A Preliminary Investigation into the Impacts of Artificial Lighting on Selected Fish and Benthic Species in Maltese Waters

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Abstract

The increasing recognition of Artificial Light at Night (ALAN) as a potential concern for biodiversity is grounded in a growing body of research highlighting its impact on animal behaviour, migration, reproduction, and biological interactions. In the Maltese Islands, there has been a noticeable rise in the prevalence of ALAN over the years. Despite this trend, the quantification and exploration of the consequences of artificial light on marine biota in the Maltese Islands remain understudied. This research aims to address this gap by investigating the effects of ALAN on multitrophic marine communities. Utilising a Baited Remote Underwater Video (BRUV) equipped with artificial lighting, the study focuses on two distinct local sites within the Ċirkewwa harbour that are characterised by comparable communities and depth, but different lighting conditions. The experimental design involved the replication of the study in both space and time, capturing the response of the communities to a pulse disturbance, represented by introduced light from the BRUV. This response was examined both with and without the additional stress induced by an ongoing press disturbance, represented by the already-present ambient light. The study establishes a direct link between artificial light and the local abundance of both predatory and prey fish in areas influenced by varying light conditions. The main findings from the study were that the plausible occurrence of habituation, especially in response to the press light disturbance. Predatory species, as exemplified by *Trachurus trachurus*, demonstrated a strategic utilisation of light conditions, particularly in high-light intensity situations. These predatory fish exhibited 'station holding' behaviour, actively maintaining their position within the illuminated area—an energy-intensive choice that is favoured in environments where a clear net energy gain is apparent. In contrast, species at a lower trophic level, such as *Apogon imberbis*, experienced a decline as light intensity increased. Additionally, species like *Sepia officinalis* and *Hermodice carunculata* exhibited a distinct preference for darker environments. These findings collectively underscore the complex responses of marine communities to varying light conditions.

Acknowledgements

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List of Acronyms

AI	Artificial Intelligence
ALAN	Artificial Light at Night
BRUV	Baited Remote Underwater Video
CCT	Correlated Colour Temperature
EU	European Union
IBI	Index of Biological Integrity
LD	Light-Dark
Lm	Lumen
LPAG	Light Pollution Awareness Group
MMI	Multimetric Indices
MSFD	Marine Strategy Framework Directive
nMDS	nonmetric Multidimensional Scaling
PCO	Principal Coordinates Ordination
PERMANOVA	Permutational Multivariate Analysis of Variance
PRIMER	Plymouth Routines in Multivariate Ecological Research
PVC	Polyvinyl Chloride
ROV	Remotely Operated Vehicle
SIMPER	Similarity Percentage
ULOR	Upward Light Output Ratio
UVC	Underwater Visual Census

1. Introduction

1.1 Study Rationale

The increase of global light emissions in recent times has brought about the need for an urgent evaluation of their impacts on the behaviour, ecology, and physiology of several organisms. Numerous species exhibit daily cycles or strong scototaxic behaviours that may be susceptible to disruption if natural lighting conditions or cycles are disturbed. Artificial Light at Night (ALAN) refers to scenarios where artificial light alters natural light-dark cycles, including light intensities and wavelengths (Dominoni & Nelson 2018). ALAN is progressively acknowledged as a potential threat to biodiversity, primarily due to an expanding body of research demonstrating its impact on animal behaviour, migration, reproduction, and biological interactions. This occurs through the modification of the temporal patterns of organisms' engagement in activities such as foraging, hiding, and resting (Bolton et al., 2017). ALAN's influence on the temporal availability of activities can disrupt evolutionary dynamics between predators and prey (Minnaar et al., 2015). Since there is less competition and predation during the dark, darkness is essential for many nocturnal species to get food and mates, but ALAN depletes this resource (Duffy et al., 2015). Moreover, given the growing human use and development of the coastal zone, organisms inhabiting shallow coastal or intertidal systems could face escalating risks from ALAN (Pulgar et al., 2019). Additionally, the diminishing availability of naturally dark spaces poses a threat to species using such environments for rest and recovery, potentially leading to a loss of refuge for these species (Gaston et al., 2013).

Predicting ecosystem reactions to environmental change on a global scale is crucial to scientific and political agendas worldwide. Thorough understanding of this component is necessary to predict the future status of ecosystems and, as a result, to direct efficient adaptive management of ecosystems (Díaz et al., 2019). However, generating broad predictions proves challenging through singular-site experiments or observational studies due to the context-specific nature of responses and/or the nonstationary character of environmental changes (Angeler et al., 2020). While most studies on ALAN have focused on terrestrial organisms and ecosystems, investigations into the effects of ALAN on marine ecosystems remain sporadic. In recent years, there has been a noteworthy emergence of experimental networks to solve this issue. These networks apply the same treatments at different sites, which allows for the creation of statements that can be applied generally to the average and variable responses to different kinds of environmental change (Duffy et al., 2015).

As of the current policy framework in the European Union (EU) related to marine communities, there is a notable gap in explicit considerations for ALAN and its impact on fish. The existing policies primarily focus on broader aspects of marine conservation, sustainable fisheries, and habitat protection but lack specific provisions addressing the implications of ALAN on fish populations.

1.1.1 Local Context

A study by Caruana et al. (2020) showed that the Maltese Islands have seen a significant increase in the incidence of artificial lighting at night throughout the years (Figure 1.1). Despite such statistics, the impact on biodiversity and ecosystems in a local context has not been thoroughly researched. One of the only keystone studies undertaken locally to-date stemmed from the EU LIFE Yelkouan Shearwater project and described the impact of artificial light on *Puffinus yelkouan*, a seabird that has a large breeding colony at Rdum tal-Madonna (L'Aħrax tal-Mellieħa) (Raine et al.,2007). The rapid increase in ALAN, often attributed to inadequateplanning in certain areas among other factors, underscores the need for a comprehensive examination of its consequences on community assemblage in Maltese waters. However, the impact and related corollaries of artificial light on marine biota in the Maltese Islands has not yet been quantified or studied.

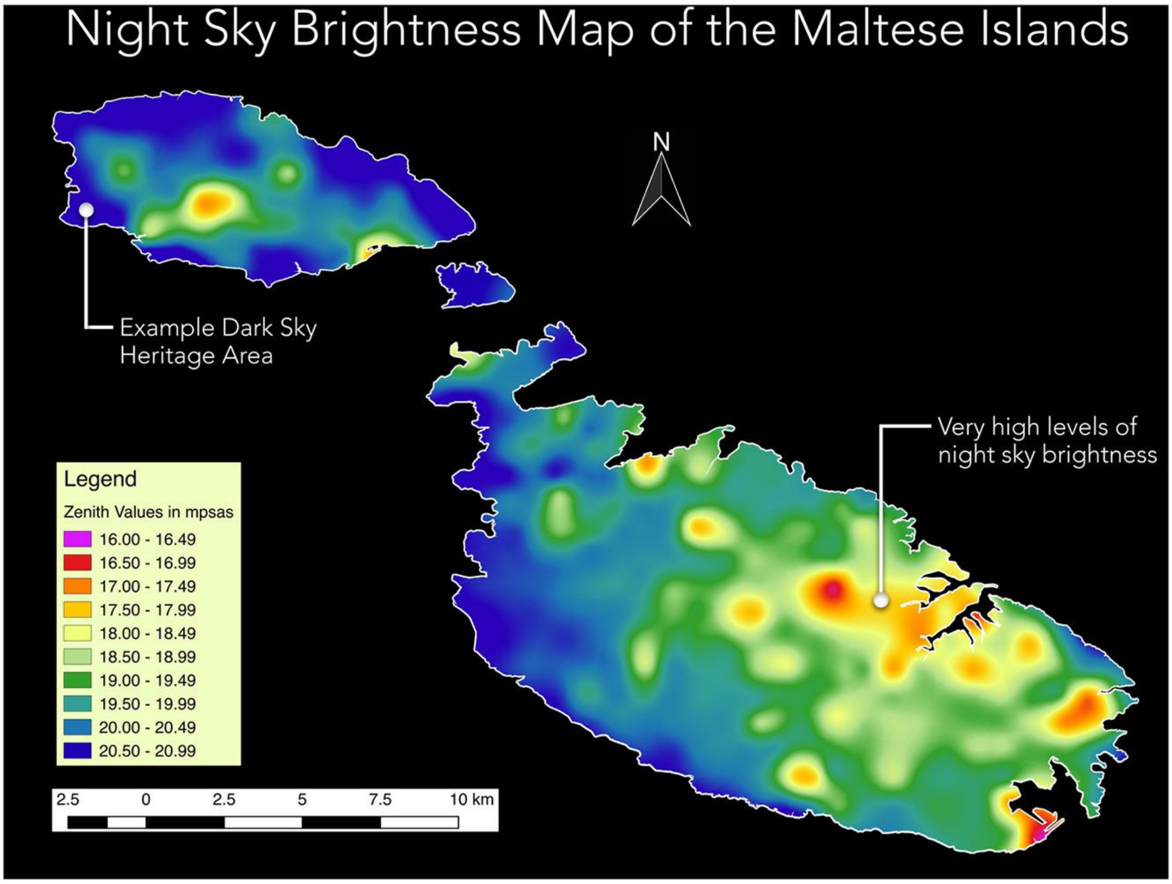


Figure 1.1 *showing a Night Sky Brightness Map of the Maltese Islands*

(*Caruana et al., 2020)*

1.2 Study Scope

The aim of this research is to study the effects of ALAN on multitrophic marine communities using a Baited Remote Underwater Video (BRUV) equipped with artificial lighting.

The experiment was replicated in two different local sites that have comparable communities and depth. Therefore, it was replicated in space and time to record the communities' response to a pulse disturbance (introduced light from the BRUV) both with and without the additional stress induced by an ongoing press disturbance (already-present light). Red light was used to observe the marine communities so as to have a baseline for the community assemblage without the addition of the pulse light disturbance. Moreover, two different white light intensities ('Low' and 'High') were used as the pulse light disturbance to assess the differences, if any, in community assemblage. Furthermore, this study aims to identify inter-species variations in predation patterns under different light conditions, discerning which species may exploit or be hindered by specific light regimes. By uncovering the differential impacts of light on various species within multitrophic marine communities, this research aims to provide a foundation for informed decisions in the placement and design of artificial lighting. The findings could also pave the way for more thoughtful and environmentally conscious decisions to be made in the future when it comes to urban design and lighting installations, as certain species may experience distress due to particular light conditions.

Given the scarcity of empirical evidence in the specific context of Maltese waters, such a study contributes valuable insights for local conservation efforts and informs future coastal planning decisions. Furthermore, by thoroughly analysing the intricate relationships between artificial light and marine communities, this research aids in the development of sustainable management strategies that prioritise the preservation of Malta's unique marine environment.

Based on related literature (refer to Section 2), the hypothesis posited was that the introduction of the pulse ALAN disturbance would increase nocturnal fish activity, particularly predatory behaviour. Consequently, it was anticipated that this increase in predatory behaviour bring about alterations in assemblage structure. This study provides one of the initial empirical pieces of evidence from the marine ecosystem in Maltese waters, illustrating the direct influence of ALAN on fish behaviour, community structure, and the subsequent trophic repercussions through predation.

2. Literature Review

Section **2.1 (Light as a Cyclic Cue for Rhythmicity in Organisms)** will primarily focus on how natural light plays an essential role for rhythmicity in marine organisms. This will further detail the chronobiology aspect relating to light and put emphasis on such rhythmicity. Section **2.2 (Artificial Light)** will then constitute Subsections **2.2.1 (Impact on Marine Biota)** and **2.2.2 (Local Context)**. Section **2.2.1 (Impact on Marine Biota)** will detail the ramifications of artificial light on marine organisms by focusing on their feeding efficiency and activity (movement). By highlighting the behavioural changes in organisms brought about by artificial light, the project rationale is further strengthened.

2.1 Light as a Cyclic Cue for Rhythmicity in Organisms

In order for organisms to thrive in a changing environment, biological rhythms are essential. Numerous physiological and biochemical processes must be coordinated temporally with the environment as well as with one another within the body in order to maintain homeostasis. As a result, the body's timing systems are necessary for normal behavioural, physiological, and psychological functioning (Bailey et al., 2014).

Several species exploit the different sun and moon cycles to modify their physiology and behaviour. In chronobiology, a biological rhythm is defined as “any recurrent, endogenous cycle (behavioural or physiological) that persists in the absence of geophysical or environmental temporal cues” (Bailey et al., 2014, p. 1). Light serves as one of the most crucial "Zeitgebers" for almost all organisms (Meisel et al., 2002). Therefore, it can be stated that the main physical mechanism that creates regular environmental cycles is provided by the sun and moon cycles, although the precise form of these cycles relies on the habitat and its physicochemical characteristics. Astronomical photocycles describe fundamental rhythmic patterns in environments that are exposed to light, which marine organisms can make use of. Position, particularly water depth, but also proximity to the shore, has a significant influence on the total quantity and spectrum quality of the light (Häfker et al., 2023).

2.1.1 Biological Rhythm of Fish

Biological rhythms are present in all living organisms at various levels, ranging from molecular to organismal. These rhythms allow organisms to anticipate and synchronise with cyclic changes in their environment, regulating their biological functions over different time scales (Meisel et al., 2002).

The majority of fish species that have been studied so far seem to support a highly decentralised model of fish circadian biology, which features independent, light-responsive circadian pacemakers in all tissues and most cells. Numerous hormonal cues, such as melatonin rhythmic cues, may also affect tissue-specific, daily oscillations. It is interesting to note that all cells and tissues in vitro appear to have their clocks set to the same phase by the environmental light signal. A potential outcome in vivo is that light sets all of the body clocks to the same phase, and then different hormonal/neural inputs make minor modifications to this timing, resulting in tissue-specific phasing of rhythms (Frøland Steindal & Whitmore, 2019).

2.1.2 Diurnal and Circadian Cycles

Strong diurnal and circadian regulation of gene expression has been observed under laboratory settings in diverse species of algae. This suggests that this circadian function is essential for a healthy environment (Coesel et al., 2021). Other functions of the circadian clock include helping to control population behaviour in many algae and although on a much smaller scale than zooplankton, phytoplankton also exhibits diurnal vertical migrations. Endogenous cycles of cellular aggregation and bioluminescence have also been documented (Gaten et al., 2008).

Moon-related cycles are also common. A multitude of diatom species have been found to exhibit migratory rhythms in intertidal sediments that are synchronised with tidal and diurnal cycles, and the diatom *Seminavis robusta* has been found to exhibit semidiurnal (12-h) gene expression periodicity (Bilcke et al., 2021). These observations likely reflect adaptations to the tides in coastal ecosystems. Another activity in algae that is influenced by lunar cycles is sexual reproduction. According to lunar or semilunar periodicity, multicellular algae have long been shown to exhibit synchronised gamete release patterns (Frenkel et al., 2014).

Algae's circadian clock, like that of other species, is entrained by cyclical environmental cues. Blue and red light appear to be crucial in entrainment, according to physiological investigations (e.g., Poliner et al., 2019). Numerous blue-light-absorbing proteins have been found in the genomes of algal model systems, and evidence of certain species' participation in circadian control has been provided. Contrarily, only a few species of diatoms have been reported to exhibit red/far-red light-sensitive phytochromes, presumably because of the high attenuation of long wavelengths underwater (Fortunato et al., 2016).

2.2 Artificial Light

Changes in biological rhythms brought on by the environment, neurological conditions, or disease states can have detrimental effects on both the health of an individual and a group (Bailey et al., 2014).

The rise in global light emissions has emphasised the necessity for immediate assessment of their effects on the behaviour, ecology, and physiology of organisms. Many species display daily cycles or strong behaviours in response to darkness (scototaxis), which could be affected if natural lighting conditions or cycles are disrupted. Artificial Light at Night (ALAN) refers to situations where artificial light disrupts natural light-dark cycles, light intensities, and wavelengths. ALAN is now acknowledged as a potential threat to biodiversity, supported by a growing body of studies demonstrating its impact on animal behaviour, migration, reproduction, and biological interactions (Pulgar et al., 2019).

2.2.1 Visual Perception and Response of Marine Biota to Artificial Light

Vital behaviours such as feeding, schooling, and migration of teleosts, which make up 96% of the entire fish population, are contingent on specific levels of light intensity.

The teleost eye's visual cell layer comprises two types of photoreceptors, namely rods (scotopic) and cones (photopic), each with distinct light sensitivity thresholds (Atta, 2013). These photoreceptors adjust their location within the eye in relation to the light source in response to changes in light intensity. When light intensity increases above the cone threshold, the eye assumes a light-adapted condition in which the rod cells extend and the cone cells contract. On the contrary, when light intensity falls below the cone threshold level, the cones extend and the rods compress. These photoreceptor alterations are directly proportional to the light intensity logarithm, a finding that remained consistent with initial studies on the topic such as that conducted by Ali (1959). The ability of fish to school and feed is therefore correlated with these rod and cone thresholds (Nightingale et al., 2006).

Fish reactions to light can be categorised into two types: reactions to luminance (the intensity of direct glare from an environment light) and reactions to illumination (the quantity of light per unit area incident on nearby objects). According to studies of fish under experimental conditions, this fundamental response varies even within species, depending on various parameters. Such parameters may include the characteristics of both the respective fish and light (such as duration, intensity, and spectrum), as well as the surrounding environment (Nightingale et al., 2006).

The foraging strategy of a given fish species and the duration of the lighting also has an influence of the behavioural response to light of said species. Strobe lights, for example, produce extremely brief light which heavily contrast to the usual flickering light in underwater habitats that brought on by wave, cloud, and solar conditions. The brief length of a strobe's quick flashes results in sharp contrasts in light intensity that are too abrupt for any retinal adaptation to occur. In contrast to the control and post-treatment periods, Kim & Mandrak (2017) indicated that Common Carp *(Cyprinus carpio)* and Brown Bullhead *(Ameiurus nebulosus)* kept much further away from the strobe light when it was flashing. Similar responses were in Largemouth Bass *(Micropterus salmoides)*, Rainbow Smelt *(Osmerus mordax)*, Zebrafish *(Danio rerio)*, Yellow Perch *(Perca flavescens)*, Coho Salmon *(Oncorhynchus kisutch)*, Chinook Salmon *(Oncorhynchus tshawytscha)*, and Atlantic Salmon *(Salmo salar)* (refer to Hamel et al., 2008; Mesquita et al., 2008; Richards et al., 2007; Sullivan et al., 2016).

2.2.2 Impact of Artificial Light on Marine Biota

Studies have found that artificial light at night alters predator-prey interactions and fish predation increases with the presence of night illumination (Bolton et al., 2017; Czarnecka et al., 2019; Nelson et al., 2021).

The following represent case studies relating to the impact of artificial light on fish behaviour:

- Czarnecka et al. (2019) tested the effects of artificial light at night on the Eurasian perch (*Perca fluviatilis*) and its feeding habits on gammarids (*Gammarus fossarum*) in relation to simple and complex freshwater habitats. Through a series of laboratory experiments, Czarnecka et al. (2019) determined that **fish predation** on gammarids in freshwater ecosystems **was enhanced by artificial night-time illumination**. The fish were therefore **much more active during artificially illuminated nights** than at dusk, with habitat type having minimal effect on their swimming activity. The study also concluded that the Eurasian perch (*Perca fluviatilis*) was **equally efficient as a predator in lit nights as it was in dark nights**. Moreover, complex habitats were an effective sanctuary to prey only when combined with complete darkness. Night-time darkness is therefore typically a crucial indication for movement decrease and rest in perch (Czarnecka et al., 2019, as cited in Craig, 1977).

