveys cannot be conducted in depths exceeding 40 metres on recreational equipment or without additional training;
3. analysing images is time-consuming and requires expertise, or training and specialised software (Van Rein et al., 2011).

During Photoquadrat surveys a square with specific dimensions — in this study 50x50 cm — is placed over the (benthic) area of interest. Operated by a scuba diver, the quadrat ensures a consistent scale for analysing benthic organisms. A camera is used to capture overhead images of the area within the quadrat, often mounted on a frame to maintain a fixed distance from the surface. However, in this study, technical and cost constraints necessitated the use of two different underwater cameras (a GoPro 12 and an Olympus Tough TG4, both with underwater housings), without a fixed frame.

To avoid biased sampling, the placement of the PQ was randomised during dives. Therefore, the starting point was chosen randomly and five fin kicks are counted between each of the PQ. The aim was to try to stay on the same depth on each of the side of the shipwrecks. Due to time restrictions under water and for the whole research project PQ were only taken on the smooth surfaces of the shipwrecks, cryptic environments (structures, ropes, windows, inside etc.) were therefore avoided.

A total of 15 PQ samples were collected per dive, with five samples taken from each of the starboard and port sides and five from the deck — two at the bow and three at the stern. Table 3.2 shows the sequence of the photoquadrates taken. Surveys started on the starboard side (PQ 1-5), then two PQ were taken on the bow side of the deck (PQ 6-7), followed by the five PQ on the port side (PQ 8-12), and finally the last two PQ were taken on the stern side of the deck (PQ 14-15).



Figure 3.7: Survey divers placing the PQ and taking a picture of the PQ on the deck of the shipwrecks.

Table 3.2: Survey layout for photoquadrates.

Position	Starboard					Deck					Port				
PQ Frame	1	2	3	4	5	6	7	13	14	15	8	9	10	11	12

3.5 | Data processing and data analysis

The data that was collected during this study can be separated in biological data, and hydro chemical data. Analysis done with the help of photoquadrates are biological data, the information gathered with the help of the loggers is classified as hydro chemical data. Additionally, currents were calculated with the help of model data from the surface, at 10 metres depth and at 20 metres depth. Analysis was done with PRIMER, Octave and R.

3.5.1 | Biological data

Biological data from the PQ collected during research dives was used to analyse the benthic organisms and coverage on the two shipwrecks.

3.5.1.1 | Image analysis

Images taken with the PQ method were analysed to identify species to the lowest possible taxonomic level. The images were sorted by date and wreck, organisms were identified using identification guides such as Bergbauer and Humberg; Blondel; Louisy.

3.5.1.2 | Species identification and coverage calculation

For each species that could be identified and showing extensive surface growth/formations, coverage was measured in cm^2 and converted to percentage coverage. Species were marked and analysed with the help of the ImageJ/Fiji software (Schindelin et al., 2012).

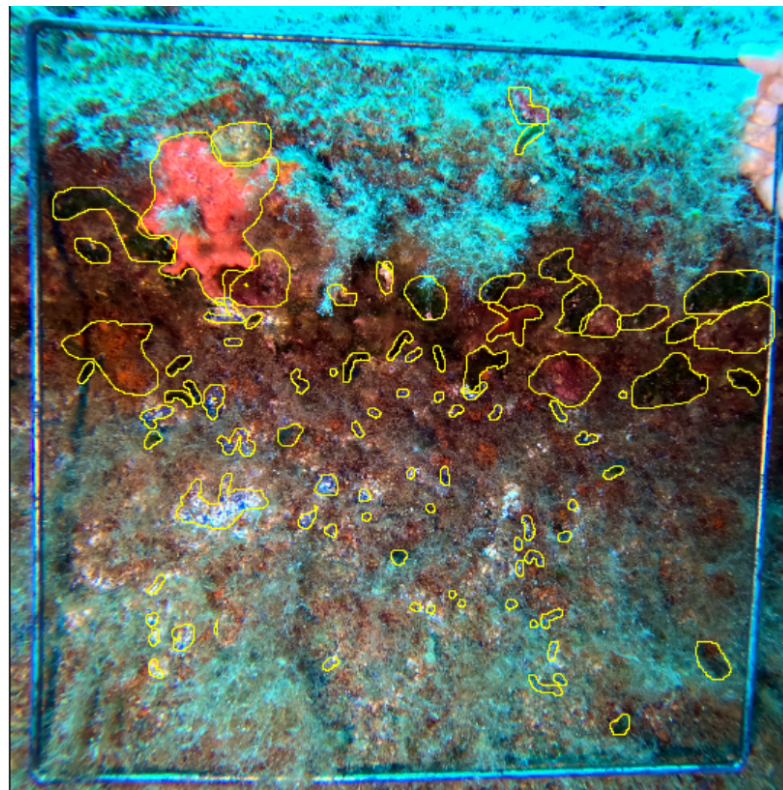


Figure 3.8: Selected regions of interest ROI) in the Fiji software from one of the MV Rozi surveys (survey 4, PQ 7). Species visible are *C. crambe*, *P. crassum*, *D. vermicularis*, *M. lichenoides*, *E. sepositus* as well as barren areas. Areas were calculated and sum subtracted from total area (2.500 cm^2) to get coverage of the most abundant species that covers most of the area – *Dictyota intricata*.

3.5.1.3 | Diversity indices

To compare the diversity of benthic organisms between the MV Rozi and the P29 different indices were used. Alpha diversity was used to measure species richness within a single habitat by counting the number of species present per wreck, beta diversity is used to measure species diversity between different habitats or ecosystems — in this case the P29 and the MV Rozi — indicating how different species composition is. The Shannon Index (H') takes abundance and evenness of species within the habitat into account (Magurran, 2021).

3.5.1.4 | Multivariate analyses

Several multivariate analyses were conducted using Primer v7 and PERMANOVA+ software packages (Anderson, 2017) to further understand the diversity and distribution of species upon the shipwrecks. To do so, a raw data matrix was constructed using the Count-N values for each identified species per photoquadrat, which was further processed and analysed. To equalise the contributions of species with different abundances, a dispersal weighting was applied, followed by a square root transformation. This transformed matrix showing the abundances of species was used to create a Bray-Curtis Index into a triangular distance matrix (Clarke et al., 2006).

With this transformed data multivariate analysis such as Non-metric Multidimensional Scaling (NMDS), Principal Component Analysis (PCA) and Similarity Percentages (SIMPER) were conducted. NMDS is a non-parametric method that is used to help visualise the possible similarities between two or more groups. The PCA uses orthogonal transformation to convert a set of observations of possible correlated variables into a set of values of linearly uncorrelated variables (Paul & Anderson, 2013). A SIMPER analysis was done to analyse the contribution of individual species to the overall similarity between groups.

3.5.2 | Hydro chemical data

Hydro chemical data was calculated in four categories: temperature, dissolved oxygen (DO), pH and conductivity/salinity. Three HOBO data loggers have been placed in the water for approx. four months, all three of them measured temperature next to the other values. Software used for calculation was GNU Octave (version 9.2.0) (G. N. U. Octave Developers, 2023).

Conductivity/salinity data was transferred into salinity values using PSS-78, the Practical Salinity Scale 1978 (Lewis, 1980). Each logger collected data hourly, these val-

ues were calculated to get an average value per day, which further was visualised with a time scale.

3.6 | Model data

To gain information about water currents in the study area, time series data was extracted for a single grid cell over the Ćirkewwa Marine Park from a Mediterranean model with a resolution of 0.0416 degrees (approximately 4 km), at depth of 0m, 10m, and 20m. This product is a forecast for the Mediterranean Sea developed by CMEMS (Clementi et al., 2023). It provides 3D fields for key parameters such as temperature, salinity, as well as zonal and meridional velocities. It also has 2D fields such as sea surface height, mixed layer depth, and bottom temperature. The data is conducted every hour and extends from 13th November 2023 till 30th October 2024.

Additional to these current models, temperatures from different depths are compared with the measured data from the loggers placed close to the P29 and the MV Rozi for the study period.

Results

4.1 | Species community composition

A total of 25 taxa were recorded and identified on the shipwrecks, the species list can be found in Table 4.2. Of these species, two could only be identified to genus level, two to family level and one could be placed into the right class but not further. A total of 12 algae species was found, from which four belong to the phylum Chlorophyta — green algae, including Prasinodermatophyta — seven to the phylum Ochrophyta — brown algae — and one species of Rhodophyta — better known as red algae. Two representatives of the mollusca phylum were identified; a gastropod and eggs from the squid *Loligo vulgaris*. Next to that, three different species of polychaeta from the phylum annelida were present during this study, as well as six different families of sponges (Porifera - Demospongiae), one species of corals — the endemic *Cladocora caespitosa* — and one member of the echinodermata phylum, *Echinaster (Echinaster) sepositus*. In Figure 4.1 a PQ of the P29 and one of the MV Rozi is visible.

Not all the 26 identified species were observed on both shipwrecks. Three species were solely observed on the P29 surveys namely *Dictyota implexa*, *Ochrophyta sp.*, and *Terebellidae sp.* More unique species were observed on the MV Rozi shipwreck. These species are: *Padina pavonica*, *Sarcotragus fasciculatus*, *Cladocora caespitosa*, *Echinaster sepositus*, *Cratena peregrina*, *Dictyopteris sp.*, *Loligo vulgaris* (eggs) and *Cerithiidae sp.*

Additionally, to the species taxa, a category 'barren' and a category 'sediment' was created. Under the 'barren' category, spaces that have been blank, rusted or where paint was visible were included. As 'sediment' mainly areas on the deck without growth of algae or other organisms was included that appeared to be sand or substances of similar origin.

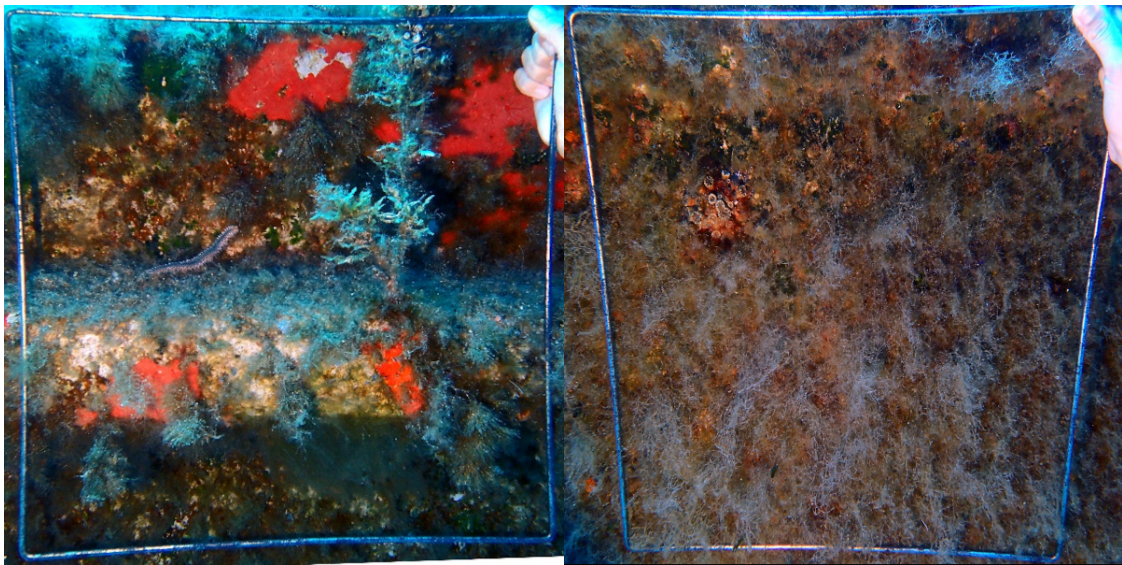


Figure 4.1: Examples for photoquadrats (50x50 cm) on the P29 (left) and the MV Rozi (right)..