- Marchesan et al. (2005) examined the impact of artificial light with varying intensity and wavelength on the behaviour of four fish species: European seabass (*Dicentrarchus labrax*), common grey mullet (*Mugil cephalus*), gilthead seabream (*Sparus auratus*), and striped bream (*Lithognathus mormyrus)*. The experiments were conducted in controlled laboratory conditions. In the first set of experiments, the researchers adjusted light intensity in discrete steps ranging from 0.2 to 68 $\mu E\ s^{-1}\ m^{-2}$. In the second set, the authors used different colour filters to shift light from shorter to longer wavelengths of the visual spectrum and vice versa. The common grey mullet and gilthead seabream exhibited the strongest attraction to light, particularly when the intensity varied. The striped bream was **attracted to light** at all intensity levels. However, the European seabass showed no strong preference for or aversion to light. The grey mullet also displayed a **positive response to monochromatic stimuli**, especially with shorter wavelength lights, which similarly affected the European seabass.

- A study conducted by Pulgar et al. (2019) examined the impact of ALAN on the rockfish *Girella laevifrons*, an ecologically important intertidal species in the Southeastern Pacific littoral. **ALAN exposure led to increased oxygen consumption and activity levels** when compared to the control group. Additionally, natural activity cycles were disrupted in the ALAN-exposed fish. These findings indicate potential long-term effects on fish populations and intertidal communities in ALAN-affected areas.

The aforementioned case studies focus solely on ALAN and its effects on fish behaviour in terms of predation and activity due to the nature of the study being conducted. However, it is also important to note that ALAN and changes in LD cycles also affect hormone production and release in fish. A study conducted by Brüning et al. (2015) proposed that light plays a central role in regulating melatonin rhythm in European perch (*Perca fluviatilis)* as results show that the presence of ALAN impaired the rhythm. Based on indirect evidence, fish pheromone may also function as a zeitgeber for circadian rhythms in fish (Baghel & Pati, 2015).

2.3 Underwater Observation

Fish populations and communities are studied for several purposes such as evaluating abundance, population dynamics, habitat connections, community composition, and biological diversity patterns. In the past, surveys of demersal marine species were typically carried out using various mobile and fixed fishing equipment such as bottom trawls, longlines, and pots. At present, the preferred method for surveying shallow-water fishes is visual direct-counting (Edgar et al., 2004), hence being one of the methods used for this study.

The most suitable UVC (Underwater Visual Census) method will differ based on the specific circumstances, taking into account the study objectives, logistical limitations, and characteristics of the target species (such as density, patchiness, size, mobility, behavioural response to divers, and camouflage), as well as factors like the expertise of data gatherers, water clarity, depth, habitat type, and environmental conditions such current speed, and wave exposure (Edgar et al., 2004).

2.3.1 Remotely Operated Vehicles and Baited Remote Underwater Video

Remote underwater video systems are non-extractive sampling methods that offer the advantage of conducting surveys in deep-water environments. However, they also have their own inherent biases and limitations.

Among these techniques, Baited Remote Underwater Videos (BRUVs) have emerged as one of the most prevalent methods and have become established as a standard means for documenting the relative abundance of fish across various habitats. Using bait also enables the observation of specific species, including those that are typically hesitant to approach divers (Langlois et al., 2018).

ROVs have also emerged as an alternative method for studying fish communities, especially in artificial structures such as pipelines. The oil and gas industry frequently employs ROVs to examine underwater infrastructure for maintenance and assessment purposes. Advances in ROV design and cost reduction have now made such operations more accessible for scientific research. As will be discussed further in Section **2.3.2 (Sampling Bias in Observation Using Underwater Vehicles)**, the behavioural inclinations of fish toward ROVs are predominantly based on factors such as the impact of lighting, noise originating from electrical components and propulsion systems, as well as the dimensions of the ROV (Schramm et al., 2020).

Whitmarsh et al. (2017) highlights the findings of four studies (refer to Harvey et al. 2007; Bernard and Götz 2012; Dorman et al. 2012; Hannah and Blume 2014) that specifically investigated the effects of using BRUV systems in comparison to ROVs without bait. The studies found four main advantages to using baited systems in relation to:

- **Statistical power and replicates**
    - The use of bait led to an increase in similarity between duplicates, resulting in improved statistical power. This implies that baited systems offered more dependable and consistent data for analysis.

- **Species composition**
    - Baited systems attracted and increased predatory and scavenger fish. However, it is important to note that the populations of fish that are herbivorous and omnivorous were unaffected. The overall ecosystem dynamics in the investigated environments could therefore be impacted by this change in species composition.

- **Detecting changes**
    - Baited replicates were better at detecting differences between various habitat types. The use of bait may therefore improve a study's ability to detect changes and variances in marine ecosystems.

- **Abundance and measurement accuracy**
    - According to one study (Hannah and Blume 2014), bait substantially enhanced the number of deep-water demersal fishes. A further benefit of using bait was that it attracted fish to the observation devices, improving the accuracy of both the length measurements, and species identification.

2.3.2 Sampling Bias in Observation in UVC Surveys

Every method used to assess fish populations has its inherent biases. As an example, the behaviour of several fish species was found to be influenced by the presence of trawls, depending on ambient illumination (Ryer & Barnett, 2006).

Underwater vehicles enable direct visual surveys, offering several advantages compared to traditional fish-sampling equipment. Nevertheless, the biases linked to these surveys have not been thoroughly quantified and are largely unrecorded or anecdotal (Stoner et al., 2008).

2.3.2.1 Bias Associated with Underwater Vehicles

Distinct fish species may behave differently when introduced to equipment. Underwater vehicles may therefore not have much of an impact on some fish species, whilst heavily affecting others. Some stimuli that are associated with underwater vehicles are summarised in Table 2.1 below:

Table 2.1 *showing stimuli associated with underwater vehicles and their observed effects and reactions*

Stimuli	Observed Effects and Reactions
Artificial light	Experiments related to artificial lighting do not consistently produce uniform results. For example, Trenkel et al. (2004) reported significant adverse reaction to a lighted ROV in the roundnose grenadier (*Coryphaenoides rupestris*), while the review compiled by Nguyen & Winger (2019) indicates that the use of light in fishing has evolved into one of the most effective, approaches for capturing commercially valuable species on an industrial level due to their positive reaction.
Sound	Fish movements in response to an approaching vessel may be intricate, involving both movements towards and away from the vessel as the sound field properties around it change (Handegard & Tjøstheim, 2005).
Vehicle motion and water displacement	Vehicle motion and water displacement can influence fish behaviour through visual or tactile stimuli in the immediate vicinity. However, certain species may be sensitive to low-frequency pressure waves and may react to motions even when at significant distances (Stoner et al., 2008).
Vehicle speed	Trenkel et al. (2004) found that the density of all recorded species within the conducted study (cods of the family Moridae and northern cutthroat eels) declined as vehicle speed increased, despite some species having opposite reactions to light.
Chemicals	Chemical compounds linked to underwater vehicles, such as lubricants and cleaners, can also impact attraction or avoidance responses in fish. The olfactory sensitivity of fish allows them to detect these chemical cues from considerable distances, depending on the relative current direction (Stoner et al., 2008).

2.3.2.1 Bias Associated with BRUVs

When conducting an UVC using a BRUV, employing different **types of bait** introduces another variable within sampling initiatives (Dorman et al., 2012). Consequently, standards ought to be established for the type, quantity, and delivery of bait. Physical properties of the bait, including its durability and moisture content, will ultimately govern factors such as **soak duration**, the **area of dispersion**, and the **persistence of the bait signal**, hence why bait should be standardised for the period of study (Whitelaw et al., 1991).

The study conducted by Dorman et al. (2012) recorded one-hour dual-view video footage with four distinct types of bait (pilchards, cat food, falafel mix, and absence of bait as a control) at locations both within and outside a targeted fishery closure zone. The results conclude that various bait varieties impact the sampling of reef fish communities in relation to both their abundance and composition. The substantial quantity of individuals lured by cat food and pilchards implies that these bait varieties might possess effective **bait trail characteristics** (such as high moisture content and consequent dispersion potential). Other aspects to consider include the **breakdown of bait** caused by the initial species, the occurrence of the "**chumming effect**" (which intensifies odour release and consequently draws more species), and the impact of **benthic topography** and **current speeds** on the velocity and course of the bait trail.

Additionally, it was found that apart from changes in fish assemblage, bait type must be chosen in relation to other impacting parameters. In the aforementioned study for example, the falafel mixture produced a bait trail that created a disturbance as it significantly diminished visibility and impeded the identification of species. Similarly, cat food generated an observable trail, although this did not impede the identification of species or measurements as frequently as the falafel mixture did.

2.3.3 Limitations and Challenges in Underwater Fish Surveys Through Recorded Observations

For the purposes of this study, surveying will be done through the analysis of recorded footage. Jarina et al. (2019) describe how images taken underwater are prone to various distortions in comparison to those taken on land, primarily because of the unique characteristics of the water environment. Underwater images are therefore susceptible to diffraction, polarisation, absorption, scattering, colour loss, and light attenuation.

When a camera is focused on a target, its lens concentrates on the target's texture and emits veiling light. This light can be affected by particles, marine snow, and various types of scatterings, such as Rayleigh, Mie, Back, forward, and multiple scatterings. Large, suspended particles also cause diffraction of the veiling light. Additionally, artificial light used for capturing images in dark underwater areas also undergoes Rayleigh and Mie scattering. As a result, Rayleigh scattering leads to hazy images, while Mie scattering results in blurry, murky, and faded images (Jarina et al., 2019). Such distortions are summarised in Figure 2.1 below.

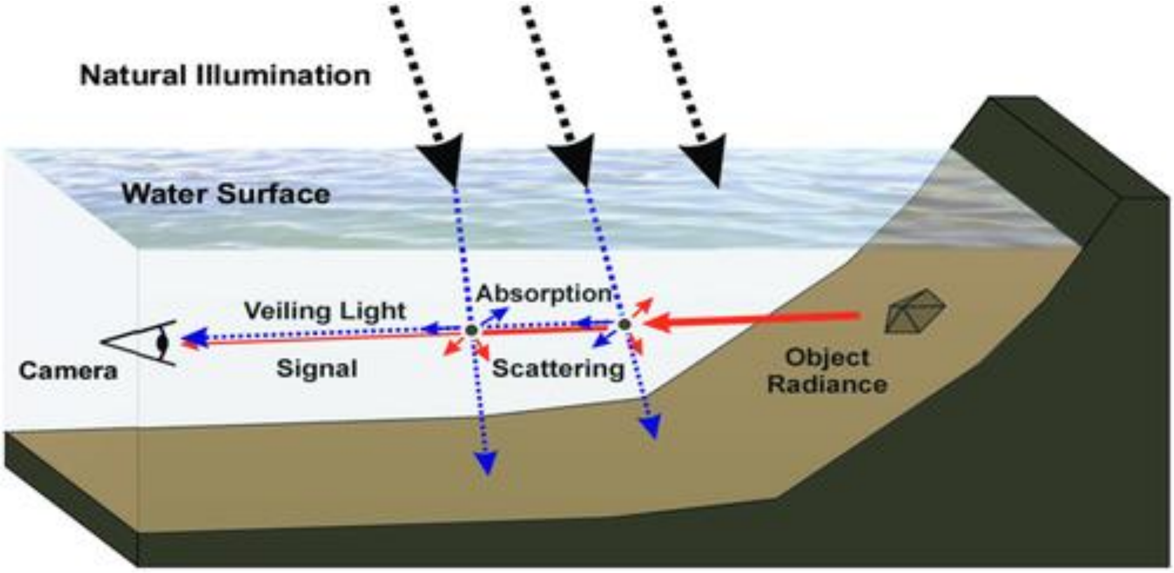


Figure 2.1 *showing various forms of distortions in underwater imagery*

(Garcia et al., 2017)

Underwater images also face challenges due to varying colour absorption. This is primarily due to the fact that each colour possesses its unique wavelength and frequency. Since the deployment of the camera for this study will primarily be in shallow waters, it is best to refer to the 50% absorption section between zero- and ten-meters depth in Figure 2.2 for the relevant light absorptions and their penetrating levels.

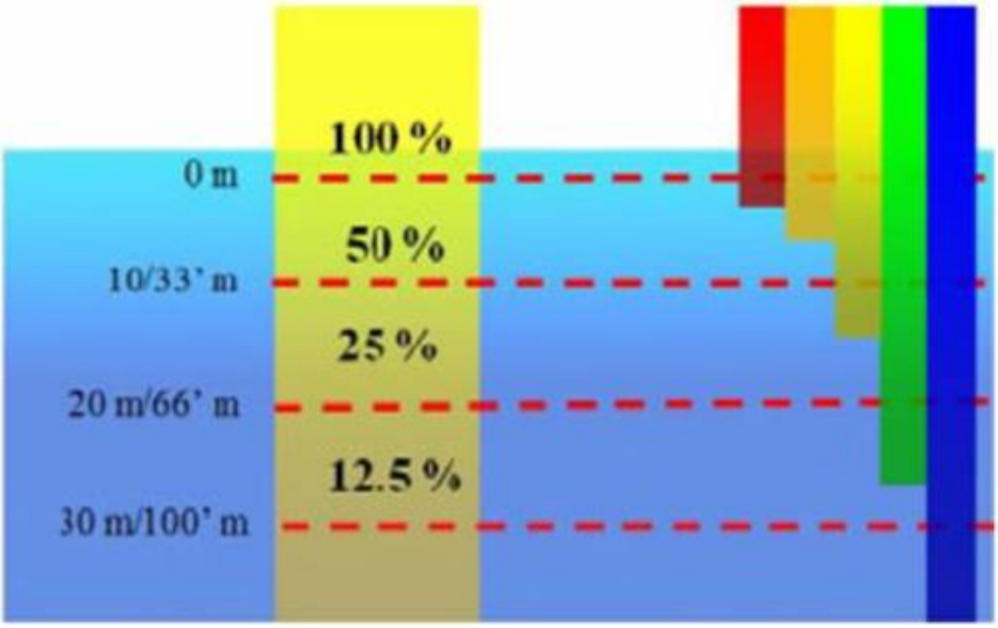


***Figure 2.2** showing colour absorption and penetration levels in water*

(Chiang & Chen, 2011)

In addition to these issues, underwater images are also affected by other challenges like focus, magnification, and motion (Jarina et al., 2019).

Spampinato et al. (2014) discuss the effects and conditions that frequently appear in underwater settings, which make video analysis challenging. These have been summarised in Table 2.2, with an additional contribution from Yang et al. (2021).

Table 2.2 *showing the effects and conditions that frequently appear in underwater settings*

Effects and Conditions	Description
Abrupt and gradual shifts in lighting	The brightness and contrast of the photos or footage are significantly reduced when there is less light, making it necessary to take light transitions into account. Additionally, during analysis, periodic gleaming in underwater imagery must be considered (Spampinato et al., 2014).
Unfavourable weather conditions	The weather can undergo rapid changes, such as sudden cloudiness and storms. The aforementioned conditions result in having less visual contrast in the imagery, which makes it difficult to detect and track any targets clearly (Spampinato et al., 2014).
Murky water	When examining fish movements in their natural environment over a given timeframe, the water's clarity may fluctuate due to drift and the presence of plankton. Targets that aren't fish could be misidentified under these circumstances (Spampinato et al., 2014).
Seagrass and algae on camera lens	The quality of the footage may be compromised due to algae and grime on the camera lens. Moreover, seagrass suspended in the water column may inhibit the field of view of the ROV (Spampinato et al., 2014).
Periodic and multimodal background	Particularly if using algorithms for fish detection, handling background motions and variations may be challenging. The algorithm must be robust enough to handle any arbitrary changes in the scene. To prevent the detection of moving non-fish objects, periodic motions (such as plants impacted by flood-tide and drift) must also be considered (Spampinato et al., 2014).
Low resolution	The process of image acquisition may be affected by noise and distortions arising from the camera's optical and electronic systems, which leads to a lower image resolution (Yang et al., 2021).

***Table 2.2** cont.*

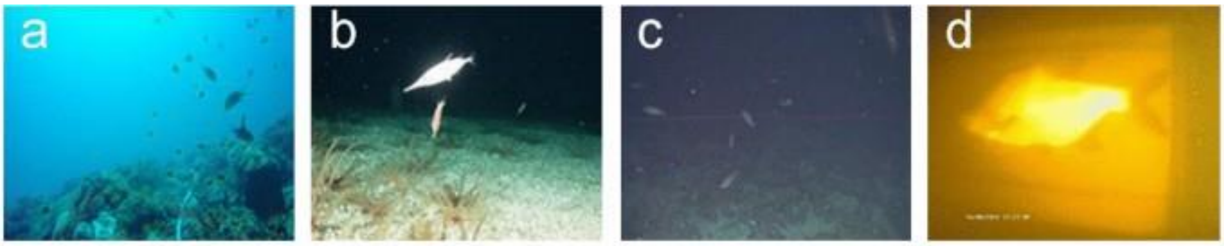
Complex background	The image background encompasses numerous non-fish objects that closely resemble fish, thereby diminishing the accuracy of detection (Yang et al., 2021).

Yang et al. (2021) also discuss the main challenges associated with underwater observation and also include most of the effects and conditions mentioned by Spampinato et al. (2014) (mentioned in Table 2.2). Yang et al. (2021) furthermore discuss the inherent characteristics of fish that may pose a difficulty in relation to detection and identification. Table 2.3 below details the types of inherent fish characteristics as described by Yang et al. (2021). These challenges may be applied for manual detection and analysis, but particularly when using algorithms and machine learning models.

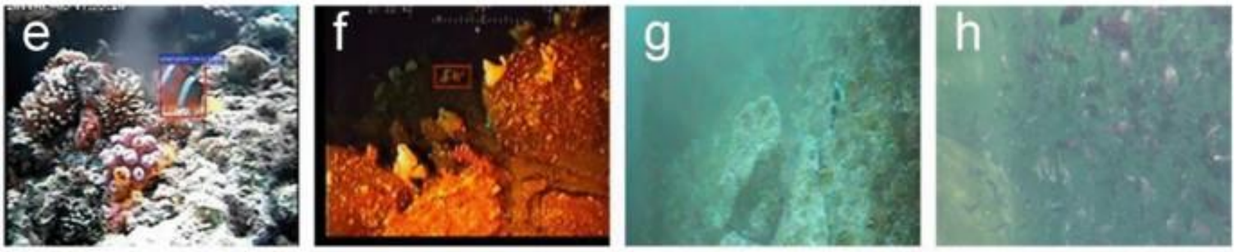
Table 2.3 *showing the inherent characteristics of fish that may pose a difficulty in relation to detection and identification*

Adapted from Yang et al. (2021)

Inherent Characteristics of Fish	**Description**
Intra-class variations	Instances of objects within various fish categories exhibit intra-class appearance variations, with each category appearing to be remarkably similar (refer to Figure 2.3 (i))
Colour similarity between fish and background	Fish undergo colour changes during their growth as an adaptive response to environmental fluctuations. The similarity between the colour and texture of fish and their background poses significant challenges in fine-grained fish classification and their precise segmentation from the surrounding environment. This resemblance may therefore hamper the accuracy of fish detection.
Deformation	The dynamic movements of fish result in changes in the characteristics of their appearance, making tracking susceptible to failures. When fish posture and shape change during motion due to their non-rigid nature, it becomes increasingly difficult to properly track the given target.
Scale change	The target's size in the image is determined primarily by the distance between the camera and the fish. This variable scale factor directly impacts the effectiveness of fish detection and counting. When the fish appears smaller in the image, it incorporates excessive background information, leading to detection errors. Conversely, when the fish appears larger, a single image may fail to encompass multiple targets which results in updating failures in the target tracking model.
Occlusion and disappearance	The target may experience occlusion or momentary disappearance during its movement, leading to tracking failure and subsequent loss of the target in the following frame.



(a)Small objects, Scale [11] (b) Deformation (c) Low illumination, High noise (d) Low visibility, Blur



(e) Complex background (f) Color similarity between fish (g) Camouflage foreground (h) Clutter, Occlusion, High-density scene

(i) Inter-class variations: four different categories [164]

Figure 2.3 *showing the emerging challenges in fish detection*

(Yang et al., 2021)

Figure 2.4 below summarises the challenges discussed in Table 2.2 and Table 2.3.

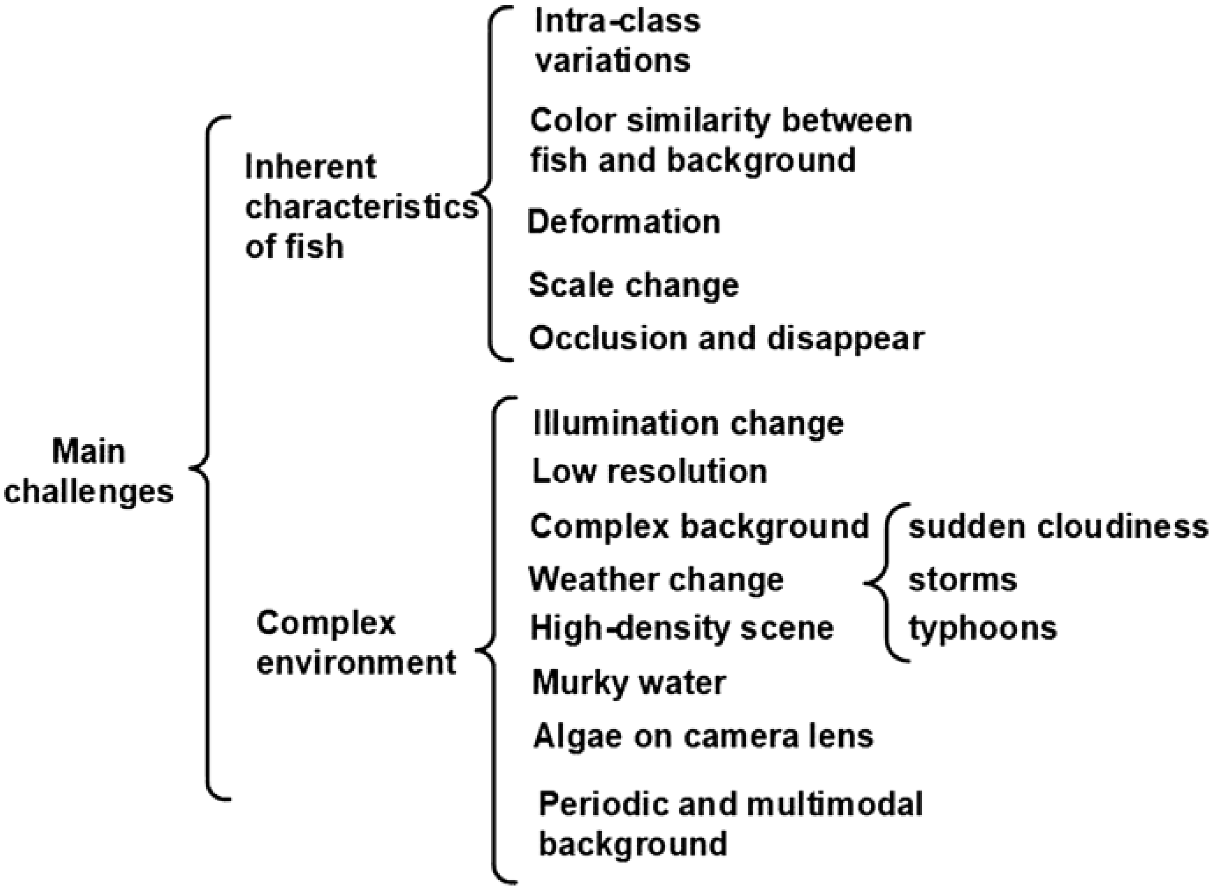


Figure 2.4 *showing the taxonomy of challenges in fish detection*

(Yang et al., 2021)

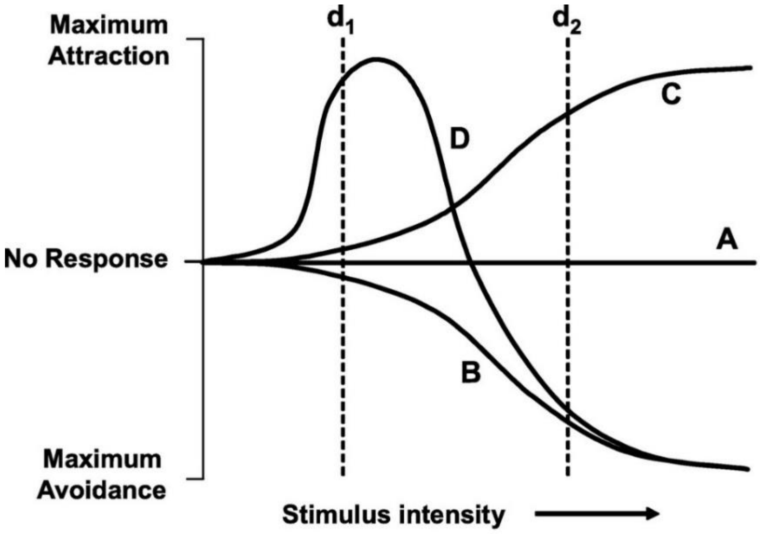
2.3.3 Analysis of Recorded Underwater Footage

Different techniques have been employed to identify and measure fish reactions. These methods encompass informal observations, structured assessments of movements across perspective grids, as well as experiments conducted in field and laboratory settings that are specifically designed to examine particular stimuli (Stoner et al., 2008). Such methods may be used when examining recorded underwater footage to denote which behavioural parameters are affected through specific stimuli, which in the context of this research refers to artificial light.

2.3.3.1 Fish Reaction to Underwater Vehicles

Stoner et al. (2008) describe primarily two methods for the detection and evaluation of fish reactions to underwater vehicles. These behavioural assessments may also be applied for differing contexts, such as the purposes of this study. The assessments include both basic movements and distance sampling and detection functions.

- **Basic movements**
  - Several studies have described fish movements in simple descriptive terms, while other studies have provided detailed accounts of the percentages of fish displaying various behaviours. Examples include a semi-quantitative method that categorised the response of fish into five distinct categories (Adams, 1995), which may be presented on a response curve as shown in Figure 2.5.



Response type	Distance from vehicle d_1	d_2
A	No bias	No bias
B	Small bias	Large negative bias
C	Small bias	Large positive bias
D	Large positive bias	Large negative bias

Figure 2.5 *showing a response curve to a stressor*

(Stoner et al., 2008)

- **Distance sampling and detection functions**
    - Conducting surveys to determine fish density using a line transect or distance estimator can furnish valuable insights for understanding fish movements in response to potential stressors.

2.3.3.2 Fish Metrics

The Fish4Knowledge initiative employs real-time video streams from ten underwater cameras situated in Taiwan's coral reefs. The primary objective of this project is to create an automated system that integrates data capture, video analysis, fish detection, and classification, thereby facilitating marine biologists in studying fish populations, behaviours, and interactions (University of Edinburgh, 2011). Through the analysed video footage, several plots and graphs are created to visually explain the data further. These include:

- **Fish counts week by week over a year**
- **Distribution of the aforementioned counts over daylight hours**
- **Distribution of particular fish species in relation to total fish count**
- **Number of occurrences of a particular species**
- **Comparison of parameters in different locations**

(EMBS Galway, 2014)

Roset et al. (2007) reviewed various quantitative measures have been used to establish specific biological criteria. The Index of Biological Integrity (IBI) has gained widespread application in evaluating the environmental quality of aquatic habitats through the analysis of fish assemblage data. Despite all being Multimetric Indices (MMI), multiple versions of the IBI have been created with characteristics adapting to different regions and habitat types. These indices encompass crucial aspects of community ecology, including **taxonomic richness**, **composition of habitats and trophic guilds**, **individual well-being**, and **abundance**. The majority of these indices follow the **reference condition approach** which entails evaluating an ecosystem exposed to potential stress in comparison to a reference condition representing a natural or near-natural state.

The application of such MMIs are extensive and relevant to several potential stressors. As an example, a study undertaken to assess whether mines exert a regional stress influence on fish made use of several fish metrics. Concomitantly, the study also attempted to characterise any associations between mines and stream fish communities in multiple streams within three ecoregions. The study conducted by Daniel et al. (2015) pursued three key objectives:

- An investigation was carried out to establish correlations between stream fish communities and the occurrences of distinct categories of mining activities within their respective catchment areas. The fish communities were analysed using a variety of functional features as well as other pertinent indicators due to the wide geographic extent.

- A comparison of the responses of specific fish metrics to varying mine densities across different regions was made to assess the uniformity of identified associations.

- A comparison was conducted between the reactions of three chosen fish metrics that were known to exhibit adverse responses to mining, to the effects of urban, agricultural, and impermeable land covers within the same geographical regions. This was done to understand better whether mines are a regional source of stress to fish.

The metrics used in the study were classified into six distinct categories according to how they reflected different ecological aspects and factors relevant to management decisions. These categories encompassed: (1) **aspects of reproductive patterns and life history**, (2) **preferences for habitat**, (3) **trophic ecology**, (4) **assemblage diversity and evenness**, (5) **ability to endure human-induced disruptions**, and (6) **game species recognised by authorities**.

2.3.3.3 Metrics Relevant for BRUV Studies

When using BRUV systems (discussed in Section **2.3.1 Remotely Operated Vehicles and Baited Remote Underwater Video**), further analysis, in addition to the previously stated parameters in Sections **2.3.3.1 (Fish Reaction to Underwater Vehicles and 2.3.3.2 Fish Metrics)**, may be extracted from the footage. Cappo et al. (2001) present findings from preliminary investigations into the relative benefits, sampling effectiveness, and data constraints of surveys conducted using BRUVs. The BRUVs in the aforementioned study were deployed and retrieved using the same method as fish traps, utilising polypropylene rope, and attached marker buoys. For each deployment, the time, depth, latitude, and longitude were recorded. The examination of each recording then yielded the following information:

- **Identification of the habitat type at each deployment site**
- **Time taken for the BRUV system to settle on the seabed** (TOB)
- **The moment when the first sighting of a particular taxa occurred** (TFS)
- **The initial feeding activity of taxa within the field of view** (TFF)
- **The highest number of occurrences of each taxa observed simultaneously on the entire recording** (MaxN)
- **Detailed observations of intraspecific and interspecific behaviour of each taxon**
- **Time taken for all the bait was completely consumed, if such an event occurred**
- **Estimations of fish sizes located directly above a scale bar on the bait canister**

The authors of the study chose MaxN as a convenient under-estimator of the fish count in their BRUVs sample. Based on the notion that the abundance of fish is linked to how frequently they are spotted among replicates within a sequence (n/N BRUVs) and the time elapsed before the first sighting (TFS - TOB), in addition to MaxN, a valuable indicator of abundance for each species could be calculated as follows:

Abundance Indicator = mean MaxN (n/N) / mean (TFS - TOB)

For a species that is typically observed very early and appears in large numbers within the field of view in every BRUVS replicate of a sequence, this indicator will be high. Conversely, species that are seen infrequently, on a few replicates, and in small quantities will yield a low indicator value (Cappo et al., 2001).

Moreover, to address the issue of double counting, tallies of the maximum count (MaxN) of individuals of a particular species seen over the recording duration can be used. In the context of monitoring, comparative investigations have proposed that the use of MaxN might be "hyper-stable" (i.e., it might not accurately represent significant shifts in actual abundance) in cases of high fish abundance, where the field of view becomes saturated. As a result, alternative metrics like MeanCount have been suggested. However, MaxN remains the prevailing and widely accepted metric, offering the most suitable option for standardisation across different sampling initiatives (Bouchet et al., 2018).

3. Methodology

3.1 Study Area

The Ċirkewwa harbour, located at coordinates 35.98333°N latitude and 14.33333°E longitude, lies along the northwest coast of Malta, marking the northernmost point of the island. This harbour is recognised by the United Nations Code for Ports and Other Locations (UN/LOCODE) as MTMRF, also referred to as MARFA. Operating from the Ċirkewwa Ferry Terminal, regular vehicle ferries provide a vital link to the Mġarr port in Gozo. Additionally, organised boat trips, including visits to Comino and other guided excursions, are conducted from Ċirkewwa primarily during the summer season. Moreover, Ċirkewwa is also one of the most popular SCUBA diving locations in the Maltese islands. Other notable features include the presence of a hotel, namely Paradise Bay Resort Hotel, and other establishments within the catering industry in close proximity of the harbour area.

Within the study area, two sites were chosen for the purposes of this study, which were denoted as "Reef" and "Harbour" (Figure 3.1). The notable distinction between the sites predominantly resides in the influx of artificial light each site receives, given that the majority of other environmental variables (such as sea surface temperature, weather conditions, biodiversity, among others) are not expected to differ much across both study sites due to their proximity to one another.

In relation to light, the terms *press*, and *push* disturbances are to be used. A press disturbance signifies a continuous exposure to light, as seen with the persistent illumination from sources like restaurant or streetlights in the 'Harbour' habitat. In contrast, a push light disturbance involves the introduction of light from the BRUV.

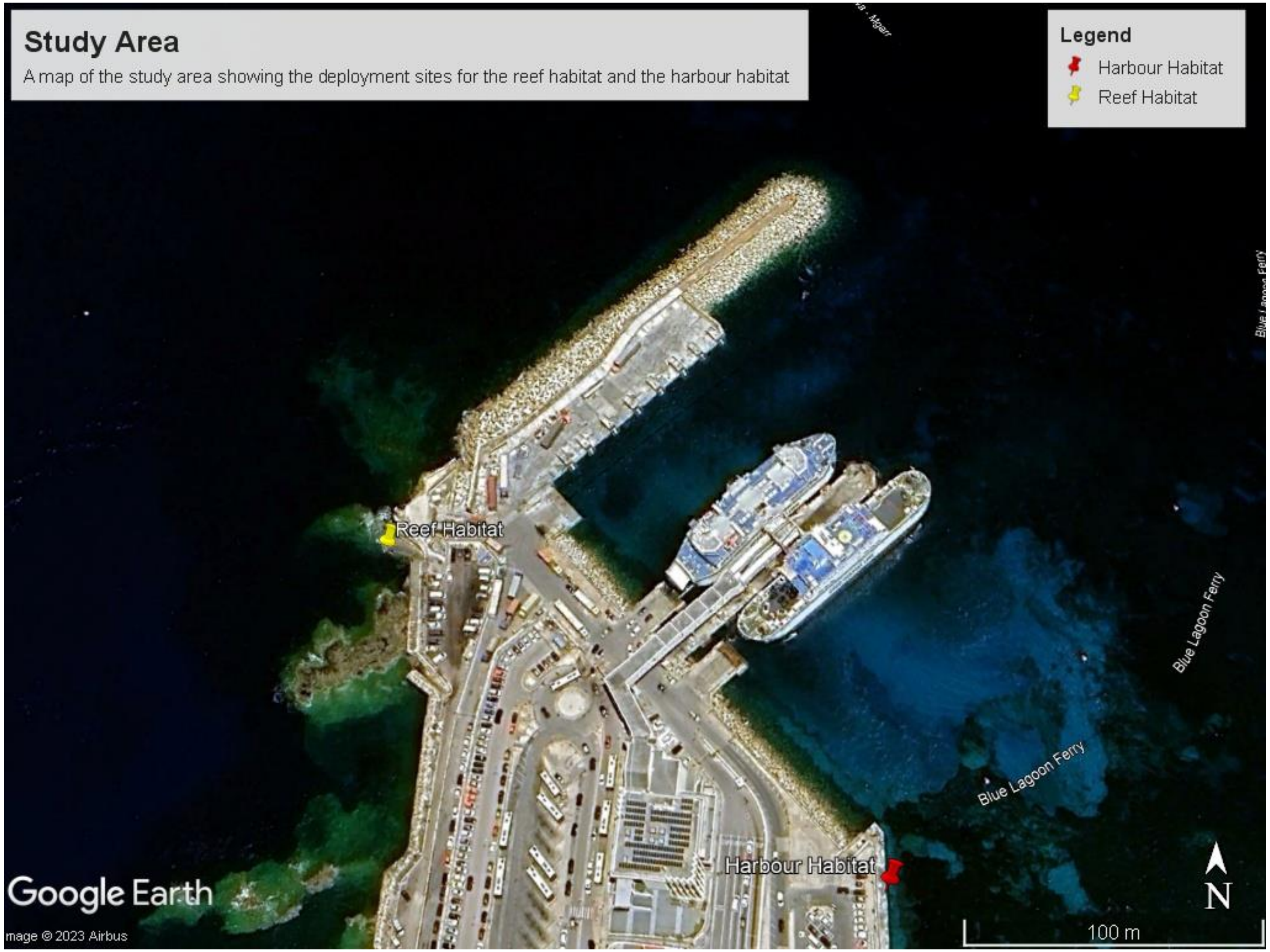


Figure 3.1 *showing a map of the study area*

(Google Maps, 2023)

3.1.1 Reef (Dark)

The selection of the site was based on the putative absence of artificial light characterising the same, thereby enabling a comparative analysis with the light-polluted harbour site (refer to Section 3.1.2 Harbour). The photometer reading at the deployment location (35.989253°N, 14.327892°E) within the site was in fact that of 0 Lm.

The site is exposed to the prevailing north-westerly winds which occur on an average of 20.7% of all the days in a year (Galdies, 2011). According to the Marine Strategy Framework Directive (MSFD) habitat classification, the operational site of the BRUV was recording is classified as shallow sublittoral with its topography being denoted as coastal water (Environment and Resources Authority, 2015). At the site, the prevailing seabed composition is a rocky reef substrate that features substantial rocky outcroppings of a horizontal orientation. From the collected data it was clear that the extensive rocky reefs support various coral and algal species. *Posidonia oceanica* meadows were also present in the surrounding areas.

The site (shown in Figure 3.1) is located within the Ċirkewwa Marine Park (shown in Figure 3.2). The Ċirkewwa Marine Park receives funding from the Malta Tourism Authority and is executed by Nature Trust FEE Malta. It aims to establish a versatile zone that seeks to conserve the existing habitats and species in the region while concurrently fostering sustainable tourism. The Ċirkewwa Marine Park is encompassed within the Natura 2000 site MT 0000112 and includes three primary diving locations (Environment & Resources Authority, 2023). The park's ecological significance is mostly attributed to varied geomorphological characteristics, healthy *Posidonia oceanica* (Neptune seagrass) beds, and the presence of species of global conservation significance (Ċirkewwa Marine Park, 2023).

Figure 3.3 *showing the designated Ċirkewwa Marine Park conservation area*

(Ċirkewwa Marine Park, 2023)

Figure 3.2 *showing the surrounding area as seen from the deployment point within the Reef habitat*

3.1.2 Harbour (Already-Present ALAN)

The site was chosen in an artifical light-polluted area along the north-easterly quay that serves as a departure point for the Blue Lagoon Ferry. The photometer reading at the deployment location (35.988169°N, 14.329861°E) within the harbour site was 80 Lm, going up to 145 Lm when charter boats pass by.

The site is exposed to north-easterly winds which show no dominance on the Maltese Islands (Galdies, 2011). According to the Marine Strategy Framework Directive (MSFD) habitat classification, the operational site of the BRUV was recording is classified as shallow sublittoral with its topography being denoted as coastal water (Environment and Resources Authority, 2015). The prevailing seabed composition at the recording site was a rocky reef substrate, with some sparsely-distributed sand pockets in adjacent regions. Within proximity to where the BRUV apparatus was placed, the terrain was also interspersed with sizeable rocks and debris. *Posidonia oceanica* meadows were also present in close proximity to the operational monitoring site.