4.2 | Biological data

4.2.1 | SIMPER

With the help of Similarity Percentages (SIMPER) analysis, the most abundant species found on the wrecks was detected within a threshold of 70%, as can be seen in Figure 4.6. Most common on both sites is *Dictyota dichotoma* var. *intricata* (C. Agardh) Greville, 1830, with a slightly higher abundance on the MV Rozi shipwreck. The encrusting red algae *Mesophyllum lichenoides* (J.Ellis) Me.Lemoine, 1928, is common on both wrecks but significantly more abundant on the P29 shipwreck. The same pattern is observed for *Sargassum* sp. C. Agardh, 1820, *Crambe crambe* (Schmidt, 1862), and *Palmophyllum cras-sum* (Naccari) Rabenhorst, 1868. Despite some blank spots found on the P29 due to ongoing paint erosion, most barren spots were recorded on the MV Rozi. The amount of sediment found on the wrecks is comparable between the two sites, suggesting that sediment coverage is not the primary factor influencing species distribution.

4.2.2 | PERMANOVA

To detect which factors are playing a significant role in the possible difference between the wrecks, a Permutational multivariate analysis of variance (PERMANOVA) was conducted (see Table 4.1). Looking at the results, comparing the shipwrecks as well as the

different positions along these, there seem to be a significant difference between these two factors, with values of 0.0001, meaning highly significant. The factor 'month', with a $P(\text{perm})$ of 0.0004, seems to be less significant, but a difference can be seen, nevertheless. When looking at differences between more than one factor, shipwreck and position appear to show the most difference with a $P(\text{perm})$ value of 0.0001. Least significant in this comparison are the factors month regarding to position.

Table 4.1: PERMANOVA analysis showing significant differences between the factor's month 'Mo' shipwreck 'Sh', and position 'Po', as well as the relation between month and shipwreck 'MoxSh', month and position 'MoxPo', shipwreck and position 'ShxPo', and all three factors together 'MoxShxPo'. Significance is shown with the help of $P(\text{perm})$ values. The smaller the number, the higher the significance with the highest significance being 0.0001.

Source	df	MS	$P(\text{perm})$
Mo	3	2447.5	0.0004
Sh	1	36166	0.0001
Po	2	41442	0.0001
MoxSh	3	1663.5	0.0119
MoxPo	6	760.72	0.5693
ShxPo	2	3456.6	0.0001
MoxShxPo	6	1376.4	0.0157

Table 4.2: Diversity of Sessile Marine Biota observed on the shipwrecks.

Phylum	Class	Family	Genus	Species
Chlorophyta	Ulvophyceae	Dasycladaceae	Dasycladus	vermicularis
		Halimedaceae	Flabellia	petiolata
		Polyphysaceae	Acetabularia	acetabulum (Linnaeus)
Ochrophyta	Phaeophyceae	Dictyotaceae	Dictyopteris Dictyota	sp. dichotoma var. intricate (C. Agardh) Greville, 1830
Ochrophyta	Phaeophyceae	Dictyotaceae	Padina Dictyota	pavonica implexa
			Sargassum	sp.
		Sargassaceae	Sporochnus	pedunculatus
		Sporochnaceae		sp.
Prasinodermatophyta	Palmophyllophyceae	Palmophyllaceae	Palmophyllum	crassum
Rhodophyta	Florideophyceae	Mesophyllumaceae	Mesophyllum	lichenoides
Mollusca	Cephalopoda Gastropoda	Loliginidae	Loligo	vulgaris (eggs)
		Cerithiidae		sp.
		Facelinidae	Cratena	peregrina
Annelida	Polychaeta	Amphinomidae	Hermodice	carunculata
		Sabellidae	Sabella	spallanzanii
		Terebellidae		sp.
Porifera	Demospongiae	Agelasidae	Agelas	oroides
		Crambeidae	Crambe	crambe
		Hymedesmiidae	Phorbas	tenacior
		Irciniidae	Sarcotragus	spinulosus
		Irciniidae	Sarcotragus	fasciculatus
		Spongiidae	Spongia	(Spongia) officinalis
Cnidaria	Hexacorallia	Cladocoridae	Cladocora	caespitosa
Echinodermata	Asteroidea	Echinasteridae	Echinaster	(Echinaster) sepositus

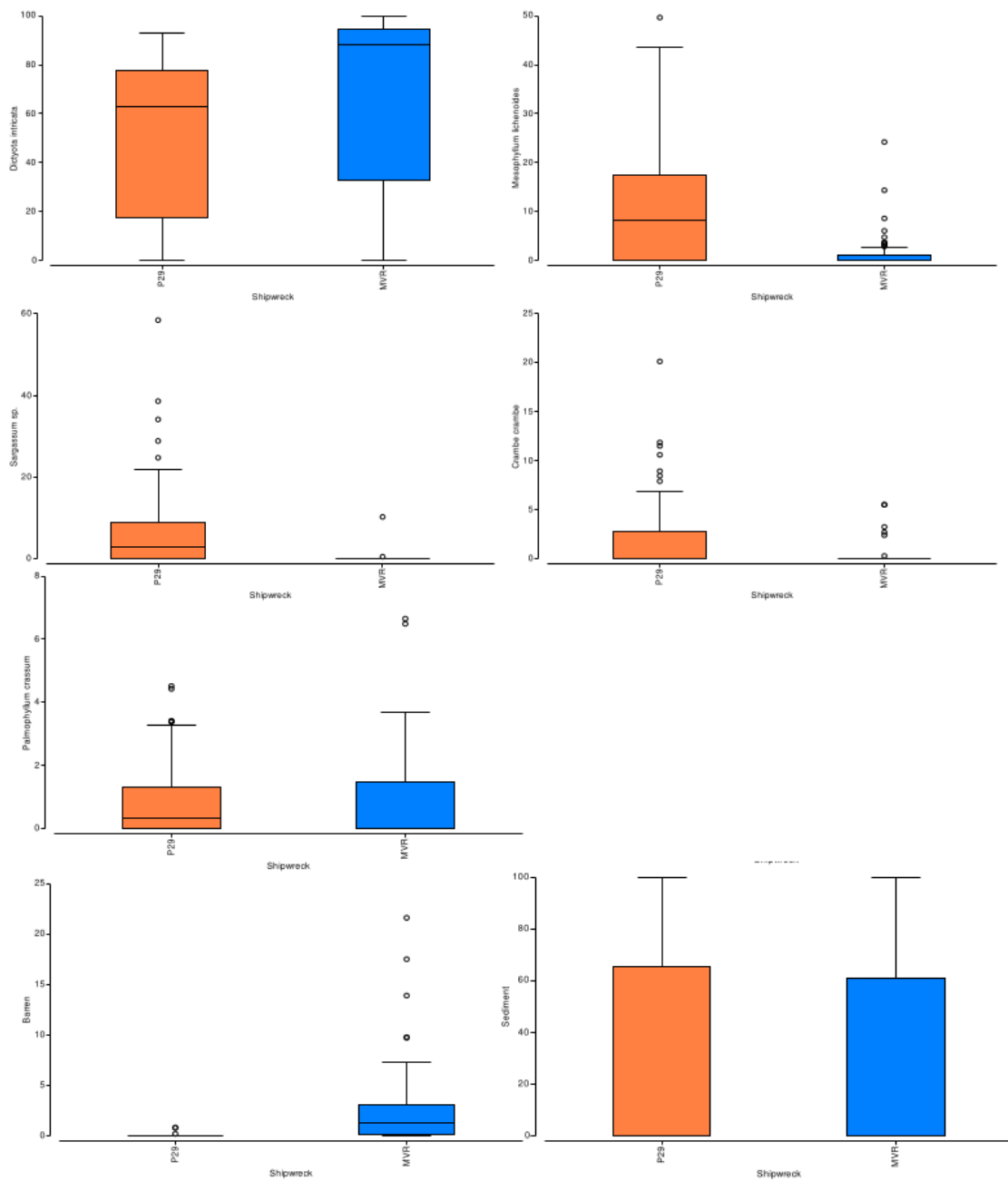


Figure 4.2: Boxplots illustrating the abundance in percentage of different species on the two shipwrecks — P29 and MV Rozi — with the help of a SIMPER analysis.

4.2.3 | Pairwise

With the help of the pairwise PERMANOVA factors between the sites are compared to find significant differences between them. Looking at table 4.3 there seems to be a significant difference between the P29 and the MV Rozi on all three different sides of the wrecks ('starboard', 'deck', 'port').

Table 4.3: Showing the results of the pairwise PERMANOVA test with focus on the factor 'Shipwreck' comparing different factors for the P29 and the MV Rozi (MVR) in term of the shipwreck paired with position ('ShxPo'). First for the starboard side of the wrecks, then the deck and lastly the port side. Significance is shown with the help of P(perm) values. The smaller the number, the higher the significance with the highest significance being 0.0001.

Term 'ShxPo' for pairs of levels of factor 'Position'			
(i) Within level 'Starboard' of factor 'Position'			
Groups	t	P(perm)	Perms
P29, MVR	5.58	0.0001	9938
(ii) Within level 'Deck' of factor 'Position'			
Groups	t	P(perm)	Perms
P29, MVR	3.2718	0.0001	9953
(iii) Within level 'Port' of factor 'Position'			
Groups	t	P(perm)	Perms
P29, MVR	4.3553	0.0001	9947

In table 4.4 the different sides of the shipwrecks are compared within the same wreck, with results showing significant differences between each side of the P29 as well as the MV Rozi.

When looking at changes during seasons, one needs to compare the different months of the survey with each other. This has been done in table 4.5, where each of the four months are compared with each other to see if there is a difference between any of them. The results suggest that there is a significant difference between the months of May and August, but none between any of the other months.

Table 4.4: Showing the results of the pairwise PERMANOVA test with focus on the factor 'Position' comparing different factors for the P29 and the MV Rozi (MVR) in term of the shipwreck paired with position ('ShxPo'). First for the P29 then for the MV Rozi comparing the starboard and the deck, the starboard and the port and the deck and the port. Significance is shown with the help of P(perm) values. The smaller the number, the higher the significance with the highest significance being 0.0001.

Term: ShxPo			
(i) Within level 'P29' of factor 'Shipwreck'			
Groups	t	P(perm)	Perms
Starboard, Deck	7.8818	0.0001	9961
Starboard, Port	3.1024	0.0001	9946
Deck, Port	7.0302	0.0001	9957
(ii) Within level 'MVR' of factor 'Shipwreck'			
Groups	t	P(perm)	Perms
Starboard, Deck	4.9594	0.0001	9940
Starboard, Port	2.0783	0.0003	9953
Deck, Port	4.2187	0.0001	9959

Table 4.5: Showing the results of the PERMANOVA test in regards of the factors 'month' and differences between these. Significance is shown with the help of P(perm) values. The smaller the number, the higher the significance with the highest significance being 0.0001.

Groups	t	P(perm)	Perms
May, Jun	1.1933	0.2329	9950
May, Jul	1.7472	0.0107	9964
May, Aug	2.7446	0.0001	9946
Jun, Jul	0.89637	0.5694	9941
Jun, Aug	1.9441	0.0044	9964
Jul, Aug	1.3526	0.1222	9955

The metric MDS graph (see figure 4.3) shows the similarities and differences between the positions on the P29 and MV Rozi shipwrecks. The axes are labelled as MDS1 and MDS2, which represent the two main dimensions of variation in the data. Triangles represent the starboard side; squares represent the deck, and circles represent the port side. Empty symbols stand for the P29, filled ones the MV Rozi, each of the symbols stand for a photoquadrat taken – this accounts for figure 4.3, 4.4 and 4.5. Average values are represented in black. The different positions on the shipwreck show distinct groupings, indicating a significant difference in species composition between the positions (starboard, deck, port) and between the two wrecks.