The harbour site (shown in Figure 3.4) presents an advantageous environment for examining the impact of artificial light on fish and other benthic organisms due to its exposure to multiple sources of artificial illumination. The principal sources of artificial light at the treatment site include streetlights in proximity, illumination emitted by the Gozo Channel Ferry and charter boats (either stationed at the quay or traversing the area), and emissions from nearby dining establishments, particularly Porto Lounge. The estimated lux level for a charter boat in the vicinity as calculated from land (the nearby quay) was that of 144.5 lux (Figure 3.6). The artificial light originating from Porto Lounge remained consistently uniform throughout the observational period, maintaining a stable luminance level and temporal consistency. In contrast, the light disturbance sourced from charter boats exhibited a non-uniform pattern. The incidence of charter boats varied across sampling instances, with some sessions witnessing a heightened presence and others displaying a comparatively negligible presence. Moreover, the durations of charter boat presence exhibited notable fluctuations.

Figure 3.4 *showing the deployment area as seen from across*

Figure 3.5 *showing over-illumination at the Ċirkewwa harbour*

(Crymble, 2020)

Figure 3.6 *showing a charter boat docked at the quay*

3.2 BRUV Structure and Equipment

A horizontal-facing single camera BRUV that was crafted from easily-accessible materials was employed for the study (Figure 3.7). For the purposes of this study, the BRUV was stationary and placed on the seafloor during sampling.

The frame of the BRUV, which spans an area of approximately 355 mm by 400 mm, was constructed using Polyvinyl Chloride (PVC) irrigation piping. The choice of material was due to its capacity to withstand the hydrostatic pressures encountered at the control and treatment sites whilst also being economically-viable. The assembly process involved connecting lengths of PVC pipe and their associated fittings using bicomponent glue, ensuring a durable and coherent construction. Several holes were then drilled in the PVC pipes to allow water to ingress into the structure, ensuring negative buoyancy once deployed. Additionally, a two two-kilogram weights were affixed to the posterior section of the structure to enhance its stability and hold it in place while deployed. The bait arm was also constructed from a PVC pipe and measured a length of 1 m from the central point of the BRUV. During deployment, the bait arm was suspended at an elevation of 260 mm above the seabed and accommodated a bait bag attached to the end of the arm. The bait bag measured 150 mm by 120 mm and was made from woven wire mesh. A rope was also attached to the BRUV structure to facilitate the process of deploying and retrieving the apparatus.

A tray mount with underwater camera housing and two flexi arms for torch attachments was then affixed to the structure. The tray mount was equipped with a GoPro Hero 10 Action Video Camera ^{TM}, which served as the primary means for capturing video sequences. This specific model was used due to its integration of a SuperView mode, which records in an immersive wide-angle perspective. Footage was recorded at a resolution of 1080p, and a frame rate of 60 frames per second. Supplementary waterproof housing was employed to safeguard the camera while deployed. Two AL2600XWP-II (Black Molly V) torches were used during each replicate recording. Each torch has a maximum light output of 2600 Lm at 6500K colour temperature. Moreover, the torches were also equipped with integrated red LEDs to improve focus and facilitate nighttime video tasks. The beam angle of the torches is set at 120 degrees.

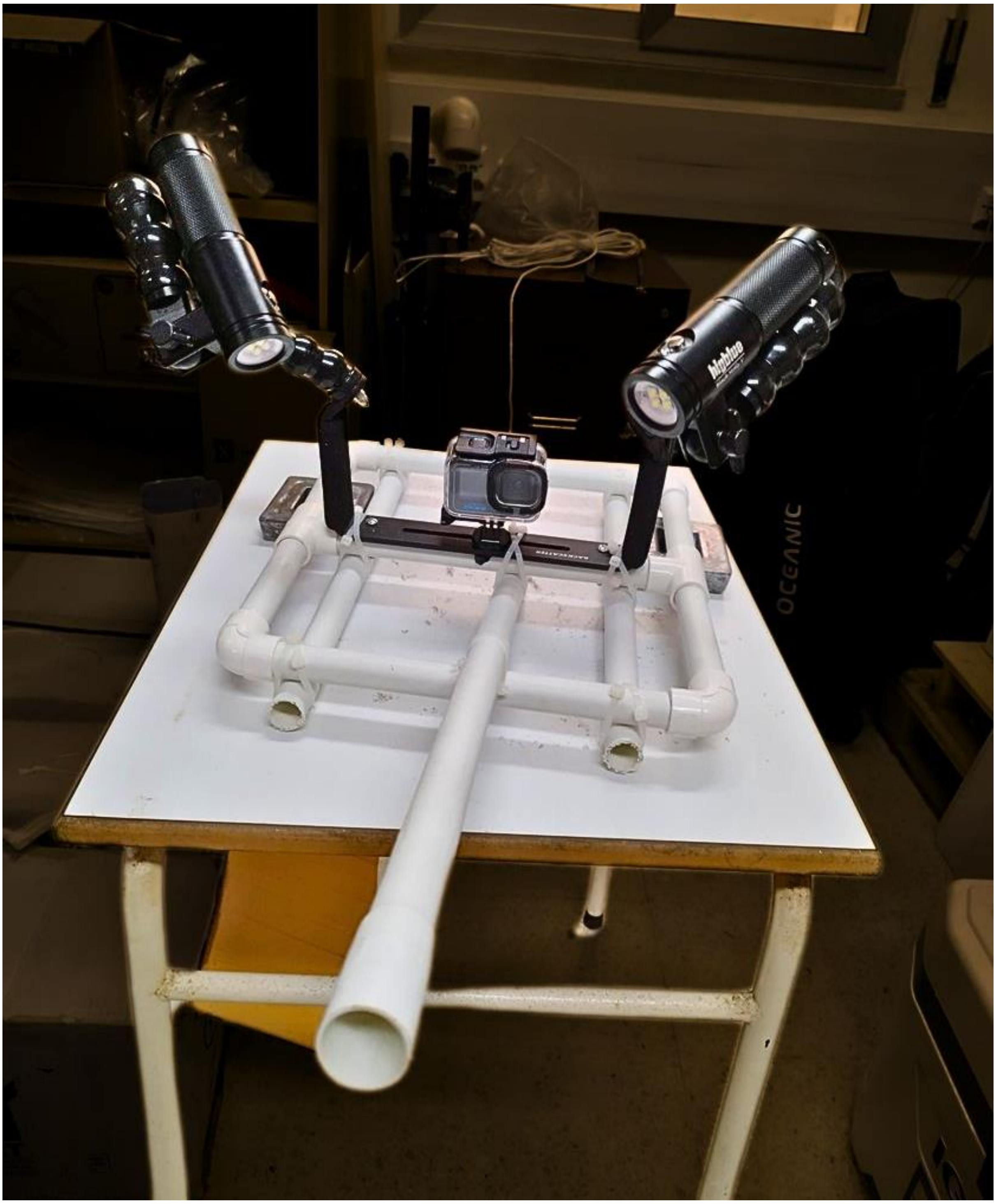


Figure 3.*7 showing the frame of the BRUV with the camera, torches, and weights attached*

Prior to each deployment, 250 grams of bait mix was enclosed in the bait bag. This included a mixture of chopped Bullet tuna *(Auxis rochei)* and European sprat (*Sprattus sprattus)* in a 4:1 ration, respectively. Both fish species have a high oil content which makes them ideal bait (Wraith et al., 2013).

Figure 3.8 *showing the preparation of the bait mix for the samples*

3.3 Monitoring Regime

During August 2023, a total of eighteen video samples were collected (3 light treatments x 2 habitats x 3 replicates per treatment). Each video sample had a duration of one hour, and the sampling protocol involved obtaining two samples per night of sampling, one from the harbour site and another from the reef site. The initial sampling consistently commenced at 10:00pm, ensuring synchronisation with the circadian rhythm of fish. Subsequently, the second sample at the alternative site was then initiated at 11:30pm and extended until 12:30am. In accordance with the specifications outlined in Table 3.1 and Table 3.2, three distinct light treatments were applied, encompassing red light, white light at low light intensity, and white light at high intensity. Each of these light treatments were replicated three times at each research site.

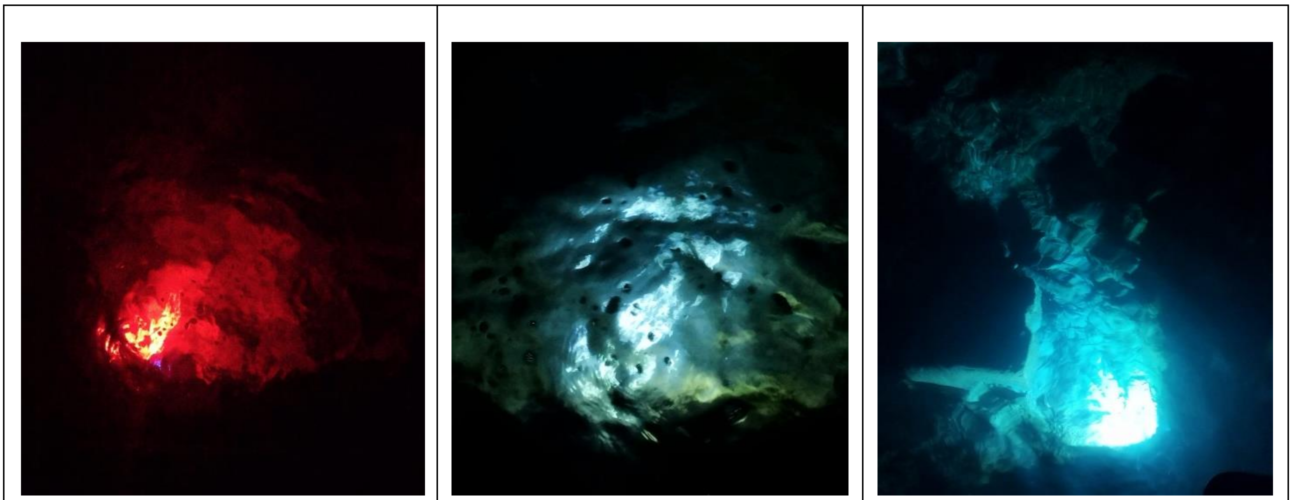
Table 3.1 *showing the light treatment categories at the reef site*

Treatment	Reef
R	[Red Light (150 Lm) x 2 torches]
L	[White Light (650 Lm) x 2 torches]
H	[White Light (1300 Lm) x 2 torches]

Table 3.2 *showing the light treatment categories at the harbour site*

Treatment	Harbour
R	[Red Light (150 Lm) x 2 torches] + [Already Present Artificial Light]
L	[White Light (650 Lm) x 2 torches] + [Already Present Artificial Light]
H	[White Light (1300 Lm) x 2 torches] + [Already Present Artificial Light]

During the first night of sampling, the photometer readings stated in Section **1.1 Study Area** were also measured. A mobile phone photometer application was used to quantify light intensity. It operates by utilising the device's camera to capture light, and then uses specialised software to process the captured images by converting pixel values into measurable light levels.



Red Light **Low Intensity White Light** **Hight Intensity White Light**

Figure 3.9 *showing the three-lighting setting used for the samples*

3.4 Observation Protocols

Each collected video sequence was examined at a playback speed of 0.4x to ensure a comprehensive and accurate assessment. The variable computed for every species in each video was denoted as "Count-N," signifying the cumulative count of individual members of a species observed during a specific monitoring duration, in alignment with previous studies (Schobernd et al., 2013; Mallet and Pelletier, 2014; Wartenburg and Booth, 2014). Count-N serves the purpose of enumerating and distinguishing all the individuals noted within the "digital transects," essentially emulating an in-situ slate-transect enumeration method. The widely used MaxN approach was not used in this study since the recordings were taken for a one-hour period. This method would have defined relative abundance as the maximum number of individuals present simultaneously in the field of view. It is widely used for its focus on relative abundance rather than accurate counting, thereby avoiding repeated counting of the same fish. However, relying on a single MaxN reading over a one-hour duration may yield misleading data regarding the average abundance of fish during that timeframe. This issue becomes apparent when deploying in an area with prolonged periods of fish absence, except for a brief passage of a large school, which was the case for some samples. In such instances, a solitary MaxN reading does not accurately represent the fish abundance over the hour-long deployment. On the contrary, Count-N meticulously identifies and tallies all individual fish that enter the video frame and this procedure was systematically applied to all the videos.

In the case of the red-light treatment, it was found that viewing the videos in grayscale facilitated the accentuation of discernible patterns, thereby enhancing the quality of the analytical process. In cases where precise species identification was not possible, a taxonomical classification at a broader level, such as genus or family, was assigned.

Among the primary challenges outlined in Section **2.3.3**, this study identified those most relevant to sampling aspects relating to the **inherent characteristics of fish** to be deformation, and scale change. The challenge arised particularly when attempting to identify fish based on their relative size (scale change) and shape (deformation), among other characteristics. The perception of size and shape varied depending on the proximity of the fish to the camera, which was at times difficult to visually perceive. intricacies highlight the importance of addressing deformation and scale change considerations in the methodology, acknowledging their potential impact on accurate fish identification in the study. Meanwhile, the most relevant challenge posed to this study relating to **effects and conditions** that frequently appear in underwater settings was murky water.

3.5 Multivariate Analysis of Community Assemblage

A data matrix was constructed using the Count-N values of the identified species for every sample. A shade plot of the raw data matrix was created to visualise the distribution of the assemblage. Shade plots offer a straightforward representation of a frequency matrix: white space indicates species that were never observed, while the degree of shading corresponds to the taxon's density (Clarke et al., 2014). To balance the contributions of species showing punctual heightened frequencies in the data matrix with those of less common species, a dispersion weighting routine from Primer 7 was employed. Subsequently, the weighted data was square-root transformed to stabilise variance and make it more consistent across different values and was then used to compute a Bray-Curtis Index (abundance) into a triangular matrix of distances. To visualise the full assemblage against the factors tested in an ordination plot, a nonmetric Multidimensional Scaling (nMDS) was used. nMDS is a robust statistical technique, that allows the visualisation of intricate multivariate datasets in a reduced number of dimensions. Assessing the adequacy of an nMDS ordination often involves examining ordination "stress," which refers to data distortion against established heuristic guidelines (Dexter et al., 2018). Bubble overlays were also applied to visualise and compare the abundances of the most statistically significant species in the dataset.

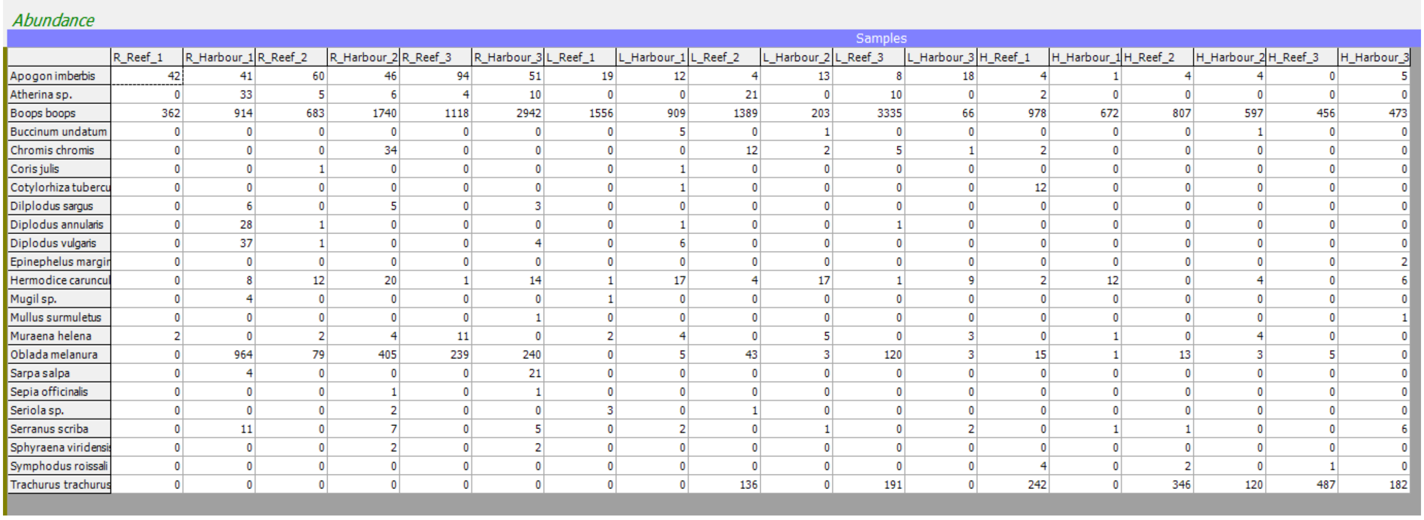
Within a multivariate analytical framework, differences were then assessed using the Primer v7 and PERMANOVA+ software packages. PERMANOVA+ is an extension module compatible with PRIMER v7, broadening the applicability of resemblance-based techniques to analyse multivariate or univariate data within the context of multifaceted sampling designs, intricate experiments, or gradients (PRIMER-e, 2023). Differences were analysed within the context of a two-factor analysis, where the factors investigated were the ‘Light Treatment’ used [LEVELS = Red Light (R), Low Intensity White Light (L), and High Intensity White Light (H)] and the ‘Habitat’ (LEVELS = Reef and Harbour). The exploration of significant distinctions between levels of the factors was then achieved through pairwise testing.

A SIMPER analysis was performed to identify the species that collectively accounted for a minimum of 70% of the assemblage within the "light treatment" factor. A Principal Coordinates Ordination (PCO) test was conducted to gain insights into the complex relationships between the different samples in the dataset. In this case, the PCO offered a valuable means of visualising and understanding the patterns of community composition and dissimilarity in response to the varying light treatments. This test reduces the dimensionality of the data and identifies the key axes that explain variation. The Shannon Index was calculated as a measure of biodiversity. This metric is important because it quantifies and provides insight into the diversity and evenness of species within an ecological community.

4. Results

4.1 Descriptive Metrics

A total of 23,955 individuals belonging to eighteen species of fish, two species of mollusc, one species of cnidarian, and one species of annelid were recorded and identified during this study from the recorded videos (18 videos of one hour each) (Figure 4.1). Of the 23,955 reported individuals, 19,200 individuals belong to the species *Boops boops.* (refer to **Appendix** for the full list of species)



Abundance

	Samples																	
	R_Reef_1	R_Harbour_1	R_Reef_2	R_Harbour_2	R_Reef_3	R_Harbour_3	L_Reef_1	L_Harbour_1	L_Reef_2	L_Harbour_2	L_Reef_3	L_Harbour_3	H_Reef_1	H_Harbour_1	H_Reef_2	H_Harbour_2	H_Reef_3	H_Harbour_3
Apogon imberbis	42	41	60	46	94	51	19	12	4	13	8	18	4	1	4	4	0	5
Atherina sp.	0	33	5	6	4	10	0	0	21	0	10	0	2	0	0	0	0	0
Boops boops	362	914	683	1740	1118	2942	1556	909	1389	203	3335	66	978	672	807	597	456	473
Buccinum undatum	0	0	0	0	0	0	0	5	0	1	0	0	0	0	0	1	0	0
Chromis chromis	0	0	0	34	0	0	0	0	12	2	5	1	2	0	0	0	0	0
Coris julis	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Cotylorhiza tubercu	0	0	0	0	0	0	0	1	0	0	0	0	12	0	0	0	0	0
Dilplodus sargus	0	6	0	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Diplodus annularis	0	28	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Diplodus vulgaris	0	37	1	0	0	4	0	6	0	0	0	0	0	0	0	0	0	0
Epinephelus margi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Hermodice caruncul	0	8	12	20	1	14	1	17	4	17	1	9	2	12	0	4	0	6
Mugil sp.	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Mullus surmuletus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Muraena helena	2	0	2	4	11	0	2	4	0	5	0	3	0	1	0	4	0	0
Oblada melanura	0	964	79	405	239	240	0	5	43	3	120	3	15	1	13	3	5	0
Sarpa salpa	0	4	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0
Sepia officinalis	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Seriola sp.	0	0	0	2	0	0	3	0	1	0	0	0	0	0	0	0	0	0
Serranus scriba	0	11	0	7	0	5	0	2	0	1	0	2	0	1	1	0	0	6
Sphyraena viridensi	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Symphodus roissali	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2	0	1	0
Trachurus trachurus	0	0	0	0	0	0	0	0	136	0	191	0	242	0	346	120	487	182

Figure 4.1 *showing the raw data denoted as 'Count-N' for every sample*

4.2 Environmental Factors

Key environmental factors that may influence the results were recorded for each sample. These include Wind Direction (degrees), Wind Speed (m/s), Lunar Phase (% illumination), and Cloud Coverage (%). Wind factors were examined for their potential impact on fish assemblage (as was suggested by Beyst et al., 2001; Milardi et al., 2019), while the lunar illumination and cloud coverage were scrutinised for their direct influence on light conditions and, consequently, fish assemblages (as was suggested by Hernández-León, 2008; Kingsford & Finn, 1997).

The bar graph shown in in Figure 4.2 illustrates the distinct impact of lunar illumination and cloud coverage on nocturnal light conditions throughout the sampling period. Lunar illumination, portrayed as a percentage, signifies the varying brightness of the moon. This becomes particularly crucial in clear skies. In contrast, cloud coverage, also depicted as a percentage, gauges the extent to which clouds obstruct lunar light. By presenting these two factors side by side, the graph highlights their independent contributions to the overall ambient light per sample.

The wind direction and speed data recorded per night of sampling shown in Figure 4.3, exhibit variability in both parameters. Wind direction values range widely, covering a spectrum from 65 to 335 degrees, indicating diverse wind patterns. The corresponding wind speed values, measured in meters per second, also vary, showcasing fluctuations between 0.514 and 3.087 m/s. This dataset suggests a dynamic and changing atmospheric environment during the sampling nights, reflecting the diverse wind conditions experienced across different measurement instances.

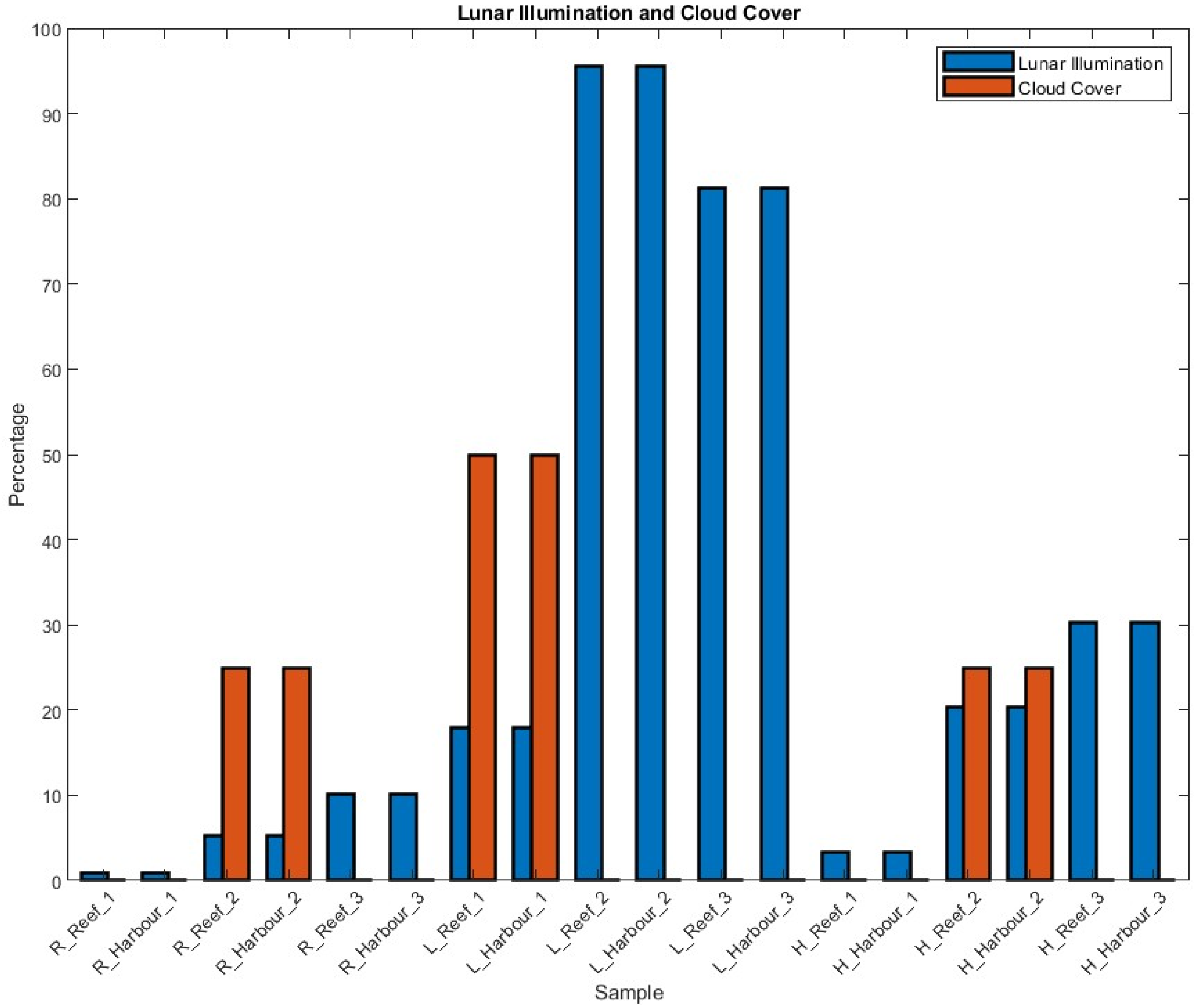


Figure 4.2 *showing the lunar illumination and cloud cover percentage for every sample*

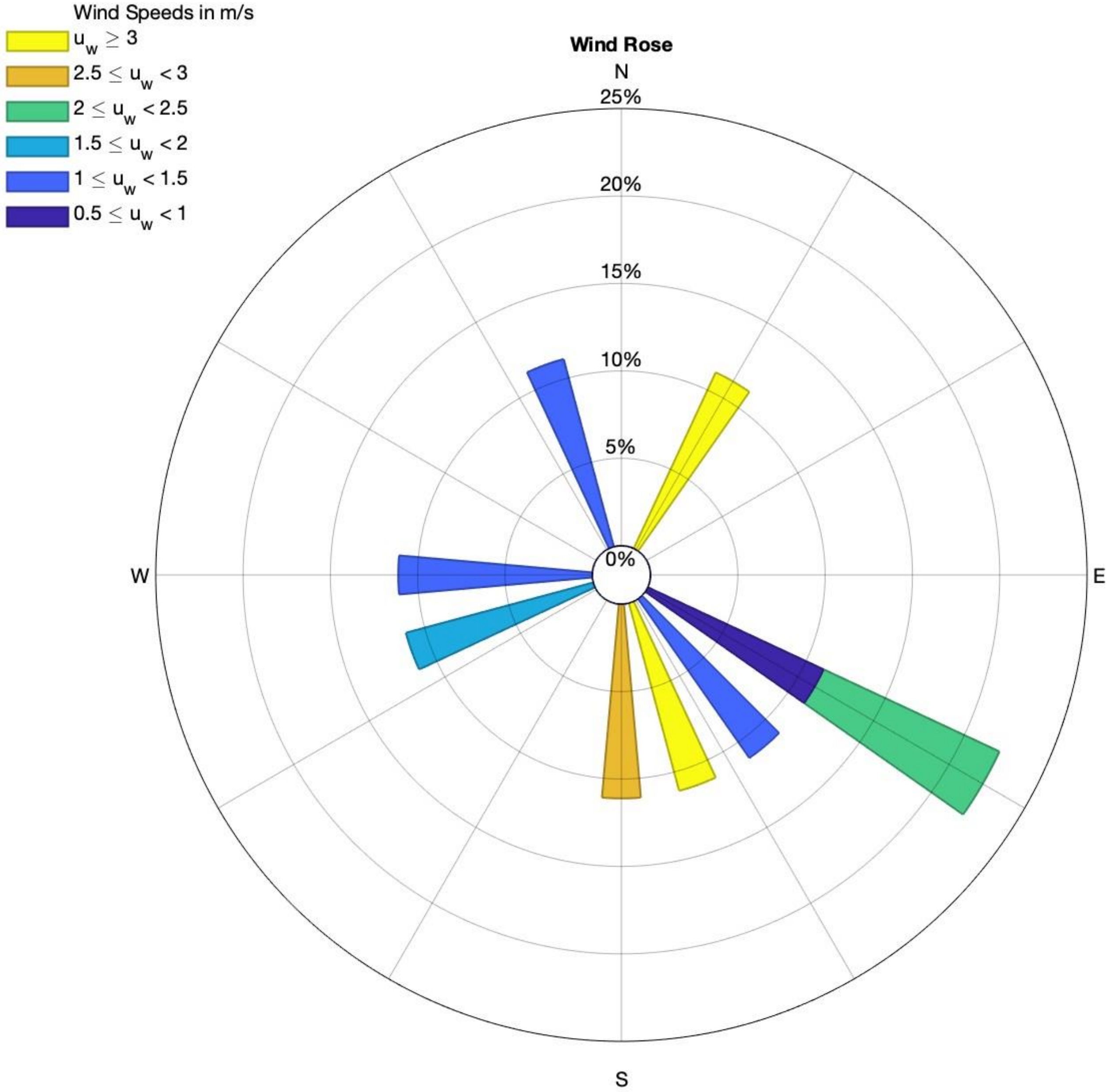
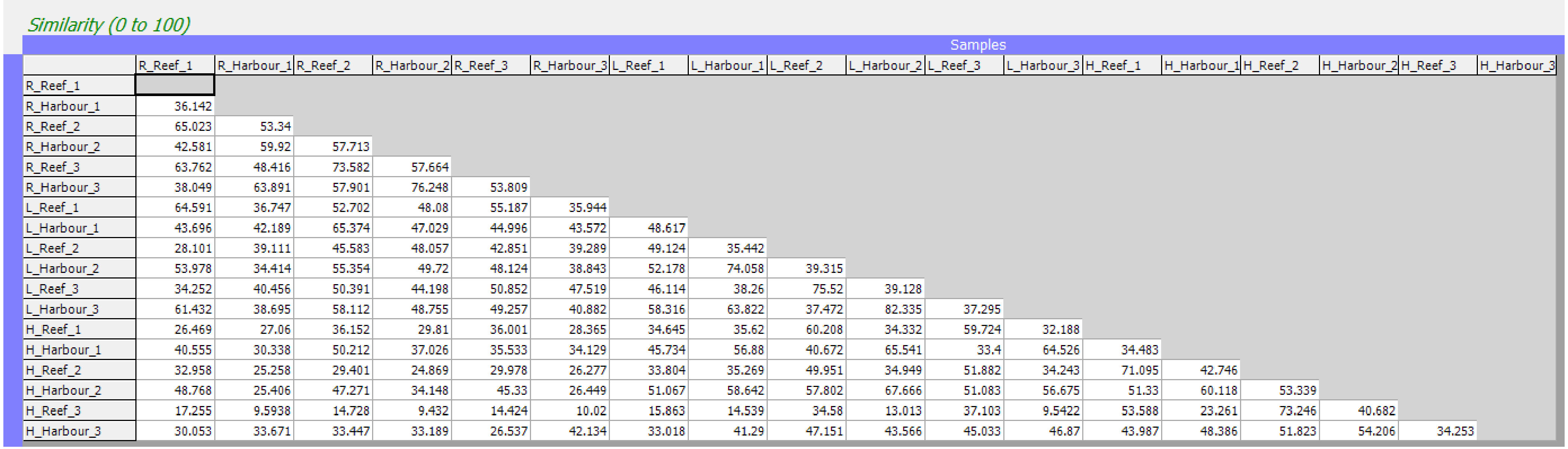


Figure 4.3 *showing a wind rose including data from all the sampling nights*

4.3 Statistical Analyses

As specified in Section **3.5 (Multivariate Analysis of Community Assemblage)**, a triangular matrix of distances for each sample was calculated using PRIMER (Figure 4.4). Apart from providing a structured and quantifiable representation of the dissimilarities or similarities between all of the samples, the triangular matrix of distances also served as the foundation for multivariate analysis techniques used, such as the nMDS, and other ordination methods.

A weighted shade plot with square root transformation was also created (Figure 4.5) as it is a valuable technique in ecological and biodiversity research. It enhances data normalisation, improves visualisation, emphasises the importance of rare species, and facilitates the interpretation and comparison of data across different conditions or treatments.



Similarity (0 to 100)

Samples

	R_Reef_1	R_Harbour_1	R_Reef_2	R_Harbour_2	R_Reef_3	R_Harbour_3	L_Reef_1	L_Harbour_1	L_Reef_2	L_Harbour_2	L_Reef_3	L_Harbour_3	H_Reef_1	H_Harbour_1	H_Reef_2	H_Harbour_2	H_Reef_3	H_Harbour_3
R_Reef_1																		
R_Harbour_1	36.142																	
R_Reef_2	65.023	53.34																
R_Harbour_2	42.581	59.92	57.713															
R_Reef_3	63.762	48.416	73.582	57.664														
R_Harbour_3	38.049	63.891	57.901	76.248	53.809													
L_Reef_1	64.591	36.747	52.702	48.08	55.187	35.944												
L_Harbour_1	43.696	42.189	65.374	47.029	44.996	43.572	48.617											
L_Reef_2	28.101	39.111	45.583	48.057	42.851	39.289	49.124	35.442										
L_Harbour_2	53.978	34.414	55.354	49.72	48.124	38.843	52.178	74.058	39.315									
L_Reef_3	34.252	40.456	50.391	44.198	50.852	47.519	46.114	38.26	75.52	39.128								
L_Harbour_3	61.432	38.695	58.112	48.755	49.257	40.882	58.316	63.822	37.472	82.335	37.295							
H_Reef_1	26.469	27.06	36.152	29.81	36.001	28.365	34.645	35.62	60.208	34.332	59.724	32.188						
H_Harbour_1	40.555	30.338	50.212	37.026	35.533	34.129	45.734	56.88	40.672	65.541	33.4	64.526	34.483					
H_Reef_2	32.958	25.258	29.401	24.869	29.978	26.277	33.804	35.269	49.951	34.949	51.882	34.243	71.095	42.746				
H_Harbour_2	48.768	25.406	47.271	34.148	45.33	26.449	51.067	58.642	57.802	67.666	51.083	56.675	51.33	60.118	53.339			
H_Reef_3	17.255	9.5938	14.728	9.432	14.424	10.02	15.863	14.539	34.58	13.013	37.103	9.5422	53.588	23.261	73.246	40.682		
H_Harbour_3	30.053	33.671	33.447	33.189	26.537	42.134	33.018	41.29	47.151	43.566	45.033	46.87	43.987	48.386	51.823	54.206	34.253	

Figure 4.4 *showing a similarity matrix computed on the basis of the Bray-Curtis similarity index*

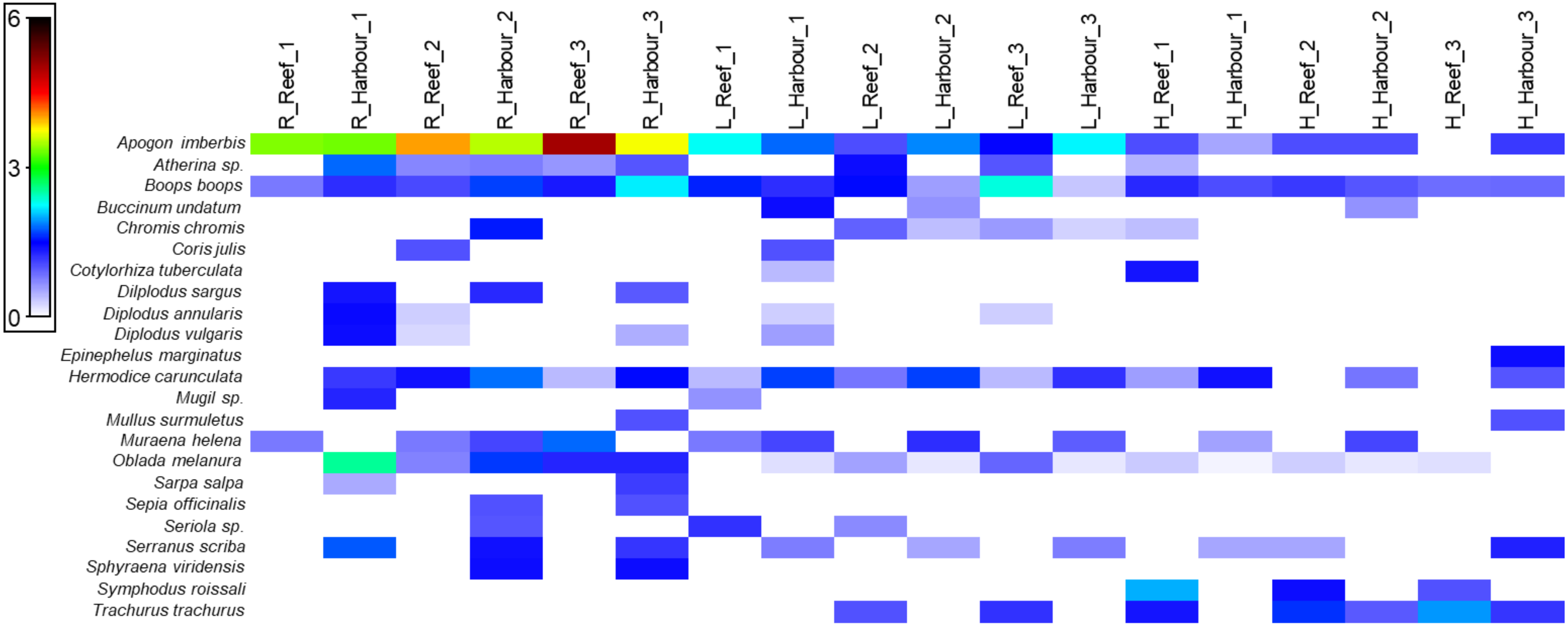


***Figure 4.5** showing a weighted shade plot with square root transformation*

In the nMDS plot shown in Figure 4.6, the proximity of the elements indicates the similarity in community composition among samples, thereby suggesting that the corresponding light conditions had relatively consistent effects on community structure. Conversely, elements positioned farther apart indicate dissimilar communities, indicating that the light treatments had a more pronounced impact on community dissimilarity within the specific habitats.

With a resulting stress value of 0.13 for the output nMDS, a relatively good representation (threshold of 0.20 as suggested by Dexter et al., 2018) of the data in a two-dimensional space was obtained. This value indicates that the configuration of points in the reduced-dimensional space is a reasonably accurate reflection of the dissimilarity structure present in the original multi-dimensional dataset.

While habitat plays a role in shaping community composition, a notable observation is that light conditions appear to exert a more significant influence. This inference is supported by the distinct cluster groupings evident in the nMDS plot which closely align with the various lighting treatments. This finding underscores the ecological significance of light as a key determinant of community composition within different habitats.