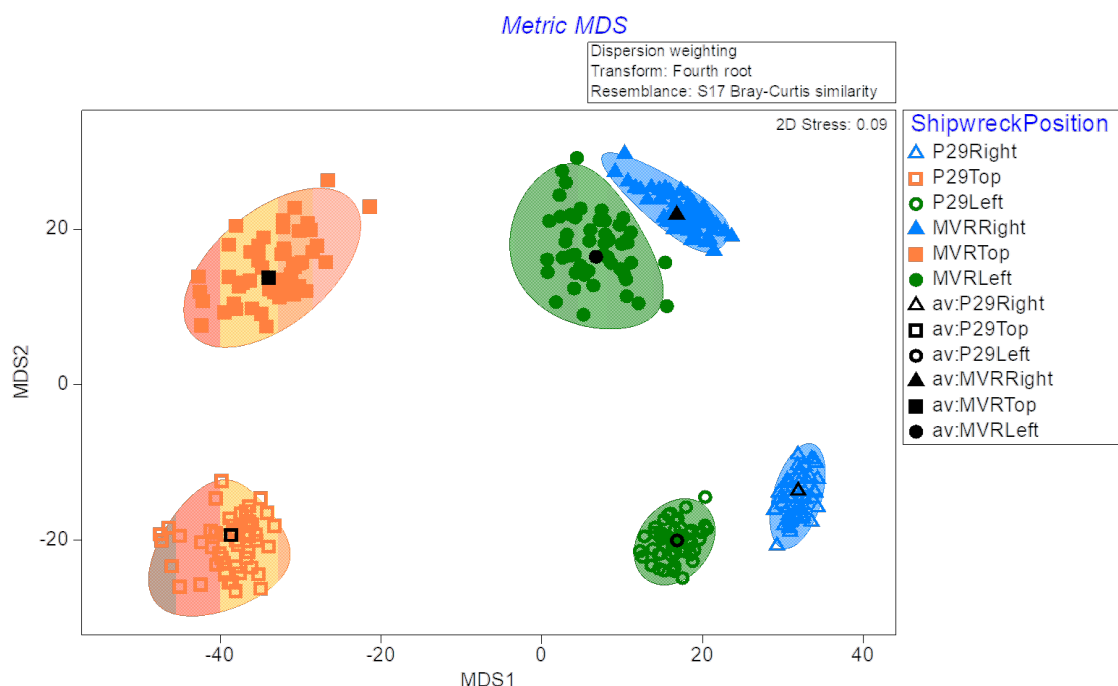


Figure 4.3: Metric Multidimensional Scaling (MDS) Plot. This plot shows the differences in species assemblages between the various positions on the P29 and MV Rozi shipwrecks. The stress value of 0.09 indicates a good representation of the data in two dimensions. Symbols for P29 are hollow, and for MV Rozi are filled. The starboard side is represented by blue triangles, the port side by orange squares, and the deck by green circles. Black symbols indicate the average values for each position. The clear separation of clusters indicates significant differences in species composition between the positions and between the two wrecks.

The ellipses around clusters of points indicate groupings or similarities among the data points. Each grouping represents similar ecological conditions or species assemblages. The stress value of 0.09 suggests that the two-dimensional representation is a

good fit and accurately shows the data's variation. The two-dimensional stress values of 0.18 suggests a fair representation of the data in two dimensions. The non-metric MDS plot (see Figure 4.4) provides a visual representation of the differences on species assemblages at various positions on the P29 and the MV Rozi shipwrecks. The plot shows distinct clustering of symbols based on the position and shipwreck. This indicates a significant difference in benthic biota between the starboard, port and deck sides of the wrecks, as well as between the two shipwrecks. The starboard sides of the wrecks (blue triangles) are distinctly separated from the port sides (orange squares) and decks (green circles). Furthermore, the fact that the data from the P29 (hollow) and MV Rozi (filled) cluster together indicates that the shipwrecks themselves, influence the assemblages of species.

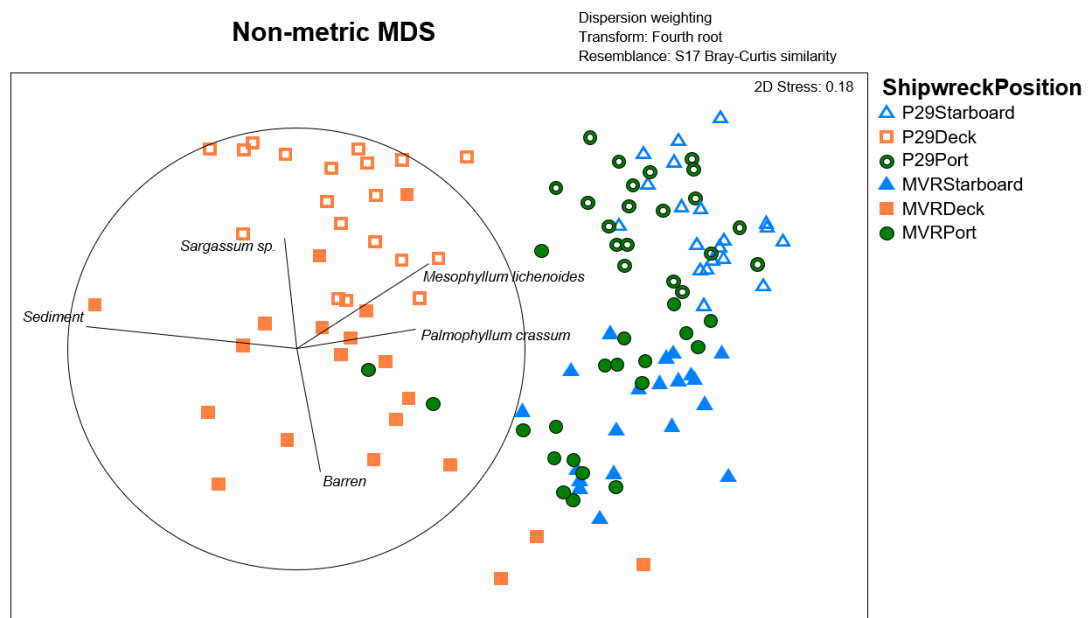


Figure 4.4: Non-metric MDS Plot. This plot illustrates the differences in species assemblages across the various positions on the P29 and MV Rozi shipwrecks, based on benthic organisms influenced by the factor 'ShipwreckPosition'. The 2D stress value of 0.18 suggests a fair representation of the data in two dimensions. Symbols for P29 are hollow, while symbols for MV Rozi are filled. Blue triangles represent the starboard side, orange squares represent the port side, and green circles represent the deck. The distinct clustering of symbols indicates significant differences in species composition between the shipwrecks and their respective positions.

4.2.4 | Principal Coordinates Ordination (PCO) analysis

From the results of the PCO analysis, we can see how the significant ecological variation is explained by the coordinates. PCO1 — which is the first principal coordinate — captures 42.2% of the variation, while PCO2 captures 22.6%. Combined, these two explain about 70% of the variation (see table 4.6).

Table 4.6: Principal Coordinates Ordination (PCO) components indicating the Eigenvalue, their contribution in explaining the assemblage variance, and the cumulative contribution.

Axis	Eigenvalue	Individual %	Cumulative %
1	96757	42.24	42.24
2	51731	22.58	64.83
3	28634	12.50	77.33
4	17812	7.78	85.10
5	15173	6.62	91.73

The scatterplot seen in figure 4.5 demonstrates the three different positions on the wrecks. Species on the starboard side (blue triangles) and deck (orange squares) are distinctly positioned, indicating unique ecological characteristics or environmental conditions in these areas. The green circles (port side) show varied distribution, suggesting differences in species composition or abundance along this section of the wrecks.

4.2.5 | PERMANOVA diversity

The PERMANOVA table of results as shown below (table 4.7) indicates a strong relation between species diversity and the factors shipwreck and position, with a highly significant p value of 0.0001. This suggests that the diversity of species is significantly influenced by both the shipwreck and its specific positions.

In contrast, the relation between different months and diversity shows a weaker significance, with a p-value of 0.021, indicating that temporal variation does have some impact, albeit less pronounced. With a p-value of 0.0001, shipwreck type is a significant factor influencing species diversity. The same can be said about position along the wreck with a p-value of 0.0001, indicating that the location on the wreck (starboard, port, deck) significantly affects the species diversity.

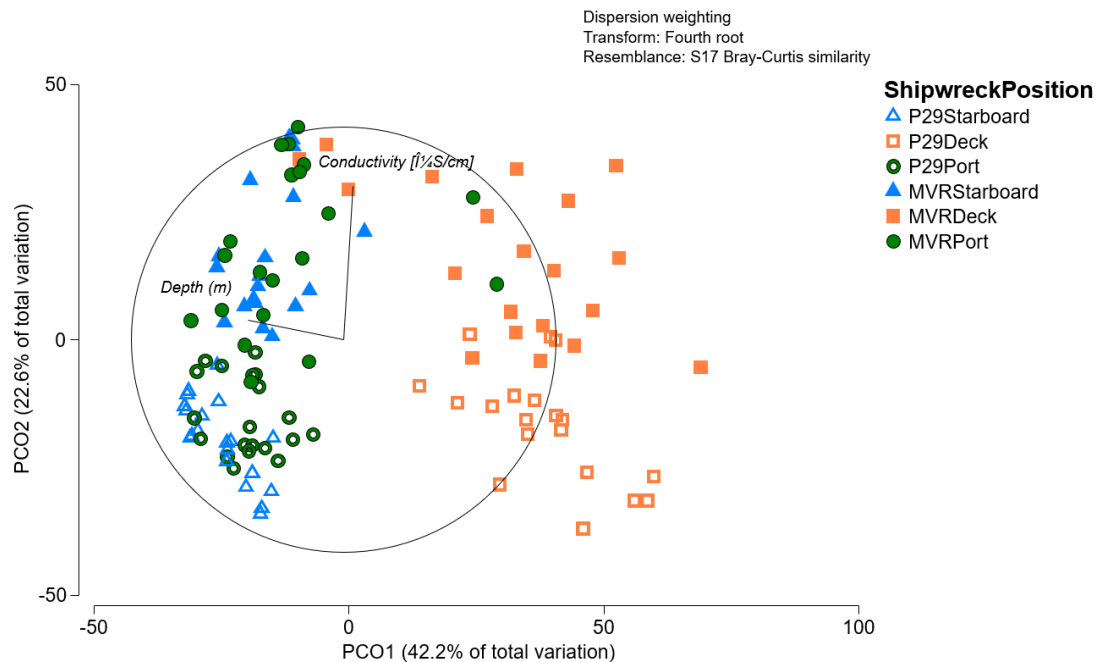


Figure 4.5: Principal Coordinates Ordination (PCO) for all photo-quadrates taken on the P29 and the MV Rozi and the three different positions, deck, starboard and port. Vectors overlay for depth and conductivity.

With a p-value of 0.21 the factor month suggests some seasonal variation in species diversity, though it seems to be less impactful compared to the other two factors.

With a p-value of 0.08 the combined factors month and shipwreck (MoxSh) are not significant, indicating that these two together do not influence species diversity. On the other hand, month and position (Mo x Po) is significant (p-value of 0.018), suggesting that seasonal changes interact with specific positions on the wrecks.

Looking at all three factors together (Mo x Sh x Po) there seems to be no combined effect of all three factors on species diversity (p-value of 0.279).

The pairwise test results for the species diversity in the four-month time period (see table 4.8) suggest that while there are some seasonal variations in species diversity, particularly between May and August (p-value of 0.006), the differences between other months are less pronounced. This information could be valuable for understanding the temporal dynamics of species abundance on the shipwrecks and may guide further ecological studies or conservation efforts.

Further, a pairwise PERMANOVA test was calculated to examine the relation between species diversity, the shipwreck, and the position on the shipwrecks. Reading the results in table 4.9, focusing on the factor 'shipwreck' in combination with position

Table 4.7: Showing the PERMANOVA table of results which categorises the differences in diversity in relation to the factors month 'Mo', shipwreck 'Sh', and position 'Po' and the combinations of such. Significance is shown with the help of P(perm) values. The smaller the number, the higher the significance with the highest significance being 0.0001.

Source	df	SS	MS	Pseudo-F	P(perm)	Perms
Mo	3	10.264	3.4213	2.634	0.021	999
Sh	1	34.879	34.879	26.853	0.001	999
Po	2	22.069	11.035	8.4953	0.001	999
MoxSh	3	7.5308	2.5103	1.9326	0.080	999
MoxPo	6	17.342	2.8904	2.2252	0.018	998
ShxPo	2	11.742	5.8712	4.5202	0.005	999
MoxShxPo	6	9.4793	1.5799	1.2163	0.279	998
Res	96	124.69	1.2989			
Total	119	238				

Table 4.8: Pairwise test showing the relation between the factor month 'Mo' in regard to species diversity. Each of the surveyed months is compared with each other. Significance is shown with the help of P(perm) values. The smaller the number, the higher the significance, with the highest significance being 0.0001.