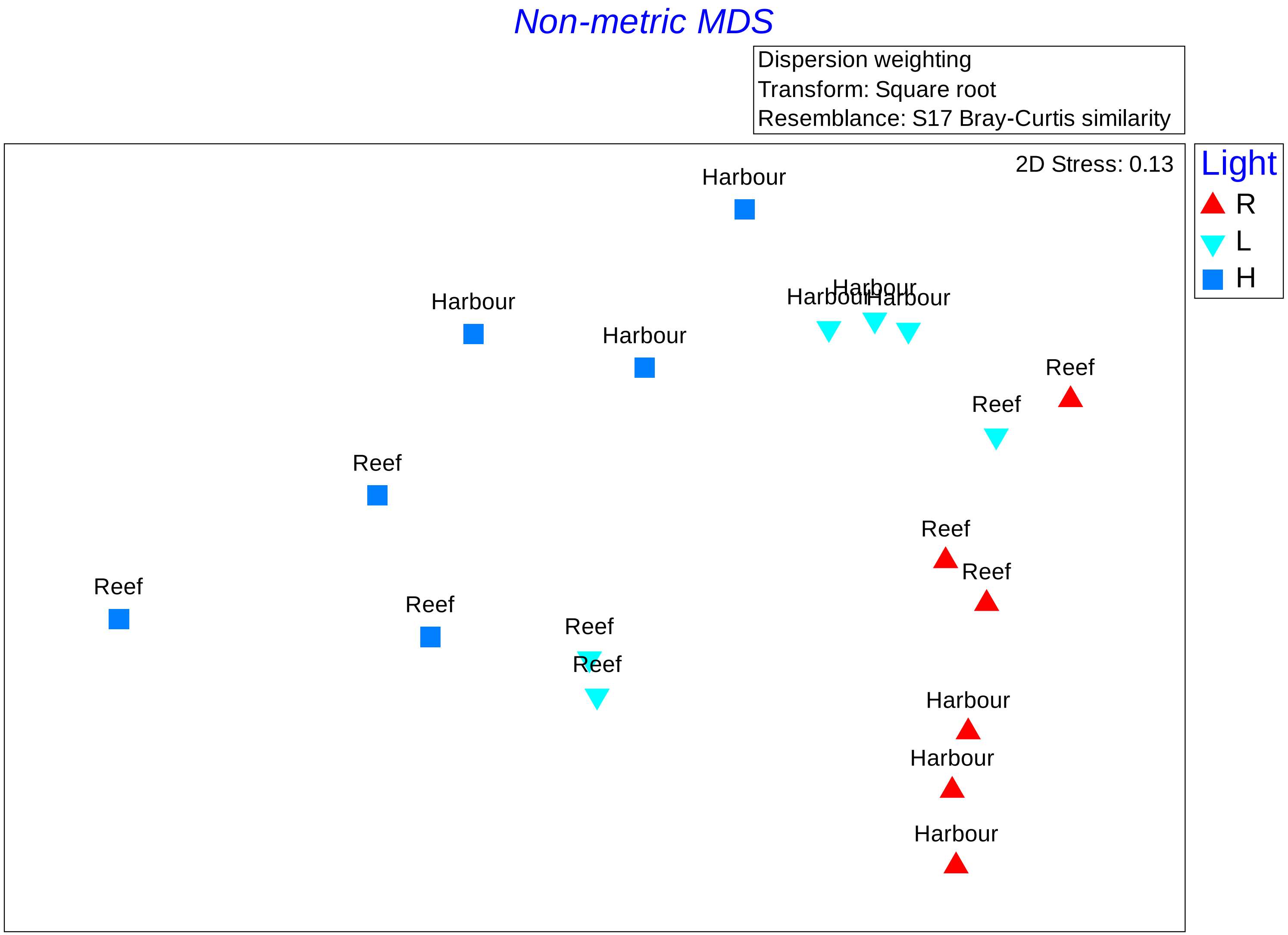


***Figure 4.6** showing a nonmetric Multidimensional Scaling plot for all the samples*

Key: R= Red light, L= Low-light intensity, H= High-light intensity

Two bubble nMDS plots were generated to assess the impact of lighting conditions and habitat types on the most notable species.

The bubble nMDS plot show in Figure 4.7 highlights the distribution and occurrence patterns of three distinct species (*Hermodice carunculata*, *Muraena helena*, and *Serranus scriba)* in relation to habitat type. *Hermodice carunculata* was observed in both the 'harbour' and 'reef' habitats. However, its abundance in the 'reef' was notably lower than in the 'harbour' environment. While it was present in all 'harbour' samples, it was absent in some 'reef' samples, indicating its preference for the 'harbour' habitat. *Serranus scriba* displayed a similar trend, with a more prominent presence in the 'harbour' habitat compared to the 'reef' habitat. However, this species was even less common in 'reef' samples, also indicating a preference for the 'harbour' environment. In contrast, *Muraena helena* demonstrated a more balanced distribution between the two habitats. However, it is worth noting that there were occasions where this species was absent in both habitat types, suggesting that its presence was not consistent across all samples.

The bubble nMDS plot show in in Figure 4.8 visualises how four key species (*Apogon imberbis, Atherina sp., Trachurus trachurus,* and *Boops boops*) respond to the varying light treatments. *Apogon imberbis* exhibits a clear preference for the 'Red Light' condition, as evidenced by its highest frequency of appearances within this lighting environment. Under 'Low Intensity' conditions, its presence remains notable but decreases, and when subjected to 'High Intensity' light conditions, its occurrences diminish even further. These observations suggest that ALAN was likely a disturbance for *Apogon imberbis* as this species had an apparent preference for subdued or 'Red Light' environments. Similarly, *Atherina sp.* demonstrates a preference for both 'Red Light' and 'Low Intensity' conditions, as it was observed in these lighting environments. However, it made only one appearance in the 'High Intensity' light conditions, suggesting a notable avoidance of intense lighting settings. *Trachurus trachurus* exhibited a strong affinity for 'High Intensity' light conditions, appearing in nearly all samples subjected to this lighting environment. While it also made occasional appearances in 'Low Light' conditions, its clear preference for high-intensity lighting is evident. Remarkably, this species was notably abundant in 'High Intensity' conditions, despite being never observed in 'Red Light' conditions. *Boops boops* displayed a noteworthy presence across all light conditions, with a particularly notable frequency of sightings in the 'Red Light' environment. While its abundance decreased with the rise in light intensity, it remained a commonly observed species in all conditions, emphasising its adaptability and consistent presence across varying light treatments.

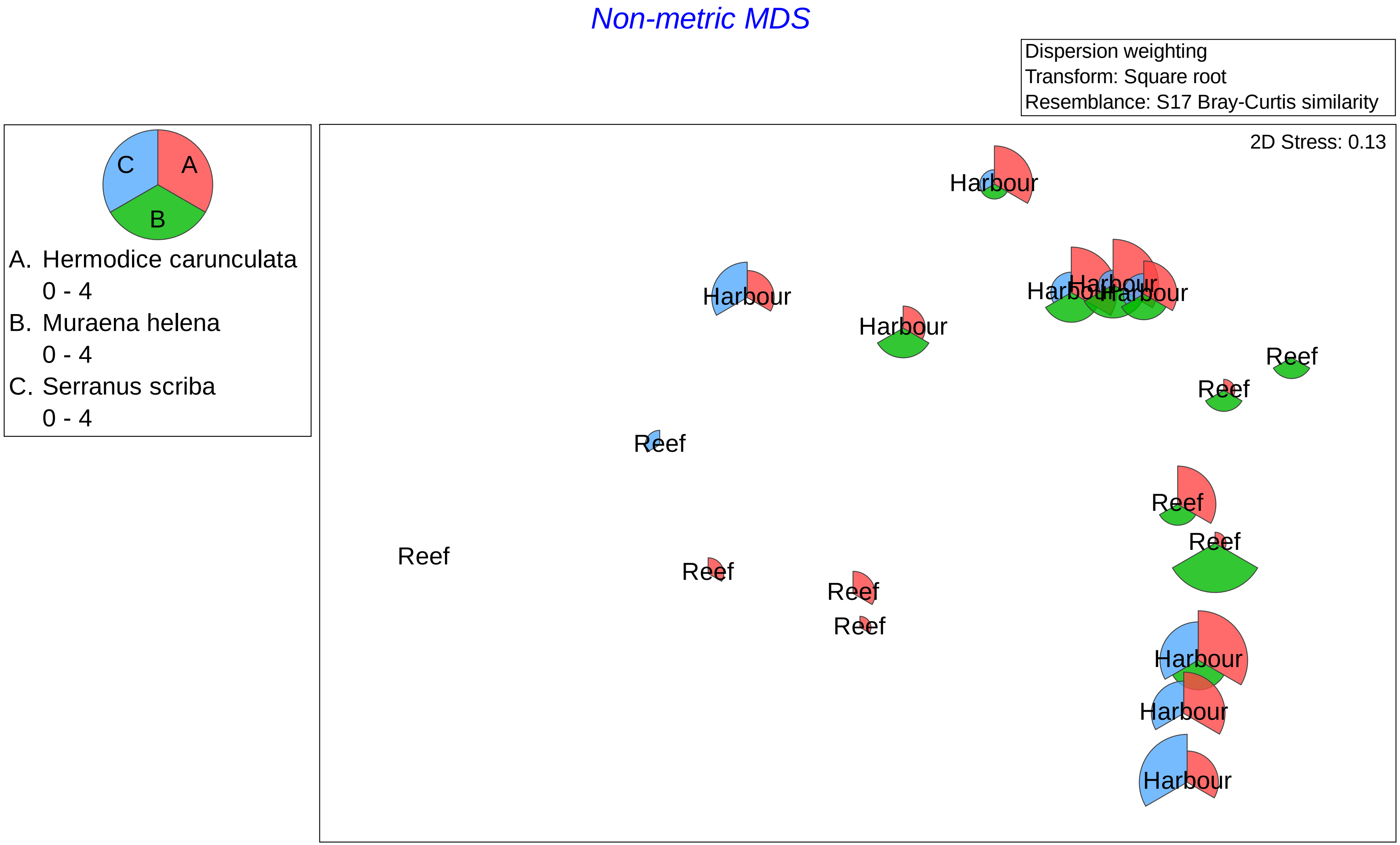


Figure 4.7 *showing a nonmetric Multidimensional Scaling plot for Hermodice caranculata, Muraena helena, and Serranus scriba in the samples*

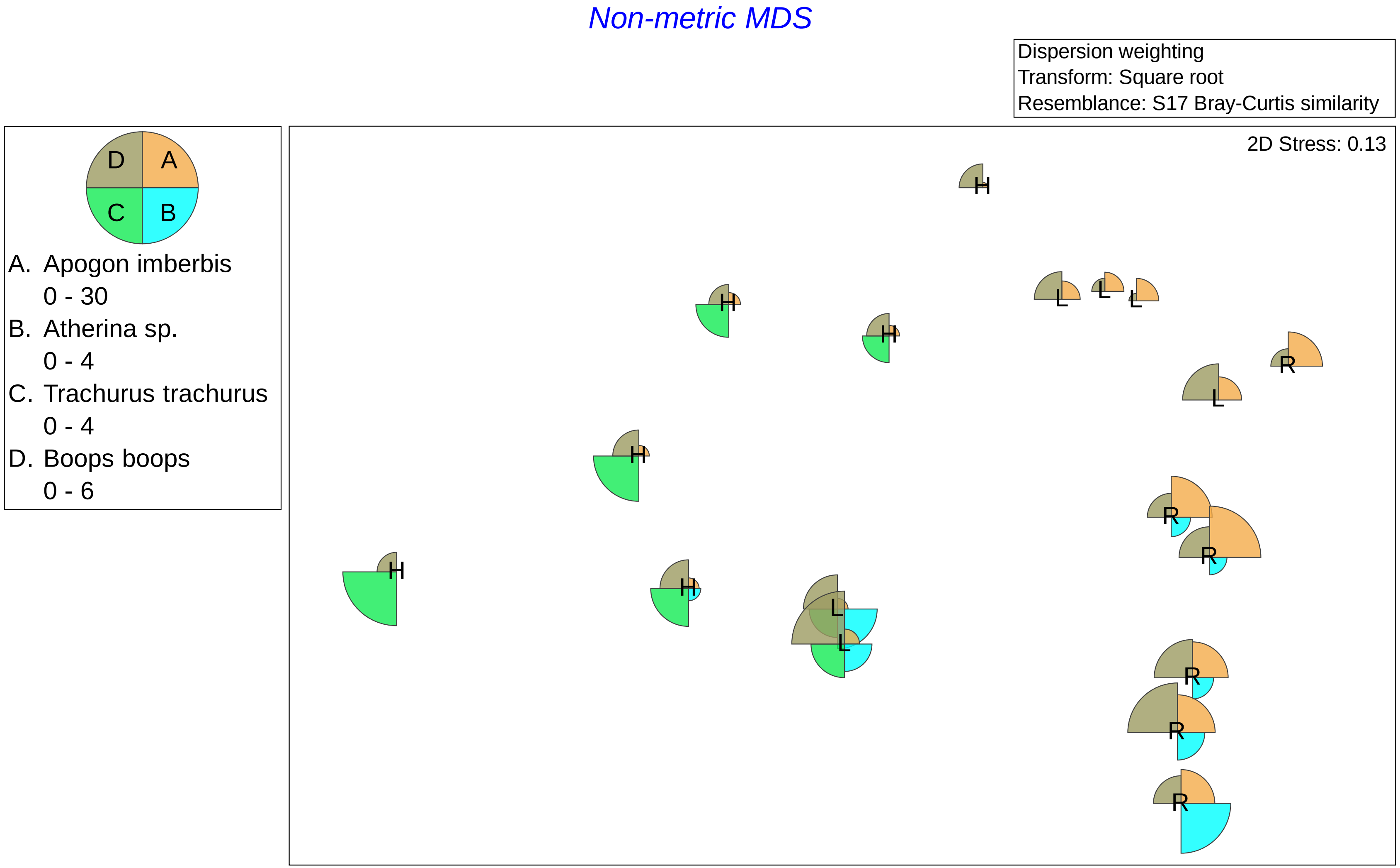


***Figure 4.8** showing a nonmetric Multidimensional Scaling plot for Apogon imberbis, Atherina sp., Trachurus trachurus, and Boops boops in the samples*

Key: R= Red light, L= Low-light intensity, H= High-light intensity

A PERMANOVA test was employed to evaluate the statistical significance of light conditions, habitat types, and their combined influence on community composition. Additionally, three pairwise *t*-tests focusing on the light treatments were conducted to assess further the statistical significance of variations in light conditions (Table 4.1).

Table 4.1 *showing a PERMANOVA for factors 'Habitat' and 'Light' with pair-wise test between levels*

(i) PERMANOVA Habitat x Light			
Source	df	MS	P (perm)
Habitat	1	4442.2	0.0005
Light	2	5370.8	0.0001
Habitat x Light	2	2742.7	0.0003

(ii) Pair-wise t-tests within different light conditions		
Treatments	t	P (perm)
Red (R), Low (L)	2.2347	0.0025
Red (R), High (H)	3.5938	0.0022
Low (L), High (H)	2.267	0.0031

i. PERMANOVA

In terms of statistical significance, the most significant factor is "Light," as indicated by its low *p*-value of 0.0001. This suggests that different lighting conditions have a highly significant impact on community composition. The low *p*-value for the factor 'Habitat' ($p = 0.0005$) also indicates a highly significant relationship between habitat types and their influence on community composition, despite being less significant than 'Light'. The *p*-value for the interaction between Habitat and Light ($p = 0.0003$) is also statistically significant. This result indicates that the combined effect of Habitat and Light conditions is a significant factor in shaping the community composition. Essentially, all three *p*-values are very low and below the conventional significance level of 0.05. This indicates a strong statistical significance, thereby emphasising that both habitat type and light conditions are important factors that influence community assemblage. The MS values also align with the low *p*-values, reinforcing the statistical and ecological importance of these factors in the study.

ii. Pair-wise test

The pair-wise *t*-tests within distinct light conditions (Red, Low, High: R, L, H) reveal the statistical significance of community composition variations under these light treatments. These pair-wise tests did not consider differences in habitat.

The *t*-value (t = 2.2347) indicates a moderate degree of dissimilarity between communities under 'Red' and 'Low' light conditions. Meanwhile for 'Red' and 'High' light conditions the *t*-value (t = 3.5938) is relatively high, indicating a substantial dissimilarity between communities. Communities subjected to 'Low' and 'High' light conditions had a *t*-value of 2.267 suggesting a moderate dissimilarity. The low *p*-values suggest that the difference in community composition between all light conditions were statistically significant. The lowest *p*-value (P (perm) = 0.0022) underscores the strongest statistical significance, showing that the differences in community composition between 'Red' and 'High' light conditions are the most meaningful.

Pair-wise tests were conducted within distinct light conditions, zooming in on the 'Reef' and 'Harbour' habitats. Table 4.2 presents the results of *t*-tests with corresponding the *p*-values for the pair-wise tests.

Table 4.2 *showing a pair-wise tests for different light conditions within the 'Reef' and 'Harbour' habitat*

(i) Pair-wise test for different light conditions within the 'Reef' habitat		
Treatments	t	P (perm)
Red (R), Low (L)	1.8964	0.101
Red (R), High (H)	3.4982	0.099
Low (L), High (H)	2.1287	0.081
(ii) Pair-wise test for different light conditions within the 'Harbour' habitat		
Treatments	t	P (perm)
Red (R), Low (L)	2.8855	0.104
Red (R), High (H)	2.506	0.093
Low (L), High (H)	1.5016	0.092

(i) Pair-wise test for different light conditions within the 'Reef' habitat

The pair-wise tests within the 'Reef' habitat revealed no statistically significant differences between Red (R) and Low (L) light conditions (t = 1.8964, p = 0.101), a slightly more notable yet also insignificant difference between Red (R) and High (H) light conditions (t = 3.4982, p = 0.099), and a discernible difference approaching significance between Low (L) and High (H) light conditions (t = 2.1287, p = 0.081). However, none of the p-values reached conventional significance levels.

(ii) Pair-wise test for different light conditions within the 'Harbour' habitat

The pair-wise tests within the 'Harbour' habitat revealed no statistically significant differences between Red (R) and Low (L) light conditions (*t* = 2.8855, *p* = 0.104), a moderately notable yet statistically insignificant difference between Red (R) and High (H) light conditions (*t* = 2.506, *p* = 0.093), and a modest difference approaching significance between Low (L) and High (H) light conditions (*t* = 1.5016, *p* = 0.092). However, none of the *p*-values reached conventional significance levels.

A SIMPER analysis was used to investigate the influence of different habitat types on community assemblages. Specifically, it examined two distinct habitats, 'Reef' and 'Harbour.' Table 4.3 highlights the specific roles played by individual species in shaping dissimilarities between these ecological settings.

Table 4.3 showing a SIMPER analysis for the factors 'Reef' and 'Harbour'

SIMPER for the factor "Habitat"			
(i) Reef			
Average similarity: 63.45			
Species	Average Abundance	Average Similarity	Contribution %
Apogon imberbis	2.13	20.89	32.93
Boops boops	1.31	15.17	23.9
Trachurus trachurus	0.79	9.43	14.86
(ii) Harbour			
Average similarity: 64.78			
Species	Average Abundance	Average Similarity	Contribution %
Apogon imberbis	2.12	18.29	28.24
Hermodice carunculata	1.35	13.28	20.51
Boops boops	1.12	9.64	14.88
Serranus scriba	0.9	5.97	9.22

i. Reef habitat

The SIMPER analysis conducted for the reef habitat has average similarity of 63.45 among samples, indicating a substantial level of community resemblance within this habitat. *Apogon imberbis* has the highest percentage contribution (32.93%) within the reef habitat, making it a key species in distinguishing the reef habitat from the harbour habitat. *Boops boops* and *Trachurus trachurus* were also found to contribute notably, with 23.9% and 14.86%, respectively, to the dissimilarity between the reef and harbour habitats.

ii. Harbour habitat

The SIMPER analysis conducted for the harbour habitat revealed an average similarity of 64.78 among samples, indicative of a substantial degree of community similarity within this ecosystem. Much like the reef habitat, *Apogon imberbis* emerges as a pivotal species within the harbour habitat, contributing the highest percentage (28.24%) and playing a central role in differentiating it from the reef habitat. Additionally, *Hermodice carunculata*, *Boops boops*, and *Serranus scriba* are also noteworthy contributors with percentages of 20.51%, 14.88%, and 9.22%, respectively, to the dissimilarity between the harbour and reef habitats.