Term: Mo			
Groups	t	P(perm)	Perms
May, Jun	1.3576	0.170	999
May, Jul	1.5714	0.085	999
May, Aug	2.589	0.006	999
Jun, Jul	0.35657	0.889	999
Jun, Aug	1.4603	0.125	998
Jul, Aug	1.384	0.162	999

(‘ShxPo’) for the P29 and the MV Rozi (MVR). When comparing the deck, no significant difference in species diversity was observed here, with a p-value of 0.62. This indicates that species composition on the decks of the wrecks are quite similar. The starboard and port side of the wrecks on the other hand, show a high significance and therefore a strong difference in species diversity — both with p-values of 0.0001. This suggests that the ecological conditions or species settlement patterns differ notably between these sides on the two wrecks.

Table 4.9: Pairwise PERMANOVA test results showing the relation of species diversity with the factor ‘Shipwreck’ comparing different positions (starboard, deck, port) for P29 and MV Rozi (MVR). Significance is denoted by P(perm) values, with smaller numbers indicating higher significance, the highest being 0.0001.

Term: ShxPo			
(i) Within level ‘Starboard’ of factor ‘Position’			
Groups	t	P(perm)	Perms
P29, MVR	4.5987	0.001	998
(ii) Within level ‘Deck’ of factor ‘Position’			
Groups	t	P(perm)	Perms
P29, MVR	0.66109	0.62	999
(iii) Within level ‘Port’ of factor ‘Position’			
Groups	t	P(perm)	Perms
P29, MVR	4.0421	0.001	999

The high significance for the starboard and port side comparisons indicates that these areas on the shipwrecks have different species assemblages. This information could be critical for understanding how different parts of the shipwreck environment influence marine biodiversity and can inform targeted conservation efforts.

4.2.6 | S and H’

Alpha diversity is indicated with S values, the Shannon index as H’ values. Species assemblages appear to have higher S and H’ values on the P29 shipwreck than on the

MV Rozi shipwreck, whereas both factors get higher into the summer, indicating a rise in species presence (see figure 4.6). Species richness (S) seems to be higher on the starboard side on both shipwrecks, as can be seen in Figure 4.7, whereas the deck has a lower value, indicating a more homogenous species abundance.

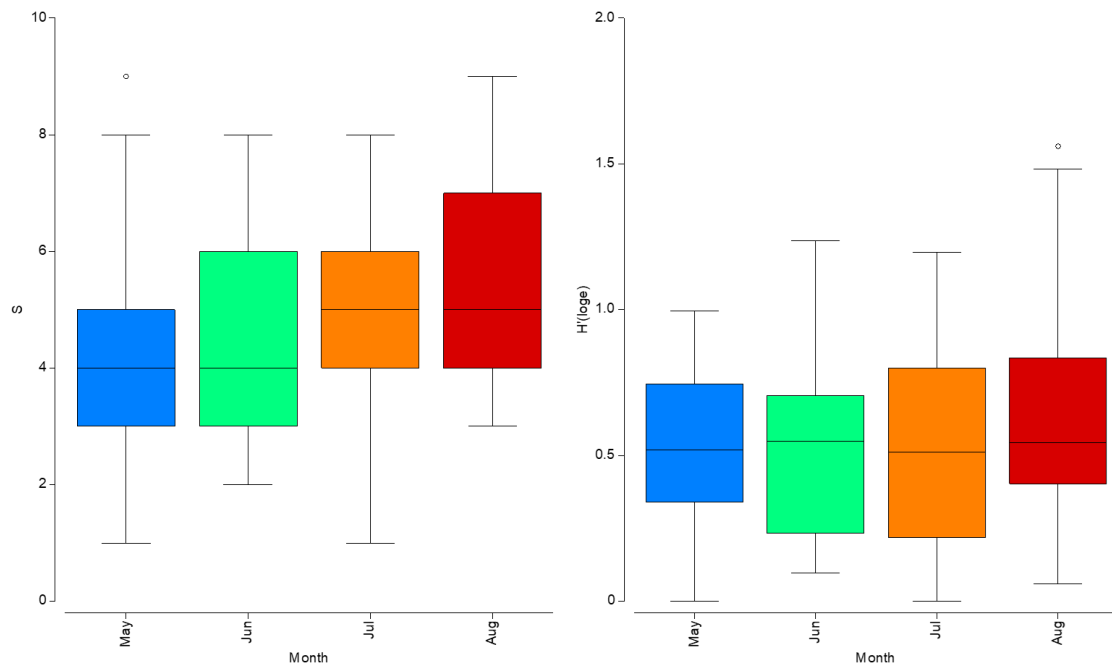


Figure 4.6: Boxplots illustrating the diversity metrics of species richness (S) and Shannon diversity index (H') for the P29 (left) and the MV Rozi (right) from May till August.

4.2.7 | Environmental data

As can be seen in the boxplot in figure 4.8, there is a wider range — between 29 to 33m — of depth on the deck of the P29, whereas the change in depth on the deck position of the MV Rozi is more consistent. The starboard and port side of the P29 have a similar depth profile, the starboard side of the MV Rozi on the other hand is deeper than the port side. This indicates that the bow of the P29 lies deeper, whereas the starboard side of the MV Rozi lies deeper. This would indicate a difference in orientation and position of the shipwrecks on the sandy bottom.

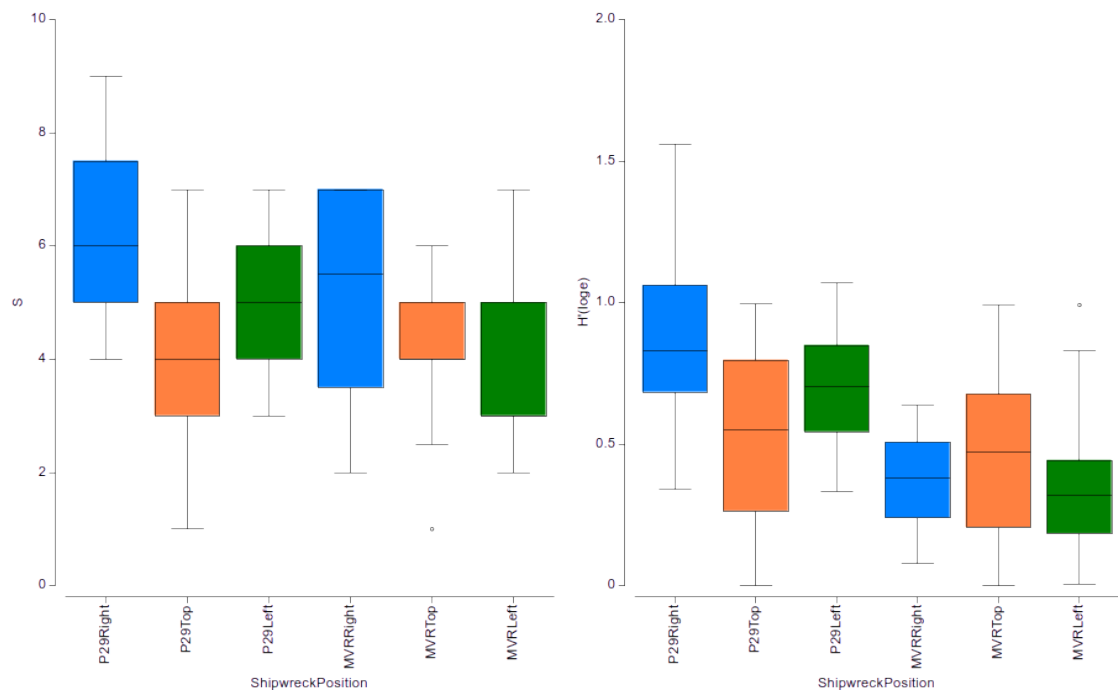


Figure 4.7: Boxplots illustrating the diversity metrics of species richness (S) and Shannon diversity index (H') for the different positions on the P29 and the MV Rozi.

4.3 | Hydro chemical data

The data loggers were in the water from 12/05/2024 to 25/08/2024. Values from the first and last days were deleted as placing and retrieving them would lead to false values. Each of the three loggers per wreck collected data hourly. These data points were summarised as daily averages to make the graphs more readable. Per hydro chemical value one graph was created, showing the timeline of both wrecks retrospectively. Data was analysed with MATLAB and OCTAVE.

4.3.1 | Temperature

Temperature measurements from each logger type were averaged and are visible in Figure 4.9. The temperatures on the MV Rozi and the P29 did not differ significantly over the measured period. The water temperature remained relatively cool during May and June, not exceeding 18°C. A noticeable increase occurred at the end of May, reaching up to 18.5°C, similar to July temperatures. In July, the maximum temperature was around 19°C, followed by a decline to 17°C around the 8th of the month. After this dip, there

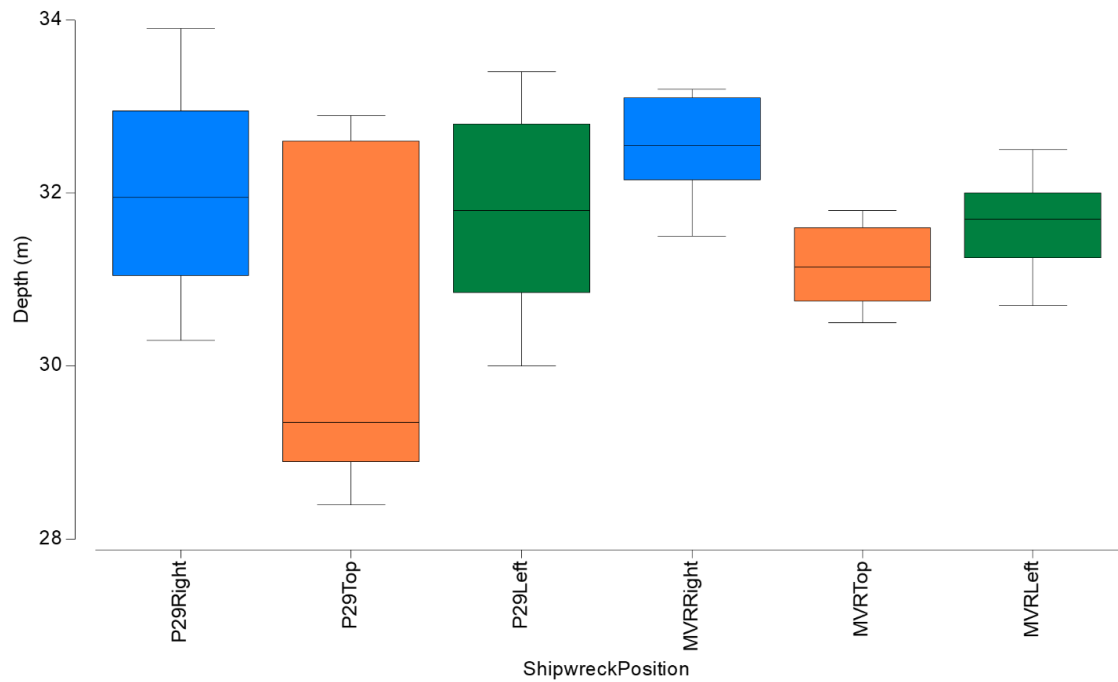


Figure 4.8: Boxplot illustrating the relationship between the position of the wrecks regarding to depth (m).

was a steady rise, with the average temperature exceeding 19.5°C by the end of August. This pattern suggests a stable thermocline was in place in shallower waters during the sampling period, typically descending into deeper waters in summer.

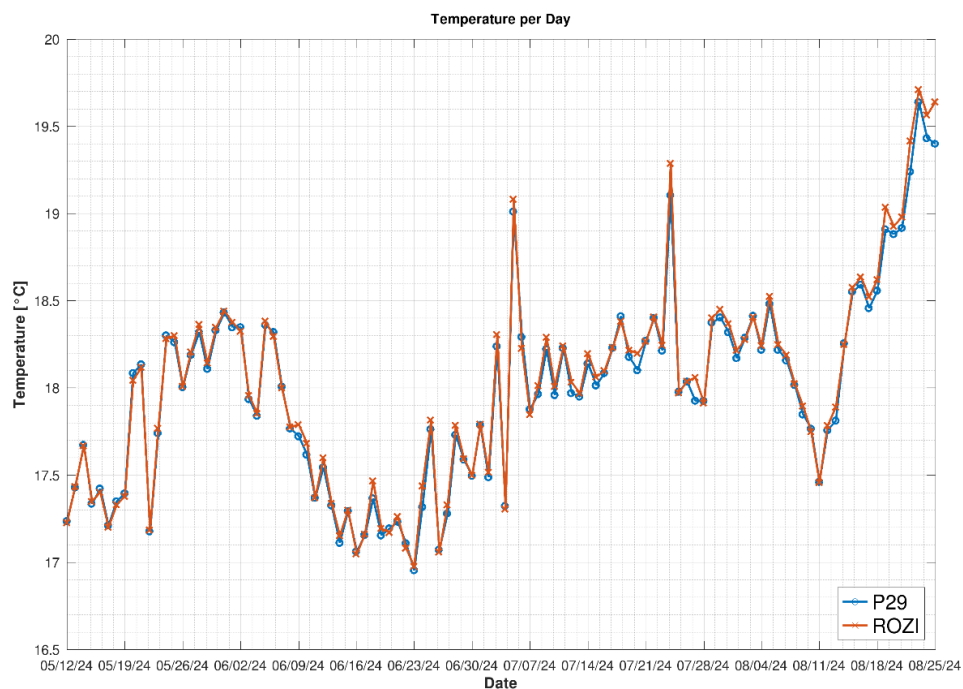


Figure 4.9: This line graph illustrates daily temperature measurements from two loggers, P29 and MV Rozi, from 12th May 2024 to 25th August 2024. The x-axis represents the dates, and the y-axis represents the temperature in degrees Celsius (°C). The graph features two data series: P29 in blue, MV Rozi in orange.

4.3.2 | pH

Shown in figure 4.10 are the average pH values per day over the measured period from the 12th May until the 25th August 2024. The lowest value did not fall below 8.05 whereas the highest value was measured between 8.2 and 8.25. When looking at the timeline of the pH values per day one can see a slight drop between the middle of May until the end of June. After this period the pH values appear to rise slightly but steadily until they reach the highest values at the end of the measured period at the end of August. Both sites — the P29 and the MV Rozi — show similar patterns, with the values measured at the MV Rozi slightly higher — meaning less acidic — than the ones at the P29. During the whole period, the values never showed the same value at the same time, indicating a possible difference in hydro chemical data — or, as the trend lines are very similar and just differ in a small fraction — a calibration error in the beginning of the measurement.

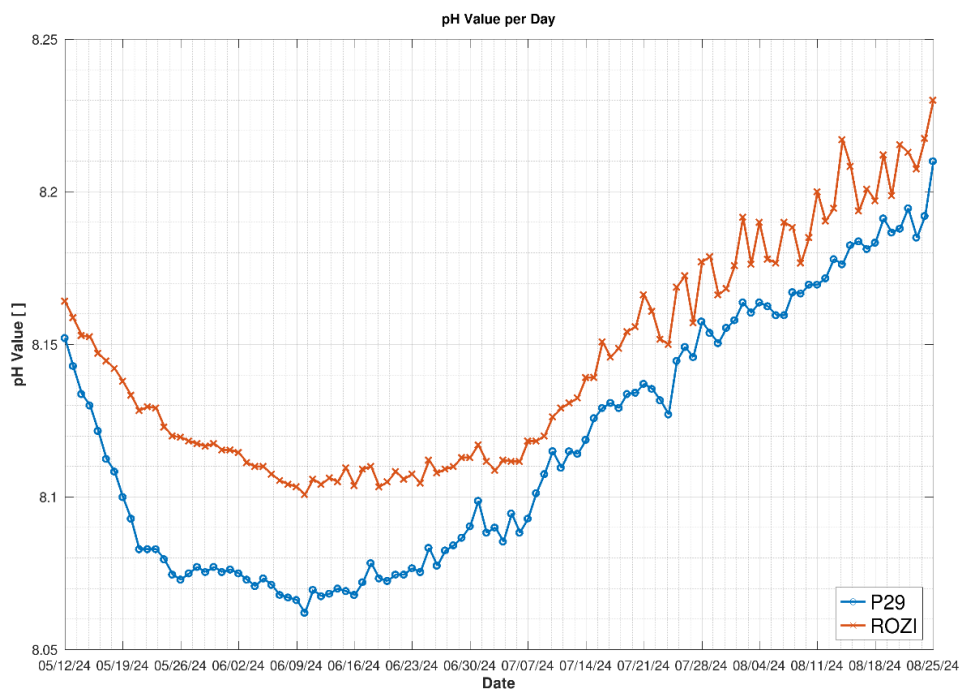


Figure 4.10: This line graph presents the average daily pH values from 12th May 2024 to 25th August 2024. The x-axis represents the dates, and the y-axis represents the pH values. The graph features two data series: P29 in blue, MV Rozi in orange.

4.3.3 | Dissolved oxygen

Values for dissolved oxygen (DO) concentration per day over the test period can be seen in figure 4.11. Best comparability between the two sites appears to be from the 12th May until the 16th June, with values slightly fluctuating between 8 and 10 mg/L with just minor differences between the two shipwrecks. Figures appear to be near enough identical during this period, with a consistent variation in values of about 0.2 mg/L. This could be accounted for, by a calibration error upon initial setup, or as a minor difference between the study sites.

Measurements on the P29 shipwreck show unusual values in what appears to be random points throughout the research, these appear to be anomalies within the dataset, firstly around the 20th June. It would be highly unlikely that the DO concentration drops by up to 6 mg/L within a couple of days around this time and in multiple other points thereafter — for example in the middle of July and beginning of August. From the 11th August onwards there seem to also be high fluctuations in the DO concentration

values measured around the MV Rozi.

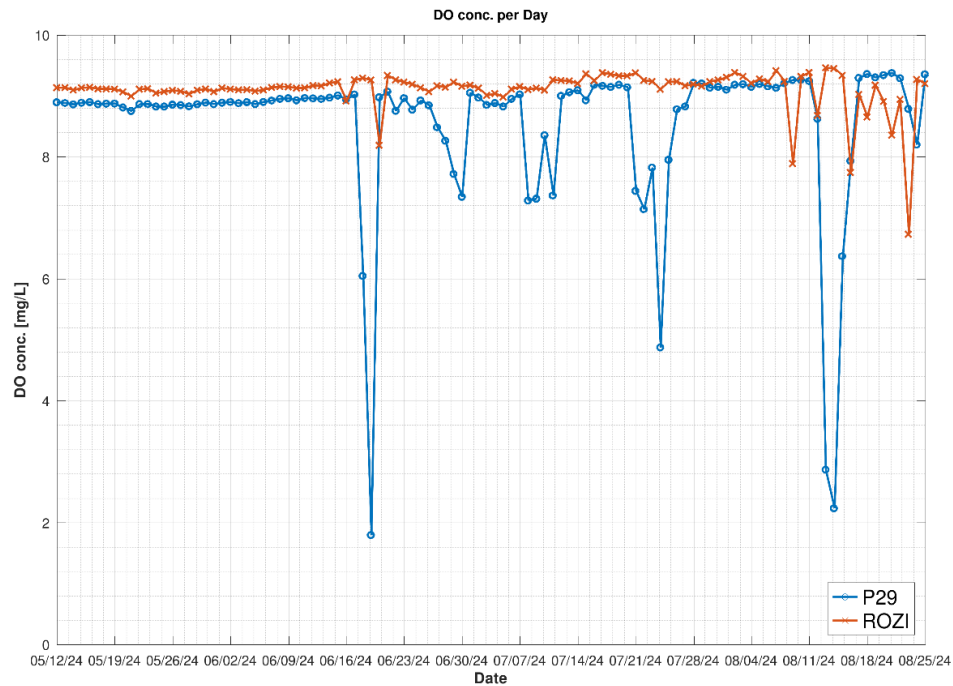


Figure 4.11: This line graph presents the average daily dissolved oxygen (DO) concentration values from 12th May 2024 to 25th August 2024. The x-axis represents the dates, and the y-axis represents the DO concentration in mg/l. The graph features two data series: P29 in blue, MV Rozi in orange.

4.3.4 | Salinity

The salinity was calculated with the help of the conductivity values using empirical relationships or formulas that account for the temperature, conductivity and sometimes pressure of the water sample. Most commonly used in the scientific community is a method based on the Particle Salinity Scale 1978 (PSS-78), which defines salinity in terms of the conductivity ratio of the sample to a standard solution of potassium chloride (KCl) (Dauphinée et al., 1981). The PSS-78 is used for oceanic salinity measurements and is suitable for conductivity ranges typically found in seawater.

The used formula is shown in equation 4.1

$$S = a_0 + a_1 R^{\frac{1}{2}} + a_2 R + a_3 R^{\frac{3}{2}} + a_4 R^2 + a_5 R^{\frac{5}{2}} \quad (4.1)$$

Where:

- $R = \frac{C}{C_{KCl}}$ is the conductivity ratio
- C : conductivity of water at 15°C
- C_{KCl} : conductivity of standard seawater with salinity of 35 PSU at 15°C

The coefficients:

- $a_0 = 0.008$
- $a_1 = -0.1692$
- $a_2 = 25.3851$
- $a_3 = 14.0941$
- $a_4 = -7.0261$
- $a_5 = 2.7081$

As done for the other loggers, the timeline for salinity shows the average values per day throughout the period of measurement. When looking at figure 4.12 there seems to be an obvious difference between the PSU values of the two shipwrecks. Values for the P29 are constantly lower than those on the MV Rozi with the highest value measured in the very beginning of the period. The values throughout are showing a declining trend, with a significant change between the middle and end of June. After this drop in salinity, the values seem to stabilise again with the steady decline still present. Looking at the MV Rozi, the salinity seems to be more stable, with fluctuations between 27 and 37 PSU during the whole time. When comparing the PSU values of the MV Rozi to the P29 and its downward trend in salinity, one could interpret an upward trend starting at the end of July with a significant drop at the end of the sampling period.

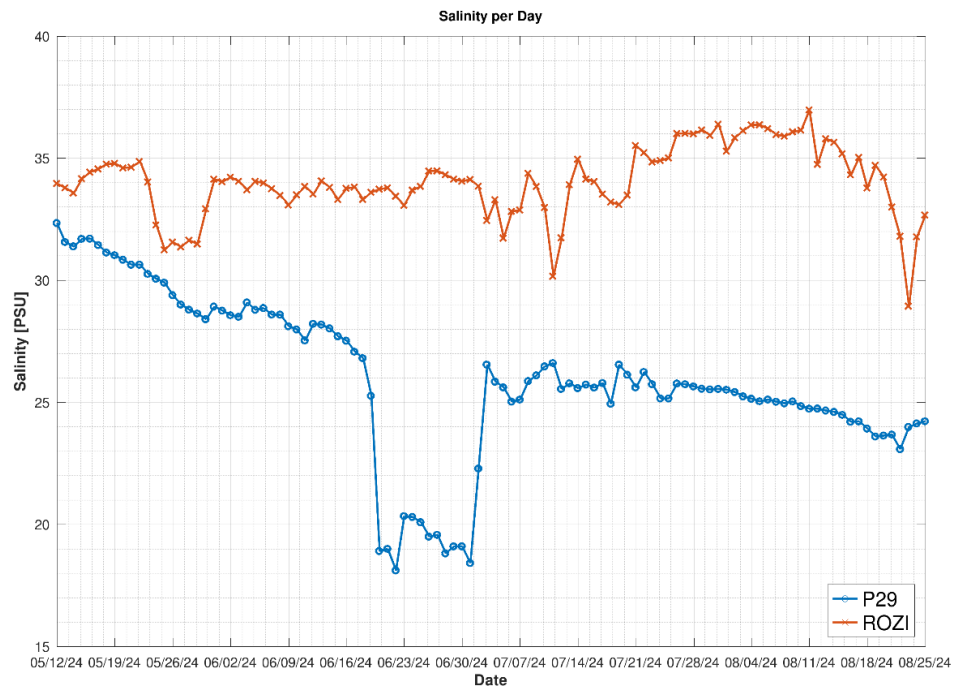


Figure 4.12: This line graph presents the average daily salinity values from 12th May 2024 to 25th August 2024. The x-axis represents the dates, and the y-axis represents the salinity values in PSU. The graph features two data series: P29 in blue, MV Rozi in orange.