In the context of this research, a SIMPER analysis was conducted to investigate the ecological impacts of different light treatments on community assemblages. Specifically, three distinct lighting conditions, Red Light (R), Low Intensity White Light (L), and High Intensity White Light (H), were examined. The SIMPER analysis outcomes for the "Light Treatment" factor provides an understanding of how individual species contribute to dissimilarities among the aforementioned light treatments (Table 4.4).

Table 4.4 *showing a SIMPER analysis for the factor 'Light Treatment' comprising Red Light (R), Low Intensity White Light (L), and High Intensity White Light (H)*

SIMPER for the factor 'Light Treatment'			
	(i) Red (R)		
	Average similarity: 67.07		
Species	Average Abundance	Average Similarity	Contribution %
Apogon imberbis	3.81	30.91	46.08
Boops boops	1.37	8.96	13.36
Oblada melanura	1.23	5.12	7.64
Muraena helena	0.73	4.6	6.86
	(ii) Low (L)		
	Average similarity: 65.16		
Species	Average Abundance	Average Similarity	Contribution %
Apogon imberbis	1.77	19.77	30.34
Boops boops	1.25	12.63	19.38
Hermodice carunculata	1.02	11.48	17.62
Muraena helena	0.67	6.46	9.91
	(iii) High (H)		
	Average similarity: 60.11		
Species	Average Abundance	Average Similarity	Contribution %
Boops boops	1.02	15.62	25.98
Trachurus trachurus	1.17	14.38	23.93
Symphodus roissali	0.74	9.18	15.27
Apogon imberbis	0.8	8.1	13.48

i. Red Light (R)

In the SIMPER analysis for the Red Light (R) condition, an average similarity of 67.07 among samples points to a relatively high level of community resemblance within this particular light treatment. *Apogon imberbis*, with an average abundance of 3.81 and an average similarity of 30.91, emerges as a key contributor with the highest percentage contribution of 46.08%. This highlights its pivotal role in distinguishing the Red Light (R) condition from other light treatments. *Boops boops*, with an average abundance of 1.37 and an average similarity of 8.96, also contributes notably with a percentage of 13.36%. Additionally, *Oblada melanura* and *Muraena helena* exhibit similarities of 5.12 and 4.6, contributing percentages of 7.64% and 6.86%, respectively, further shaping the distinct community assemblage of this light treatment.

ii. Low Intensity White Light (L)

The SIMPER analysis for the Low Intensity White Light (L) condition indicates an average similarity of 65.16 among samples, signifying a substantial level of community similarity within this lighting condition. *Apogon imberbis*, with an average abundance of 1.77 and an average similarity of 19.77, plays a significant role with the highest percentage contribution of 30.34%. *Boops boops* and *Hermodice carunculata*, with average similarities of 12.63 and 11.48, contribute notably with percentages of 19.38% and 17.62%, respectively, further delineating the unique characteristics of this light treatment. *Muraena helena*, with a similarity of 6.46, also contributes significantly, albeit less, with a percentage of 9.91%.

iii. High Intensity White Light (H)

The SIMPER analysis for the High Intensity White Light (H) condition reveals a similarity of 60.11 among samples, suggesting a high level of community similarity within this lighting environment. Despite still being a relatively high similarity, it is less than that of the other lighting conditions. This indicates a greater variance in the community composition when exposed to High Intensity White Light (H). *Boops boops*, with an abundance of 1.02 and an average similarity of 15.62, is a key contributor with the highest percentage contribution of 25.98%. *Trachurus trachurus*, with an average similarity of 14.38, also plays a significant role, contributing 23.93% to the dissimilarity between the High Intensity White Light (H) condition and other light treatments. *Symphodus roissali* and *Apogon imberbis*, had similarities of 9.18 and 8.1, contribute percentages of 15.27% and 13.48%, respectively, collectively shaped the distinct community assemblage of the High Intensity White Light (H) condition.

The following table (Table 4.5) presents the results of the PCO, highlighting the significance of each principal coordinate in explaining variation in the ecological data.

Table 4.5 *showing a Principal Coordinates Ordination indicating the variation as explained by the axes*

PCO Variation explained by individual axes			
Axis	Eigenvalue	Individual %	Cumulative %
PCO1	16368	40.31	40.31
PCO2	8389.7	20.66	60.97
PCO3	4017.1	9.89	70.87
PCO4	3549.8	8.74	79.61
PCO5	2280.9	5.62	85.22

Each PCO axis captures a specific pattern in the data, with PCO1 explaining the most significant portion of the variation. The subsequent axes then explain the remaining variation in the dataset. Collectively, they provide a comprehensive representation of the data's structure. The corresponding plot for the PCO table (Table 4.5) can be found in Figure 4.9.

Since the plot is two-dimensional only the first two axes (which account for the most significant variation) were considered. The *x*-axis represents PCO1, which primarily correlates with light conditions, while PCO2 is represented on the *y*-axis and is strongly associated with habitat types, as clearly evident from the plot. It should be noted that while the *y*-axis can be generally associated with variations in habitat types, it does not exhibit a perfect correlation.

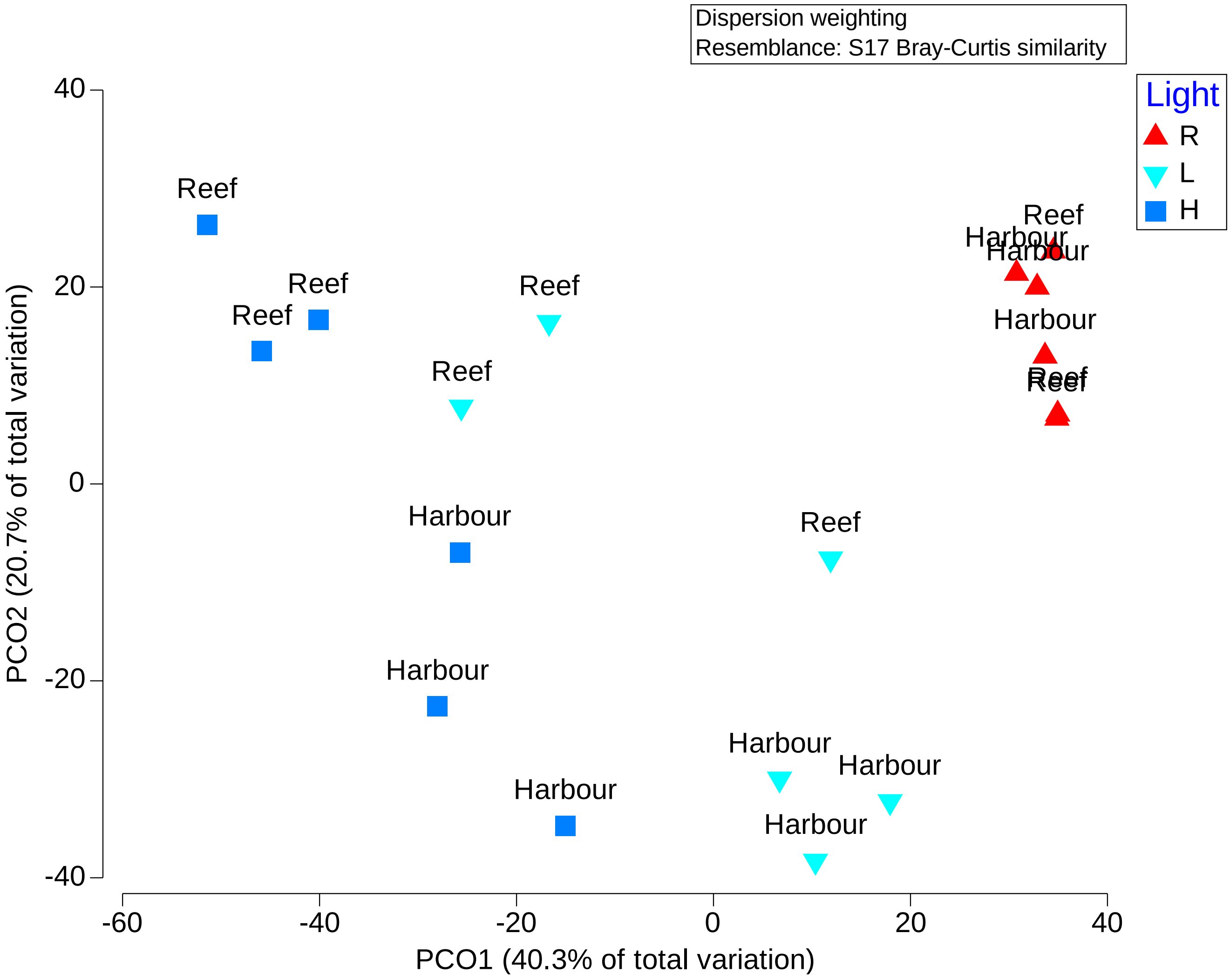


Figure 4.9 *showing a Principal Coordinates Ordination for all samples denoted by light treatment and habitat*

Key: R= red light, L= Low-light intensity, H= High-light intensity

A bubble PCO was computed using the same axes to quantify variation (Figure 4.10). This analysis focused on two species, *Trachurus trachurus* and *Apogon imberbis*, which displayed divergent behavioural patterns. *Trachurus trachurus* exhibited an increasing frequency of occurrence as light conditions intensified, particularly within the reef habitat. Conversely, *Apogon imberbis* displayed consistent presence in both the harbour and reef habitats, with a preference for the former, notably in red light conditions. Its presence was notably lower in high intensity light conditions. Additional observations from the recorded footage include that *Apogon imberbis*, characterised by its inherently curious nature, frequently swam in close proximity to the camera and spent some seconds being still in front of the camera. However, this behaviour gradually diminished in well-lit environments. Conversely, *Trachurus trachurus* demonstrated a discernible inclination to capitalise on well-lit conditions as a substantial duration of the species' observed presence was spent engaged in predatory activities.

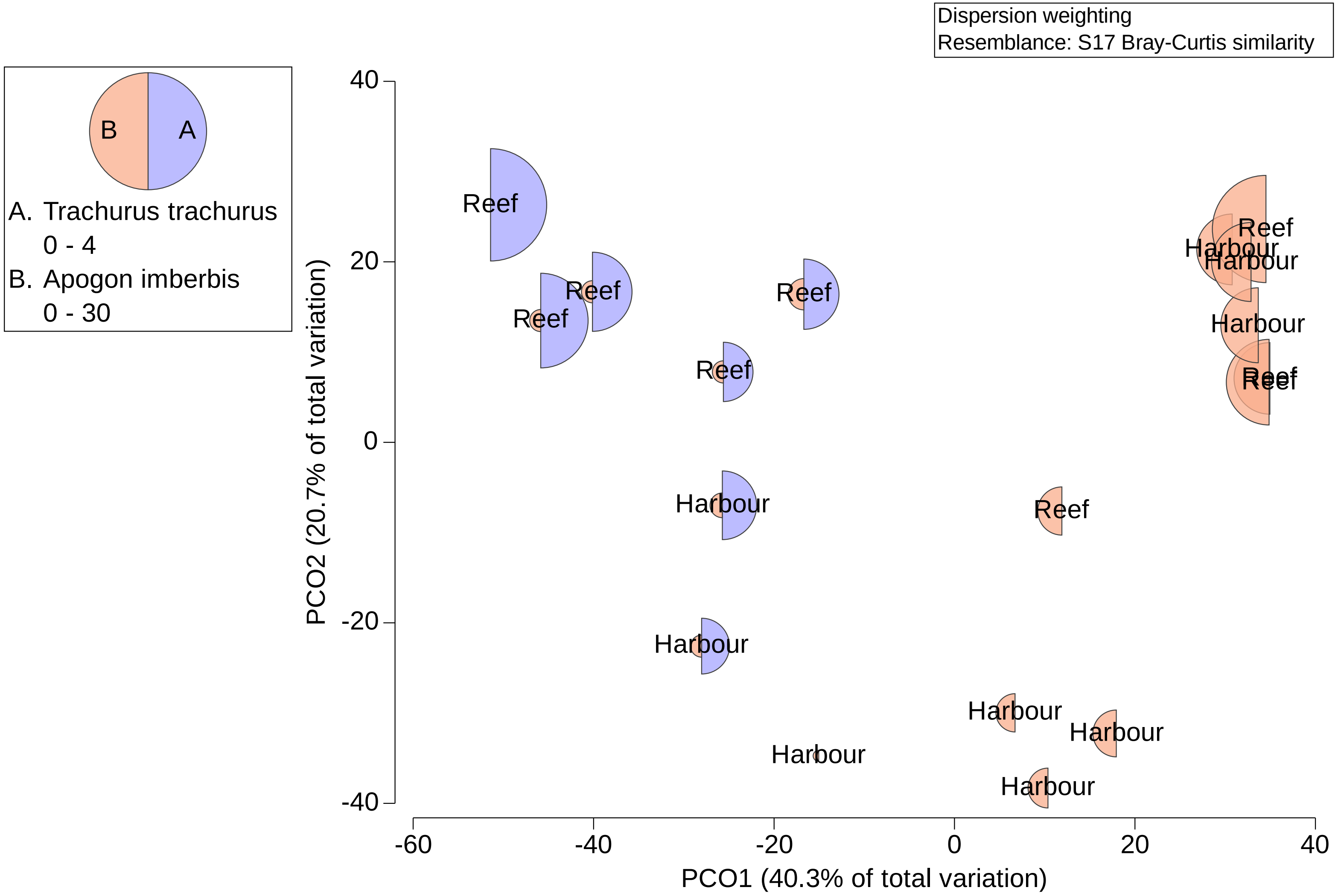


Figure 4.10 *showing a bubble Principal Coordinates Ordination for Trachurus trachurus, and Apogon imberbis for the factor 'Habitat'*

The Shannon index for biodiversity was calculated for the purposes of this study (Table 4.6). This index serves as a critical measure of the richness and evenness of species present and is a crucial indicator of species diversity within the ecological communities that are the subject of the study.

Table 4.6 *showing the Shannon index for factors 'Habitat' and 'Light'*

Shannon Index

PERMANOVA Habitat x Light

Source	df	MS	P(perm)
Habitat	1	5.0126	0.0721
Light	2	2.7638	0.2471
Habitat x Light	2	8.4177	0.0021

The non-significant *p*-value (0.0721) for the factor ‘Habitat’ suggests that differences in habitat type have a limited influence on species diversity. While there may be some variation in community diversity between habitats, it does not reach statistical significance. Similarly, the non-significant *p*-value (0.2471) for the factor ‘Light’ implies that variations in light conditions do not strongly affect species diversity. In contrast, the significant *p*-value (0.0021) for the interaction between ‘Habitat’ and ‘Light’ (Habitat x Light) indicates that the combined influence of habitat type and light conditions has a noteworthy impact on species diversity. It can also be noted that the MS values of the PERMANOVA align well with the conclusions derived from the *p*-values.

5. Discussion

5.1 ALAN as a threat to biodiversity: evolution and habituation

Hölker et al. (2010) suggested that while it is indeed a difficult task to decipher 500 million years of circadian habituation, it is noteworthy that contemporary species show a higher proportion of nocturnal species than more ancient species that have remained relatively unchanged, with the exception of amphibians. The aforementioned study therefore infers that the adoption of nocturnality could have represented a pivotal stage in vertebrate evolution, but this adaptation is currently jeopardised by widespread ALAN. This poses a threat to biodiversity by altering nocturnal behaviours, including reproduction and migration (Rich & Longcore, 2013). Fish and birds that migrate may become confused by artificial lights, which will increase their energy consumption and make it more difficult for them to navigate through space. Consequently, phenological changes and decreased migration success may result from this (Hölker et al., 2010).

Diurnal feeders may also prolong their activity in the presence of illumination, thereby heightening predation pressure on such nocturnal species. It is possible that selection brought on by light could upset the genetic composition of a population, favouring those which are not light-sensitive. Furthermore, it is acknowledged that light pollution has a major role in genetic alterations such as the loss of light-sensitive species and genotypes (Carpenter et al., 2009; Hölker et al., 2010).

However, ALAN may also bring about habituation in fish (Folkedal et al., 2010). Habituation takes place when recurrent exposure to stimuli leads to diminished responsiveness, as seen in the learning process where an initially-alarming stimulus is subsequently recognised as non-threatening (Lieberman, 1993). It has been shown that fish can become accustomed to a variety of stimuli, such as loud noises and moving objects (Fernö et al., 2006). Habituation frequently coexists with other adaptive processes such as Pavlovian learning and physiological acclimation. In a study by Bratland et al. (2010), the Atlantic salmon's (*Salmo salar*) escape response gradually decreased when food was consistently provided after an initially frightening stimulus, such as light flashes (pulse disturbance). This decline resulted from a combination of habituation and Pavlovian learning.

It is plausible that some form of habituation took place particularly relating to the press light disturbance (refer to Section **5.2 Pulse and Press Dynamics**) i.e., the already present ALAN at one of the study sites. In the samples using red light, the individual abundance count for *Boops boops* is higher in the study site with already-present ALAN than in the dark site with no ALAN for all three samples. However, in the samples with the low-intensity white light coming from the BRUV, the opposite occurred; the fish count for *Boops boops* was higher in the dark site with no already-present ALAN for all three samples. It is possible that the low intensity white light had a similar brightness, and thus, effect, as the light coming from the boats that frequently traverse the sample site and the light coming from the Porto restaurant. Whereas it is typical to have dense schooling behaviour as a predator avoidance strategy in exposed conditions such as well-lit environments (Johannesen et al., 2014), it is possible that the groups of *Boops boops* that were already used to being exposed to ALAN did not feel the urgency, or need, to exhibit this behaviour in the low-intensity light conditions.

The PCO shown in Figure 4.9 also indicates a potential occurrence of habituation in response to the press disturbance. The clustering of red-light samples in close proximity, irrespective of habitat type, is noticeable in the PCO plot. In contrast, the low-light and high-light intensity samples exhibit a more considerable variance between them, which may or may not be linked to the specific habitat conditions. The observed similarity in the red-light samples across diverse habitats suggests that the press light disturbance had a comparatively lesser impact on the fish when contrasted with the pulse light disturbance. This reduced significance may be attributed to habituation developed over time in response to the press disturbance.

5.2 Pulse and Press Dynamics

The analysis and examination of environmental disruptions and their repercussions has significantly shaped the understanding of the intricate mechanisms governing ecosystem dynamics and the assembly of resident communities (Fraterrigo and Rusak, 2008; Keitt, 2008; Murphy and Romanuk, 2012). Moreover, it has been recognised that disturbances seldom occur in isolation, necessitating the examination of responses to multiple stressors. This is particularly crucial, as the reaction to an initial disturbance can influence the susceptibility of communities to subsequent environmental stressors (Vinebrooke et al., 2004; Flöder and Hillebrand, 2012). Given this overarching comprehension, there is a notable gap in knowledge concerning the interactions among pulse and press disturbances and their impact on ecological responses within biological communities that span multiple trophic levels (Shade et al., 2012). Moreover, the existing body of literature addressing pulse and press light disturbances on fish is notably even more scarce. Only a limited number of studies have delved into the comprehensive impact of these disturbances on fish, particularly focusing on marine species. The referenced studies may possess a slightly different nature due to the aforementioned reason.

Pulse disturbances represent brief yet frequently intense environmental shifts that can yield either adverse or favourable outcomes on community biomass or abundances (Ives and Carpenter, 2007). Human interventions in climate and biogeochemical systems are expected to, and possibly already have, led to an increase in the frequency and intensity of naturally occurring pulses, such as storms, fires, and heat waves (Stocker et al., 2013). Despite their typically brief duration, pulse perturbations are increasingly recognised as potent influencers of both short- and long-term community dynamics, contingent upon the nature of the disturbance. A press disturbance on the other hand represents the category of disturbance that is anticipated and regular (Lynch & Magoulick, 2016). In the context of previous research, some studies have employed the term "press disturbance" to describe continuous and enduring alterations in environmental conditions, such as the ongoing rise in global mean temperature or ocean acidification (such as Urrutia-Cordero et al., 2021). However, within the scope of this study, both press and push disturbances will be associated with light conditions. Press disturbance will denote the consistent presence of light, such as that emanating from restaurant or streetlights, while push light disturbance will refer to the introduced light from the BRUV.