4.4 | Model data

A comparison of current direction and magnitude across depth (1.02m, 10.54m, and 19.39m) can be seen in figure 4.13 for a yearly representation and figure 4.14 for the survey period. The radial directions (N, E, S, W) represent the direction from which the current is coming, bars represent the frequency of currents from a specific direction as a percentage of total observations. The colour gradient shows current speed (and therefore magnitude) in metres per second (m/s), with darker shades indicating higher velocities.

4.4.1 | Current model data for one year

The surface current observation (1.02m depth) has a maximum magnitude of 0.95 m/s with most currents originating from the west north-west (W, NW) with frequencies of about 10%. Currents on a depth of 10.54m are coming primarily from the west, with

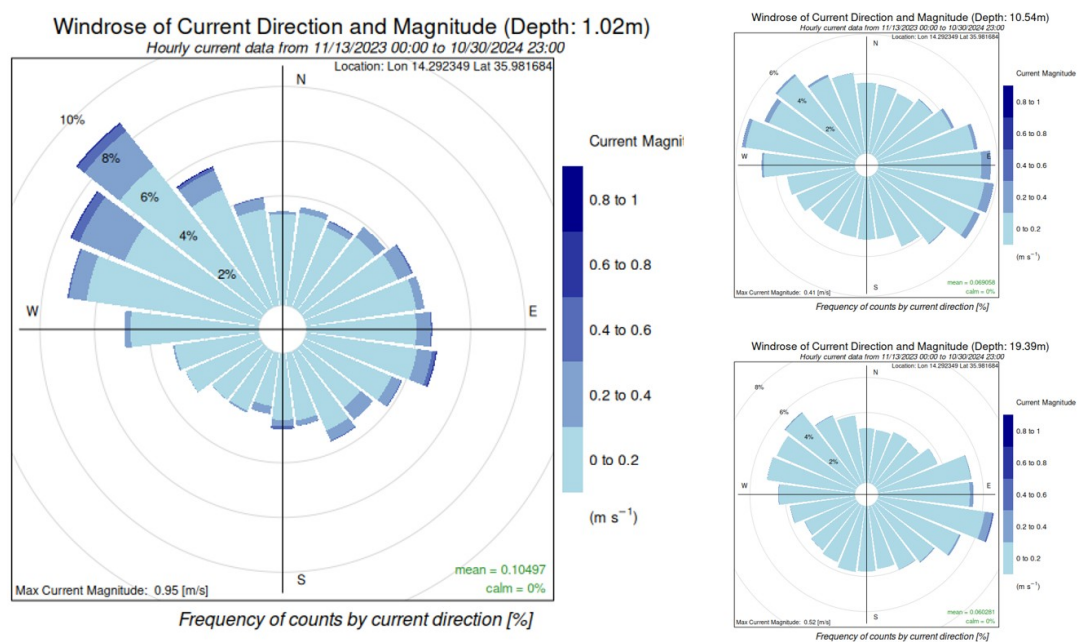


Figure 4.13: The figure presents windrose plots of ocean current direction and magnitude at 1.02m (left), 10.54m (top-right) and 19.39m (bottom-right). The data represents hourly observations and spans from 13th November 2023 to 30th October 2024 for a location at 35.981684° and longitude 14.292349°

similar directional distribution but slightly lower magnitude compared to the surface, the maximum current magnitude lies at 0.41 m/s . Deeper waters (19.39m) have a more even directional spread, mostly coming from north-west to south-east (SE), with a maximum magnitude of 0.59 m/s . Stronger currents are infrequent in all depths acquired.

4.4.2 | Current model data during survey period

Surface currents (1.02m) flow predominantly from the WNW direction, with the highest frequency of 10%, with a maximum current magnitude of 0.64 m/s . Deeper water layers in 10.54m depth show a difference in current direction, with a predominance coming from the SE with about 8% and a maximum current magnitude of 0.41 m/s . A similar pattern for current direction can be observed at a depth of 19.39m with predominate currents slightly more from east south-east (ESE), with the highest frequency of 7% and a maximum current magnitude of 0.48 m/s .

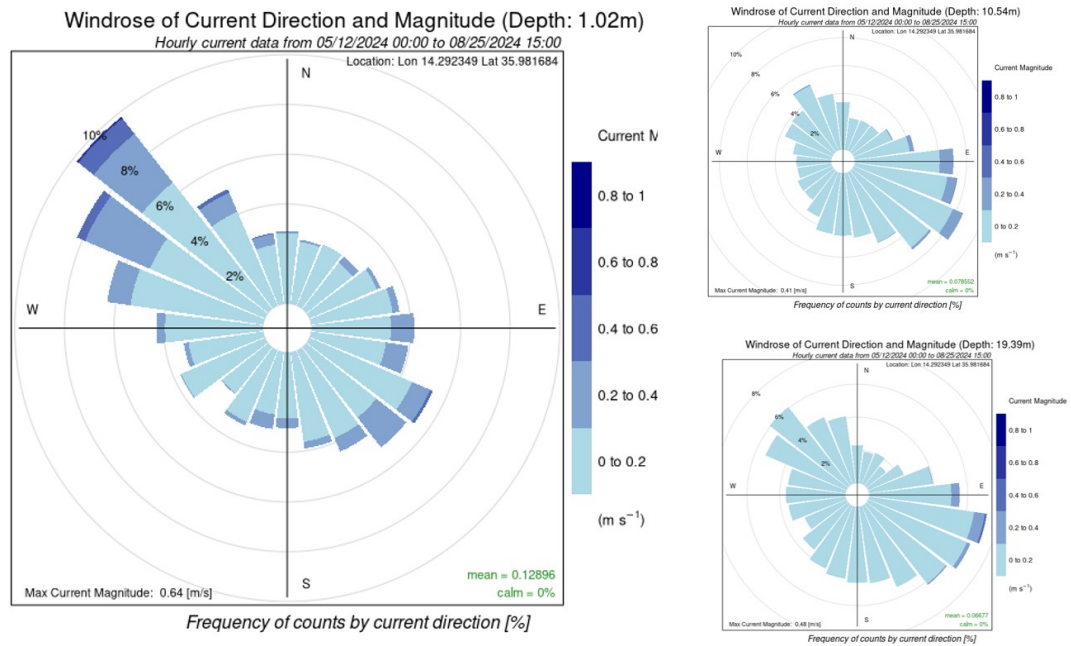


Figure 4.14: The figure presents Windrose plots of ocean current direction and magnitude at 1.02m (left), 10.54m (top-right) and 19.39m (bottom-right). The data represents hourly observations and spans from 12th May to 25th August 2024, for a location at 35.981684° and longitude 14.292349°

4.5 | Temperature mean per day

Daily average temperatures for modelled and measured data is compared in figure 4.15 over the period from mid-May 2024 to mid-August 2024. It includes data from simulated temperature from the surface, 10m depth, and 20m depth, as well as the measured temperatures from the P29 and the MV Rozi. The simulated surface temperature shows the highest variability and peaks, as expected due to direct interaction with the atmosphere. Temperatures in 10m and 20m depth are more stable with less fluctuation and lower average temperatures compared to the surface. Seasonal changes on temperatures in all depth and data can be clearly seen with a peak around early July reaching the highest values (close to 28°C). The temperatures measured with the temperature loggers on the P29 and the MV Rozi are generally stable during the study period but much lower than simulated temperatures, staying consistently between 17-19°C, indicating the thermocline still being shallower than the shipwrecks, which both lie on sandy bottom at around 30-34 metres. A noticeable sharp dip is observed in all temperature curves around early July. This might be due to heavy rainfall or even an upwelling event, or the thermocline getting slightly shallower again.

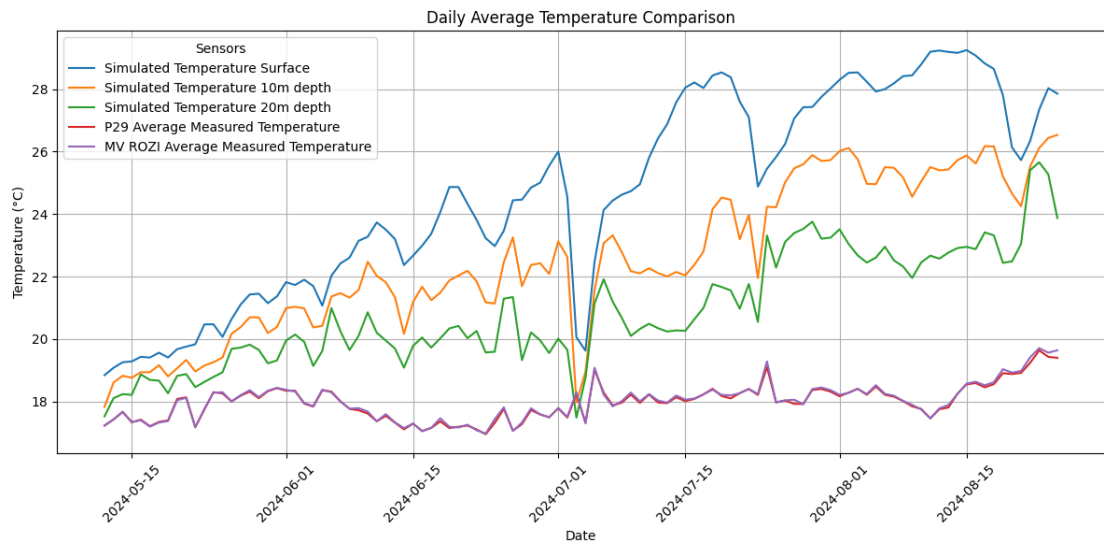


Figure 4.15: The line graph shows daily average Temperature from simulated and measured data over a period from 12th May 2024 to 25th August 2024. Simulated Temperature Surface (blue line), 10m depth (orange line), 20m depth (green line), P29 (red line), MV Rozi (purple line).

Discussion

The aim of this study was to assess the current state and differences in benthic biodiversity between the MV Rozi and the P29, two shipwrecks located in Ćirkewwa Marine Park. By exploring vessel-specific factors and oceanographic conditions, the study aimed to identify potential drivers of these differences. Data was collected via SCUBA diving, and surveys reveal notable patterns in species distribution and diversity, influenced by structural and environmental factors.

5.1 | Species Distribution and Biodiversity

Both shipwrecks show substantial benthic growth, with a wide range of species identified. However, distinct differences were obvious when looking into species composition underscoring the influence of local environmental conditions and structural characteristics on biodiversity patterns. Species such as the endemic coral *C. caespitosa*, were exclusively found on the MV Rozi, likely due to specific environmental conditions required for their growth. As corals are generally slow-growing and sensitive to environmental changes, the MV Rozi may provide a more stable and favourable habitat for these organisms. In contrast, the absence of certain non-sessile species, such as the nudibranch *C. peregrina* on the P29, could be attributed to limited survey coverage rather than true species absence.

The brown algae *Dictyota dichotoma* var. *intricata*, was found extensively on both wrecks, demonstrating its adaptability to varying conditions, with near-total coverage on the MV Rozi. Despite higher species diversity on the MV Rozi, the P29 shows greater abundance for certain species, such as the encrusting algae *M. lichenoides* and the sponge *C. crambe*. These findings highlight the close interaction between species richness and abundance, suggesting that greater diversity may correspond with more competition for resources (such as light) and space.

Apart from differences, similarities were also observed for species like the green algae *P. crassum* and sediment-associated assemblages, primarily on the decks. The ac-

cumulation of sediments on horizontal surfaces likely hampers settlement and growth of sessile organisms, further demonstrating the role of horizontal surfaces in shaping biodiversity. The accumulation of sediments likely hampers the settlement and growth of sessile organisms, with implications for community structure and composition. The presence and dominance of species such as *Dictyota dichotoma* var. *intricata* and *Mesophyllum lichenoides*, which dominated both wrecks, aligns with observations by Blondel (2010) that these species are robust colonisers in oligotrophic environments while forming a critical component of the benthic community.

5.2 | Significance of structural and positional factors

Statistical analysis revealed significant differences between the two vessels and among the different positions on each wreck (e.g. port, starboard and deck; $p=0.0001$ in table 4.3 and 4.4). These findings emphasise the critical role of structural and positional factors in shaping the (sessile) benthic biodiversity.

The MV Rozi's enclosed side walls trap more sediment on its deck compared to the more open structure of the P29. This structural variation likely affects microhabitats, providing different conditions for species settlement and growth and may explain the greater species variability on the MV Rozi. The observed differences between the two wrecks align with studies demonstrating the influence of structural complexity on biodiversity. ARs with varied microhabitats often support a broader range of species by providing niches for both sessile and mobile organisms (Paxton et al., 2017). The larger size and higher complexity in structure of the P29 appear to offer more favourable conditions for colonisation and shelter for organisms, consistent with findings from Mallet and Pelletier (2014), who point out the role of structural features in shaping underwater communities and composition.

Depth profile (figure 4.8) and orientation of the wrecks (GPS figure 3.2) further contribute to biodiversity patterns. The MV Rozi has a small depth range, with the starboard side significantly deeper than the port, and is orientated south-west, with the propeller facing towards the reef. On the other hand, the P29 spans a greater depth range (29-33m) and faces directly westwards with its bow furthest away from the reef (see figures 4.8 and 3.2).

The differences in orientation and proximity to the reef may influence the exposure to currents and light, which are critical factors for benthic organisms. Port, starboard and deck positions show differences in species assemblages, findings that might underscore the influence of light availability and orientation relative to prevailing currents.

For example, areas with greater light exposure supported a higher prevalence of photosynthetic organisms, consistent with studies by Bohnsack and Sutherland (1985). Additionally, the presence of barren zones and rust on the MV Rozi suggests a less favourable substrate, possibly linked to its 32 years under water and therefore paint erosion, a concern also noted by Mallia (2022).

5.3 | Seasonality and environmental parameters

Seasonal variation in species assemblages appeared limited during the four-month study period. Even if seasonal differences were modest, they suggest an interaction between biological cycle and environmental conditions. For example, the significant increase in diversity indices from May to August could reflect seasonal recruitment patterns, as seen in other Mediterranean studies (Fabi et al., 2011). Extended research over at least one full year would be necessary to really capture more nuanced seasonal trends, particularly given gradual changes in temperature and light, which are key drivers of marine biodiversity. Even in this short-time study, differences between May and August were visible, with the thermocline still shallower than the wrecks (see table 4.5).

The hydro chemical data showed minimal variation between the sites in temperature, salinity, and dissolved oxygen. No major differences were observed over the study period suggesting that local hydro chemical conditions do not significantly constrain colonisation processes. The pH values on the MV Rozi show more (marginally) fluctuation, which could mean that more organisms use up more oxygen due to more photosynthesis and therefore respiration. This could lead to more daily and even seasonal fluctuation in the pH measured at the MV Rozi site, which can cause even a local and generalised increase or decrease in pH. Salinity data for the P29 showed some anomalies, likely due to equipment malfunction, or heavy storms around this time if heavy winds and rains bring more freshwater input and waves could cause upwelling from deeper waters. However, these lower salinity values near the P29 may reflect differences in micro-current patterns or sedimentation rates, which could influence nutrient dynamics and algal growth (Placenti et al., 2022). Longer surveys with reliable measurements are required to establish whether such parameters significantly influence biodiversity.

5.4 | Drivers of biodiversity differences

Principal coordinate analysis (PCO; figure 4.5) highlighted two main factors of variation. PCO1 (42.2%) likely represents the influence of illumination, as the positions on the wrecks are clearly separated from each other. PCO2 may reflect vessel-specific factors, as the MV Rozi and P29 cluster separately along the axis. The MV Rozi's greater variability in species assemblages may result from structural differences, illumination patterns and its significantly longer time underwater. In contrast, the P29's more isolated location and open structure contribute to more homogeneous, but bigger species assemblages.

Patterns of the clusters suggest that different parts of the shipwrecks are home to a variety of species assemblages. Another critical driver that influences life on the shipwrecks may be sedimentation. The MV Rozi's deck is surrounded by walls, so it retains more sediment compared to the open structure of the P29. Sedimentation may affect species settlements, as well as influencing nutrient availability, microhabitat structures and illumination. With closer proximity to the reef, more sediment settles around this vessel which might even make a transition or travel for species towards the MV Rozi easier and build a migration pathway. The P29 on the other hand sits more isolated on the sand bottom, farther away from the reef which might limit migration and colonisation opportunities towards the artificial reef.

Although both wrecks are heavily colonised, the P29's complexity regarding structure provides more microhabitats, possibly accounting for the higher abundance of some species found on both wrecks (see SIMPER analysis; figure 4.6).

This study also supports the hypothesis that shipwrecks can enhance local biodiversity by acting as ecological hotspots. Similar findings have been reported for scuttled wrecks in the Ligurian Sea, where artificial reefs have enhanced both biodiversity and local tourism (Firth et al., 2023). However, the observed variations between the P29 and MV Rozi emphasize the need for careful planning in artificial reef deployments, particularly regarding vessel preparation and site selection.

5.5 | Current dynamics

Windrose plots from model data indicate that the near-surface currents are stronger and more concentrated in direction, which could indicate currents that are driven by wind (with the main wind direction in Malta being predominantly NW (2DoorsMedia, 2022)).

The difference in predominant current direction noticed in the datasets at approxi-

mately 10 and 20 metres indicates the influence of subsurface currents as the direction of the currents change slightly compared to surface currents, especially during the study period. If the currents get even stronger at depth — which can only be speculated here — and picking up from SE, both wrecks are protected by the shore and the reef itself, meaning that the main currents might not have as much influence for the assemblages on the wrecks. Even though the P29 is farther away from the reef than the MV Rozi, a strong current from NNW would affect the starboard side of both wrecks.

While the direction does change, the current magnitudes also vary with depth. The surface has the highest maximum magnitude while the deeper layers have lower magnitudes. With the main wind direction in Malta being NW, this makes perfect sense, as wind influence reduces with depth.

To make a clear conclusion, given that most of the shipwrecks lay lower than 20 metres to a maximum depth of 37 metres, currents close to the wrecks need to be studied or sediment patterns investigated to see micro current patterns along the vessels. If the P29 should be more influenced by bottom currents, the currents would transport more nutrients, organisms and their larvae (Cowen & Sponaugle, 2009) to the vessel which has a positive effect in sessile organisms development and growth.

5.6 | Recommendations for future research

While this study succeeded in highlighting differences in biodiversity between the wrecks, further research is needed to identify underlying mechanisms.

Recommendation include:

- **Extended Study Period:** A year-long study would capture seasonal variation more effectively, especially changes in temperature, light and maybe even currents, providing a more comprehensive understanding of biodiversity trends.
- **Hydrodynamic Data collection:** measurements of water currents near the wrecks could clarify their role in sedimentation and nutrient distribution.
- **Surface and paint analysis:** Testing wreck surfaces for anti-fouling paint could determine its influence on species settlement. PQ not only along the starboard and port site but even along other structures might give deeper insights.
- **Sedimentology:** Investigating sediment composition near the wrecks would reveal its impact in sessile communities.

- **Comparative Analysis:** Comparing the benthic biota on the reefs to the assemblages on the wrecks to see if there is a difference in the reefs close to the shipwrecks as well.

5.7 | Technical improvements

To make future studies more successful, standardisation of photoquadrat technique is crucial. Mounting the camera to a fixed distance and angle would ensure consistent image quality. High-resolution cameras for overview and close-up images could improve species identification and accuracy. Efficiency in data collection is critical, given safety constraints associated with dives at these depths.

Conclusion

This study underscores the role of structural complexity, sedimentation patterns, and vessel orientation in shaping benthic biodiversity on artificial reefs. Through comparative analysis of the MV Rozi and P29 shipwrecks in Ćirkewwa Marine Park, significant differences in biodiversity were observed, linked to vessel-specific factors and environmental conditions. Notably, the P29's isolation and structural features fostered greater growth of fewer species, while the MV Rozi supported higher diversity but experienced stronger competition for space.

The primary aim of this study was to assess the current state and possible differences in sessile macroflora and macrofauna between the MV Rozi and the P29 in the Ćirkewwa Marine Park, while identifying the key drivers that influence these differences. The finding of this study clearly demonstrate that vessel-specific characteristics and local environmental conditions are main drivers in shaping the biodiversity of shipwrecks.

The initial objective was to systematically collect and analyse biodiversity data on both shipwrecks with the help of Self-Contained Underwater Breathing Apparatus (SCUBA) diving, at varying points throughout the summer, focusing on the current state of the benthic assemblages colonising both wrecks. During this investigation we completed four survey dives per shipwreck, taking 15 PQ on each survey. These enabled the identification of 26 different species including 12 species of algae, six species of sponges, three species of polychaetes, two species of molluscs and one coral species.

Secondly we wanted to compare the biodiversity data from the MV Rozi and the P29 to determine if there is an existing difference in terms of species richness, abundance and overall coverage. The data collected revealed that the MV Rozi supports higher species diversity, with eight taxa only found on this vessel, whereas three unique species were found on the P29 but not on the MV Rozi. Furthermore, the data showed that the abundance and coverage of organisms was way higher on the P29 shipwreck. In general, most species were identified on both vessels with variations regarding the positions on the wrecks themselves.

The last objective was to investigate how vessel-specific factors (such as structure

and distance to the reef) and oceanographic data (such as temperature, salinity, pH and dissolved oxygen) influence biodiversity assemblage differences between the P29 and MV Rozi shipwreck. The data collected revealed that the MV Rozi supports higher species diversity, likely due to its longer time under water and proximity to the reef, which promotes colonisation. In contrast, the P29 presents greater abundance of certain species, such as the sponge *C.crambe*, highlighting the influence of its larger structural complexity and isolated position. Further, sedimentation on the deck of the wrecks is highly influenced by the difference in structure with the MV Rozi having a perimeter wall surrounding the wreck, whereas the P29 does not. This influences colonisation, as sediments prohibit growth and settlement of organisms. Another player in driving biodiversity is illumination and therefore the amount of light that is available for photosynthesis. This was inferred by the difference in growth on different positions on the wrecks. From the hydrochemical data collected, it was difficult to justify their influence on biodiversity and species richness on the vessels, as data collected from both sides was fairly similar throughout the study period.

These findings highlight the ecological impact of artificial reefs, emphasizing the trade-offs between diversity and abundance. Reduced diversity, as observed on the P29, may limit ecosystem resilience and trophic interactions, while greater diversity on the MV Rozi suggests potential for broader ecological functions, albeit with spatial limitations.

Future research should aim to extend the study period to capture seasonal and long-term trends, while integrating hydrodynamic and hydrochemical data and advanced technologies like genetic barcoding for more precise species identification. Practical recommendations, including the consideration of sedimentation and microhabitat complexity in reef design, can inform adaptive management strategies for artificial reefs globally.

In conclusion, this study provides valuable insights into the ecological dynamics of artificial reefs, emphasizing the importance of balancing structural design and environmental factors to foster diverse and resilient marine ecosystems.

References

- 2DoorsMedia. (2022). *The maltese islands - maltese islands weather*. <https://www.malteseislandsweather.com/the-maltese-islands/>
- Adi Associates Environmental Consultants Ltd. (2007, April). *Scuttling of a vessel to enhance the existing dive site at cirkewwa*. (tech. rep.). Mellieha, Adi Associates.
- Anderson, M. J. (2017, November). Permutational multivariate analysis of variance permutation. In R. S. Kenett, N. T. Longford, W. W. Piegorsch, & F. Ruggeri (Eds.). Wiley. <https://onlinelibrary.wiley.com/doi/10.1002/9781118445112.stat07841>
- Barcelona convention [Adopted in Barcelona, 16 February 1976. Available online at <https://www.unep.org/unepmap/who-we-are/barcelona-convention-and-protocols>]. (1976).
- Bartolo, A. G., Zammit, G., Peters, A. F., & Küpper, F. C. (2020). The current state of dna barcoding of macroalgae in the mediterranean sea: Presently lacking but urgently required. *Botanica Marina*, 63(3), 253–272. <https://www.degruyter.com/document/doi/10.1515/bot-2019-0041/html>
- Bergbauer, M., & Humberg, B. (2017, May). *Was lebt im mittelmeeer: Neue, erweiterte und überarbeitete ausgabe*. Franckh-Kosmos Verlags-GmbH & Company KG.
- Blondel, J. (2010, January). *The mediterranean region: Biological diversity in space and time*. Oxford University Press.
- Bohnsack, J., & Sutherland, D. (1985). Artificial reef research: A review with recommendations for future priorities. *Bulletin of Marine Science*, 37, 11–39.

- Borg, J. A., Magro, M., Schembri, P. J., & Vella, P. J. (2008). Artificial reefs project. final report of scientific studies (2004 - 2006). *Marine Ecology Research Group, Department of Biology, University of Malta*.
- Bravo, G., Livore, J. P., & Bigatti, G. (2021). Monitoring rocky reef biodiversity by underwater geo-referenced photoquadrats. *Underwater Technology*, 38(1), 17–24. <https://www.ingentaconnect.com/content/10.3723/ut.38.017>
- Clarke, K., Chapman, M., Somerfield, P., & Needham, H. (2006). Dispersion-based weighting of species counts in assemblage analyses. *Marine Ecology Progress Series*, 320, 11–27. <http://www.int-res.com/abstracts/meps/v320/p11-27/>
- Clementi, E., Drudi, M., Aydogdu, A., Moulin, A., Grandi, A., Mariani, A., Goglio, A. C., Pistoia, J., Miraglio, P., Lecci, R., Palermo, F., Coppini, G., Masina, S., & Pinardi, N. (2023). *Mediterranean Sea Physical Analysis and Forecast (CMEMS MED-Physics, EAS8 system) (Version 1) [Data set]*. Copernicus Marine Service (CMS). https://doi.org/10.25423/CMCC/MEDSEA_ANALYSISFORECAST_PHY_006_013_EAS8
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., Ballesteros, E., Bianchi, C. N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Frogia, C., Galil, B. S., Gasol, J. M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., ... Voultsiadou, E. (2010). *The biodiversity of the mediterranean sea*. <https://doi.org/10.1371/journal.pone.0011842>
- Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1(1), 443–466. <https://www.annualreviews.org/doi/10.1146/annurev.marine.010908.163757>
- Cutajar, M. (2022). *Cirkewwa Marine Park Management Plan 2022*.
- Cuttelod, A., Nieves, G., Abdul Malak, D., Temple, H., & Katariya, V. (2008). The mediterranean: A biodiversity hotspot under threat. *Nature*, 188(4752), 716–717. <https://www.nature.com/articles/188716b0>
- Dauphinée, T. M., Lewis, E. L., Ancsin, J., Phillips, M. J., Culkin, F., Smith, N., Poisson, A., Bradshaw, A. L., Schleicher, K., Perkin, R. G., & Klein, H. P. (1981). Background papers and supporting data on the practical salinity scale, 1978. *UNESCO Technical Papers in Marine Science*, 37. <https://policycommons.net/>

- artifacts/10703294/background-papers-and-supporting-data-on-the-practical-salinity-scale-1978/11610326/
- Fabi, G., Spagnolo, A., Bellan-Santini, D., Charbonnel, E., Çiçek, B. A., García, J. J. G., Jensen, A. C., & Kallianiotis, A. (2011). Overview on artificial reefs in europe. *Brazilian Journal of Oceanography*.
- Firth, L. B., Farnworth, M., Fraser, K. P. P., & McQuatters-Gollop, A. (2023). Make a difference: Choose artificial reefs over natural reefs to compensate for the environmental impacts of dive tourism. *Science of The Total Environment*, 901, 165488. <https://linkinghub.elsevier.com/retrieve/pii/S0048969723041116>
- G. N. U. Octave Developers. (2023). *Gnu octave (version 9.2.0)*. <https://octave.org/index>
- Galil, B. S. (2000). A sea under siege - alien species in the mediterranean.
- Graham, M. H., & Dayton, P. K. (2002). On the evolution of ecological ideas: Paradigms and scientific progress. *Ecology*, 83(6), 1481–1489.
- Helsinki convention [Adopted in Helsinki, 9 April 1992. Available online at <https://helcom.fi/about-us/convention/>]. (1992).
- International Maritime Organization. Updated guidelines regulating the placement of artificial reefs at sea. In: A. 26. IMO Resolution, 2009, 1024. [https://www.imo.org/en/KnowledgeCentre/IndexofIMOResolutions/Documents/A%20-%20Assembly/1024\(26\).pdf](https://www.imo.org/en/KnowledgeCentre/IndexofIMOResolutions/Documents/A%20-%20Assembly/1024(26).pdf)
- Ivkić, A., Puff, F., Kroh, A., Mansour, A., Osman, M., Hassan, M., Ahmed, A. E. H., & Zuschin, M. (2023). Three common sampling techniques in pleistocene coral reefs of the red sea: A comparison. *Geological Society, London, Special Publications*, 529(1), 223–242. <https://www.lyellcollection.org/doi/10.1144/SP529-2022-227>
- Jensen, A. C. (2002). Artificial reefs of europe: Perspective and future. *ICES Journal of Marine Science*, 59, S3–S13. <https://academic.oup.com/icesjms/article-lookup/doi/10.1006/jmsc.2002.1298>

- Jensen, A., Collins, K., & Lockwood, A. (Eds.). (2000). *Artificial reefs in european seas*. Kluwer Academic Publishers. <https://eprints.soton.ac.uk/54682/>
- Leeworthy, V. R., Maher, T., & Stone, E. A. (2006). Can artificial reefs alter user pressure on adjacent natural reefs? *Bulletin of marine science*, 78.
- Lewis, E. (1980). The practical salinity scale 1978 and its antecedents. *IEEE Journal of Oceanic Engineering*, 5(1), 3–8. <http://ieeexplore.ieee.org/document/1145448/>
- London convention [Adopted in London, 29 December 1972. Available online at <https://www.imo.org/en/About/Conventions/Pages/Convention-on-the-Prevention-of-Marine-Pollution.aspx>]. (1972).
- Louisy, P. (2002). *Europe and mediterranean marine fish - identification guide*. Les Editions Eugen UlmerUlmer.
- Magurran, A. E. (2021). Measuring biological diversity. *Current Biology*, 31(19), R1174–R1177. <https://linkinghub.elsevier.com/retrieve/pii/S0960982221010393>
- Mallet, D., & Pelletier, D. (2014). Underwater video techniques for observing coastal marine biodiversity: A review of sixty years of publications (1952-2012). *Fisheries Research*, 154, 44–62. <https://linkinghub.elsevier.com/retrieve/pii/S0165783614000356>
- Mallia, A. (2022, June). *Proposed scuttling of the m/t hephaestus at a site off ix-xatt l-ahmar, south coast of gozo to create a new diving attraction project description statement*. https://era.org.mt/wp-content/uploads/2019/11/Hephaestus-scuttling_Xatt-l-Ahmar_PDS.pdf
- Mann, K. H., & Lazier, J. R. N. (2005, December). *Dynamics of marine ecosystems: Biological-physical interactions in the oceans*. John Wiley & Sons.
- Massel, S. R. (2012, December). *Fluid mechanics for marine ecologists*. Springer Science & Business Media.
- Menna, M., Poulain, P.-M., Ciani, D., Doglioli, A., Notarstefano, G., Gerin, R., Rio, M.-H., Santoleri, R., Gauci, A., & Drago, A. (2019). New insights of the sicily channel

- and southern tyrrhenian sea variability. *Water*, 11(7), 1355. <https://www.mdpi.com/2073-4441/11/7/1355>
- Onset Computer Corporation. (n.d.-a). *Mx2501 ph and temperature data logger*. <https://www.onsetcomp.com/products/data-loggers/mx2501>
- Onset Computer Corporation. (n.d.-b). *U24-002-c salt water conductivity/salinity data logger*. <https://www.onsetcomp.com/products/data-loggers/u24-002-c>
- Onset Computer Corporation. (n.d.-c). *U26-001 dissolved oxygen data logger*. <https://www.onsetcomp.com/products/data-loggers/u26-001>
- Ospar convention [Available online at <https://www.ospar.org/convention>]. (1992).
- Paul, W. L., & Anderson, M. J. (2013). Causal modeling with multivariate species data. *Journal of Experimental Marine Biology and Ecology*, 448, 72–84. <https://linkinghub.elsevier.com/retrieve/pii/S0022098113002165>
- Paxton, A. B., Shertzer, K. W., Bacheler, N. M., Kellison, G. T., Riley, K. L., & Taylor, J. C. (2017). Meta-analysis reveals artificial reefs can be effective tools for fish community enhancement but are not one-size-fits-all, frontiers in marine science. *Coastal Management*, 45(6), 499–516. <https://repository.library.noaa.gov/view/noaa/25024>
- Pickering, H., & Whitmarsh, D. (1997). Artificial reefs and fisheries exploitation: A review of the ‘attraction versus production’ debate, the influence of design and its significance for policy. *Fisheries Research*, 31(1-2), 39–59. <https://linkinghub.elsevier.com/retrieve/pii/S0165783697000192>
- Placenti, F., Torri, M., Pessini, F., Patti, B., Tancredi, V., Cuttitta, A., Giaramita, L., Tranchida, G., & Sorgente, R. (2022). Hydrological and biogeochemical patterns in the sicily channel: New insights from the last decade (2010-2020). *Frontiers in Marine Science*, 9, 733540. <https://www.frontiersin.org/articles/10.3389/fmars.2022.733540/full>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for

- biological-image analysis. *Nature Methods*, 9(7), 676–682. <https://www.nature.com/articles/nmeth.2019>
- Silvestre. (1989). *Towards sustainable development of the coastal resources of lingayen gulf, philippines : Proceedings of an asean/us coastal resources management project workshop, bauang, la union, philippines, 25-27 may, 1988*. Philippine Council for Aquatic; Marine Research; Development ; International Center for Living Aquatic Resources Management on behalf of the Association of Southeast Asian Nations/United States Coastal Resources Management Project.
- Suarez, R., Nydia, C., Cook, M. S., Gačić, M., Paduan, J. D., Drago, A., & Cardin, V. (2019). Sea surface circulation structures in the malta-sicily channel from remote sensing data. *Water*, 11(8), 1589. <https://www.mdpi.com/2073-4441/11/8/1589>
- Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, 25(1), 1–45. <https://onlinelibrary.wiley.com/doi/10.1111/j.1469-185X.1950.tb00585.x>
- Ulman, A., Harris, H. E., Doumpas, N., Deniz Akbora, H., Al Mabruk, S. A. A., Azzurro, E., Bariche, M., Çiçek, B. A., Deidun, A., Demirel, N., Fogg, A. Q., Katsavenakis, S., Kletou, D., Kleitou, P., Papadopoulou, A., Ben Souissi, J., Hall-Spencer, J. M., Tiralongo, F., & Yildiz, T. (2021). Low pufferfish and lionfish predation in their native and invaded ranges suggests human control mechanisms may be necessary to control their mediterranean abundances. *Frontiers in Marine Science*, 8, 670413. <https://www.frontiersin.org/articles/10.3389/fmars.2021.670413/full>
- United Nations Environment Programme. (2021). *Biological diversity in the mediterranean: Unep/map*. <https://www.unep.org/unepmap/resources/factsheets/biological-diversity>
- Van Rein, H., Schoeman, D. S., Brown, C. J., Quinn, R., & Breen, J. (2011). Development of benthic monitoring methods using photoquadrats and scuba on heterogeneous hard-substrata: A boulder-slope community case study. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21(7), 676–689. <https://onlinelibrary.wiley.com/doi/10.1002/aqc.1224>