The study conducted by Lynch & Magoulick (2016) revealed that drying events in intermittent streams exhibit species-specific impacts on fish growth and benthic structure. Furthermore, it highlighted distinctions between the effects of pulse and press drying on periphyton in these systems. These findings carry significance, particularly as anthropogenic influence on stream drying intensifies in seasonally drying streams. The findings from their study indicate that the growth responses of Sunfish (Lepomis *megalotis*) in their investigation imply that press disturbances may exert a more pronounced impact than pulse disturbances on a species' resistance. Moreover, they also identified instances where there was a lack of distinction between the impacts of pulse and press drying on fish growth. This observation suggested that, in some cases, the process of stream drying could be significant, but the specific type of drying experienced may not be as crucial as initially hypothesised.

When investigating light press and pulse disturbances within this study, it was observed that while the distinction between the two was noteworthy, it held less significance compared to the actual intensity of light for the fish and other marine biota. In the PERMANOVA test for Habitat x Light (Table 4.1), the 'Habitat' factor mostly correlated to whether there was already-present ALAN on-site whereas the 'Light' factor correlated to the intensity of the light coming from the BRUV. The findings show that most significant factor in the PERMANOVA test was 'Light' rather than 'Habitat', despite 'Habitat' also having a significant *p*-value. The contrasting result to Lynch & Magoulick (2016) could imply that external disturbances are more important than habitat changes in shaping a fish community. It should be stated that although ALAN is a disturbance, in some cases, it was also a favourable circumstance.

Moreover, it was found that in samples employing red light, a conspicuous trend emerged, revealing a markedly greater abundance of fish within the 'Harbour' habitat, where artificial ALAN was already present (press disturbance), as opposed to the 'Reef' habitat devoid of ALAN. This discernible discrepancy suggests a compelling attraction of fish to the established press light disturbance in the 'Harbour' setting. This observation gains further support from the experimental introduction of both low-light intensity and high-light intensity pulse disturbances, during which certain species distinctly exhibited an attraction towards these altered light conditions. This consistent response across varied light intensities substantiates the hypothesis that fish exhibit discernible preferences and behavioural shifts in the presence of different light disturbances, thereby contributing to the understanding of interactions between marine communities and artificial light.

5.3 External Factors

The section on external factors delves into aspects beyond light conditions that might have influenced the study's samples in various ways. While the study considered habitat-related factors due to the pulse and press dynamics, it is essential to explore other elements pertaining to the habitat itself that could have potentially impacted the observed outcomes. Factors within the habitat, such as seabed type, fishing activities in the particular habitat, and proximity to other habitats, among others, should be noted for their potential contributions. Unlike habitat-related factors, environmental variables were challenging to maintain consistently across all samples due to inherent variations. Therefore, this section emphasises the importance of recognising and considering these external factors to comprehensively understand their potential influence on the study's findings.

5.3.1 Habitat

Topographic factors, such as relief, complexity, feature classes, and morphology, exert widespread influences on fish assemblages. Fish exhibit associations with a diverse array of terrain attributes, and these assemblages undergo variations in response to changes in the depth and aspect of bathymetric features in reef and shelf seascapes, as well as in the deep sea. Notably, fish from different seascapes demonstrate distinct responses to specific metrics, where alterations in slope (coral reefs), rugosity (rocky reefs), and slope (continental shelves, deep sea) are each correlated with shifts in assemblage composition. The simplification of terrain resulting from coastal urbanization activities (e.g., dredging) and resource extraction practices (e.g., trawling) can lead to reductions in both fish diversity and abundance (Borland et al., 2021).

A study conducted by Nagelkerken & van der Velde (2004) revealed that seagrass beds primarily served as habitats for nocturnally active nursery species, with their relative abundance closely tied to vegetation cover, predominantly seagrass. In contrast, sandy seabeds were observed to predominantly harbour diurnally active bay species with their relative abundance linked to the cover of bare sand. The study further indicated that similarities in both taxonomical and functional traits of fish species played a role in predicting whether they were more abundant in seagrass beds or sandy seabed. This finding underscores the influence of habitat type on the circadian cycles of fish, thereby emerging as a potential contributing factor to the dynamics explored in this study.

5.3.2 Environmental Factors

Variations in environmental factors could contribute to the observed differences among the samples. In particular, the study accounted for wind speed, wind direction, and lunar illumination, given the study's focus on light at night. Wind speed, while considered, was likely the least influential factor, as precautions were taken to avoid sampling on very windy days to prevent equipment breakages. Additionally, the two sample sites (as detailed in Section **3.1 Study Area)**, are exposed to different wind directions. Consequently, days with excessively high wind speeds were excluded from sampling to ensure that one site was not overly exposed while the other remained sheltered. The wind speed exhibited a low degree of variability across the samples, with the lowest wind speed recorded at 0.51444 m/s and the highest at 3.0867 m/s (Figure 4.3).

The wind direction data for the samples in this study were recorded in degrees. The wind direction values range from 65° to 335°, representing a variety of wind directions throughout the sampling period (Figure 4.3). Wind direction plays a crucial role in the study's context, as it can significantly influence underwater conditions and impact the visibility of fish species. For instance, prevailing winds can affect water turbulence, potentially influencing fish behaviour and distribution (Beyst et al., 2001). Fish tend to seek shelter or areas with less current against the prevailing wind, impacting the spatial arrangement of the fish community. Establishing a cause-and-effect relationship in this context proves challenging as the intricate interplay of light conditions and habitat characteristics takes precedence, making it difficult to isolate the individual impact of wind direction. The intertwined influence of these factors underscores the complexity of the studied environment and necessitates a nuanced interpretation of the observed outcomes.

The variations in lunar illumination and cloud cover may also present notable considerations in the context of this study on ALAN and its effects on fish communities. When lunar illumination is very high (e.g., 95.52%) and there is no cloud cover (0%), it introduces an additional input of natural light, potentially influencing the study's outcomes. This scenario deviates from the intended conditions of keeping all factors constant with the exception of introduced light from the BRUV, highlighting the challenge of controlling all variables. Conversely, when there is a lot of cloud cover (e.g., 50%), the impact of lunar illumination becomes less significant. However, when cloud cover is minimal or absent (0%), lunar illumination takes on greater relevance (Figure 4.2). This underscores the challenge of maintaining consistent lunar illumination across all samples, especially considering the limited sampling period and the primary focus on wind speed due to equipment sensitivity.

5.4 Species Specific Light Preference and Variations Between Trophic Levels

In contrast to natural daylight, ALAN introduces significant and unnatural disparities in light conditions. Fish exploit these conditions during the night for various purposes, including seeking refuge or foraging. The presence of artificial lighting and the resulting contrast with ambient conditions may impact nocturnal interactions among animals (Luo et al., 2009). It can therefore be concluded that in modified coastal ecosystems that are exposed to significant artificial light inputs, essential ecological processes such as predator–prey interactions which are crucial for the regulation of biological communities, may experience substantial impacts (Becker et al., 2013).

Responses to alterations in light conditions tend to be species-specific. It is probable that there exists a correlation between behavioural responses to artificial light and visual behaviour under natural light conditions. Variations in visual behaviour patterns may be attributed to both phylogenetic and ecological factors. Therefore, a species' unique reactions to artificial light may be influenced by environmental conditions, lifestyle, and feeding strategies (Marchesan et al., 2005).

Numerous predatory fish depend on visual cues to locate and capture prey. This insight is derived from studies leading to the formulation of visual foraging models. These models forecast that predator success diminishes with either escalating turbidity or decreasing light environments, due to a reduced encounter rate (Utne-Palm, 2002).

A study by Batty et al., (1990) found that the feeding proportion of herring (*Clupea harengus*) increased with prey density in high light intensity experiments. However, under dark conditions, elevated food availability did not elicit a corresponding increase in the feeding response. Moreover, the fish engaged in active prey hunting, as shown by faster swimming speed. Comparable findings have also been observed in walleye pollock (*Theragra chalcogramma*) and sablefish (*Anoplopoma fimbria*) (Ryer & Olla, 1999).

From the current study, it can be concluded that artificial light from nearby infrastructure, such as restaurants and streetlamps, has the potential to influence the feeding behaviour of predatory fish. This observation is consistent with the documented inclination of *Trachurus trachurus*, a predatory species, towards well-illuminated environments, where they exhibited increased predatory activity within such environments. To further substantiate such observations, *Trachurus trachurus* showed a contribution of 23.93% to the dissimilarity observed between the High Intensity White Light (H) condition and other light treatments in the SIMPER analysis for the factor "Light Treatment" (Table 4.4), making it a distinctly frequent predator under the higher light intensity conditions.

In a study conducted by Becker et al., (2013), significant alterations were noted in the behaviour of predatory fish. They found that the occurrence of 'station holding' was notably more consistent in the presence of light, indicating that these fish were actively trying to maintain their position within the illuminated area. This observation was also made in the current study with *Trachurus trachurus,* who were at times actively swimming against the current to stay within the well-lit area to prey. The duration of their stay in the illuminated area varied and also seemed to be influenced by current strength, among other factors. It is important to note that when current velocities reach 0.7 m s^{-1}, sustaining a position within the water column involves heightened metabolic expenditure. Fish will opt for these environments at elevated costs only when there is a discernible net energy gain (Hughes & Dill, 1990; Hill & Grossman, 1993). In comparison to other species in high-intensity light environments, the presence of *Trachurus trachurus* was particularly noteworthy. The presence of *Trachurus trachurus* was recorded in every high-intensity white light sample, whilst it was not present in the red light samples, and only present in two of the low-intensity white light conditions. To recapitulate the previous statements, *Trachurus trachurus* distinctly exhibited an attraction to the high-intensity light conditions and, notably, engaged in 'station holding,' indicating a discernible net energy gain from staying in this environment.

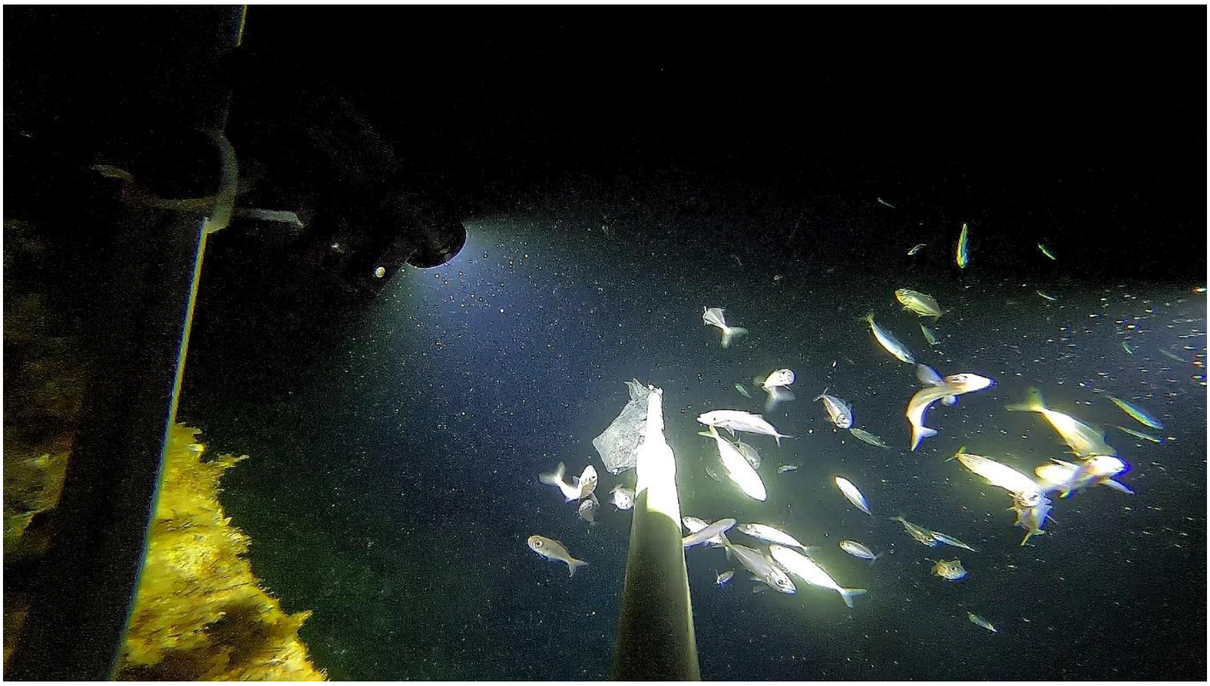


Figure 5.1 *showing Trachurus trachurus and Boops boops in high-intensity white light in the reef habitat*

Bolton et al., (2017) discovered a markedly higher occurrence of fish predation on the sessile assemblages under artificial lighting conditions compared to the naturally dark nocturnal conditions. Owing to the currents, there was always a bait trail and potentially some spillage from the BRUV's bait bag, in which the fish exhibited interest. However, the study revealed that it was specifically during illuminated nights that the fish significantly exploited the bait bag, as was already described, particularly *Trachurus Trachurus*. In the case of *Muraena helena*, its presence was not uniform across all samples. It exhibited a relatively even distribution between red light and low light samples while it was less frequent in high light intensity environments, especially within the habitat with no pre-occurring ALAN. In instances where this species was observed, it consistently attempted to tear the bait bag, displaying this behaviour irrespective of the prevailing light conditions. Notably, in the site with existing ALAN, it was concluded that the BRUV apparatus was positioned in front of the den of a solitary moray eel (*Muraena helena*) (Figure 5.2). The frequent sightings in this habitat could, therefore, be attributed to this single individual. In the dark habitat with no

ALAN, the structure of the den wasn't identified. However, it was also thought to be a single individual. This inference was drawn from the consistent appearance of a lone *Muraena helena*, displaying similar patterns and of consistently constant dimensions. Considering the occasional fishing activities in the area, it is plausible that this individual might have been captured towards the end of the sampling period, explaining its absence thereafter.

Figure 5.2 *showing the den of the Muraena helena individual in harbour habitat*

The behaviour of *Trachurus Trachurus* is highly contrasting with that of *Apogon imberbis*, as can be seen in Figure 5.3. *Apogon imberbis* is a nocturnal, sciaphytic fish that forages during the night and takes refuge during the day in dimly-lit shelters within rocky cliffs such as caves or crevices. During this time, it is often observed forming small groups or large swarms (Rastorgueff, 2020). A study by Marnane (2000) found that gathering in the same site for an extended duration could signify concentrated and predictable resources for predators, particularly for vibrant species like cardinalfishes. In response, individuals of cardinalfishes may shift from one shaded shelter to another to minimise the risk of encountering predators. This behaviour associated with predator avoidance might also be linked to their infrequent presence in environments with high light intensity, a strategy aimed at minimising the risk of predation by species of a higher trophic level.

Figure 5.3 *showing Apogon imberbis in red light in the reef habitat*

The European common cuttlefish (*Sepia officinalis*) also displayed a preference for darker environments, as it was exclusively observed under the red light settings. Notably, only two individuals were recorded during the sampling period, emphasising the importance of considering potential variations with an increased sample size. It is worth mentioning that both sightings occurred within the 'Harbour' habitat. These findings align with the results of Sykes et al.'s (2014) study, which demonstrated that the lowest light intensity (100 lux) of three distinct intensities fostered higher growth and survival rates, as well as lower energetic costs for *Sepia officinalis*. This light intensity corresponds closely to the approximate light value in the 'Harbour' habitat, suggesting optimal conditions for this species. Furthermore, the observed behaviour of *Sepia officinalis*, such as 'station holding,' indicates that this environment provided a net energy gain (Figure 5.4).

Figure 5.4 *showing a Sepia officinalis individual in red light in the harbour habitat*

A study conducted by Rosa et al. (2022) supports the notion that *Hermodice caruncculata* exhibits a preference for lower light levels. This potential preference is substantiated by both laboratory and in situ observations. In Barbados increased *Hermodice carunculata* activity and frequency of coral predation events were documented during crepuscular and night periods, contrasting with fewer observations at midday. Genovese and Witman (2004) demonstrated that foraging activity and abundances of *Hermodice carunculata* were higher during crepuscular periods. These findings collectively contribute to a growing body of evidence supporting the bearded fireworm's inclination toward lower light conditions. In alignment with the observations made by Rosa et al. (2022), the conducted study further substantiates the preference of *Hermodice carunculata* for lower light levels. Notably, when exposed to red light, a total of 55 instances of *Hermodice carunculata* were recorded. Similarly, in samples with low light intensity, there were 49 occurrences, while high light intensity conditions yielded only 24 instances. The correlation between the results and those documented by Rosa et al. (2022) highlights the consistency of *Hermodice carunculata*'s behaviour across varied environmental contexts.

In the research conducted by Becker et al. (2013), there was an increase in the frequency of observations for the more abundant density categories of small shoaling fish (<100 mm) during nights when a floodlight was turned on. This increase was likely attributed to an improved foraging environment for these smaller fish as the phenomenon of marine plankton being drawn to artificial light has also been well-documented in the literature (Spooner 1933; Wiebe et al. 2004), serving as a potential mechanism to aggregate prey for small planktivores. Kalogirou et al., (2010) classified the bogue *Boops boops* and the damselfish *Chromis chromis* as schooling planktivorous fish species. The assemblage of these two species, as noted in the study, were therefore compared to assemblage of the small shoaling fish in the study by Becker et al. (2013). From the recorded footage it was noticed that these two species of fish were shoaling in all light conditions, however they were more abundant in the red and low light conditions. However, from the footage captured in the high light intensity samples, a notable observation was that *Boops boops*, in particular, avoided approaching the camera apparatus because of the *Trachurus trachurus* engaged in 'station holding' for an extended period during the sampling. Consequently, *Boops boops* were counted when they passed in the background, yet they were less conspicuous since they were further away from the camera and positioned in an area with reduced light. It is important to note that the possibility of schooling in greater densities by *Boops boops* may have therefore been overlooked due to these factors.

6. Conclusion

6.1 Summary and Implications

During August 2023, a total of eighteen video samples were collected with a BRUV to assess the effects of ALAN on multitrophic marine communities (with particular emphasis on fish assemblages) in two different local sites that have comparable biotic communities and depth, but different light conditions. The experiment was replicated in space and time to record the communities' response to a pulse disturbance (introduced light from the BRUV), both with and without the additional stress induced by an ongoing press disturbance (already-present light). Each video segment was one hour in duration, and the sampling procedure consisted of acquiring two samples per night—a harbour sample and a reef sample. The initial sample began at 10 pm and was concluded at 11 pm, while the subsequent sample consistently started at 11:30 pm and continued until 12:30 am. Employing a pulse disturbance approach, three distinct lighting regimes—red light, low-intensity white light, and high-intensity white light—were implemented. Each lighting treatment was duplicated three times at each site.

Each collected video sequence was examined at a playback speed of 0.4x to ensure a a thorough and precise evaluation. The parameter computed for each species in every video was labelled as "Count-N," representing the total count of individual members of a species observed. In instances where a precise species identification proved challenging, a broader taxonomical classification, such as genus or family level, was assigned.

The key discussion points from the study can be summarised as follows:

1. There is a plausible indication that some form of habituation occurred, especially in response to the press light disturbance. Despite the typical anti-predation behaviour of schooling, *Boops boops* in the low-light intensity of the harbour did not exhibit this behaviour.

2. Predatory species, such as *Trachurus trachurus*, strategically utilised light conditions to their advantage, particularly in high-light intensity situations.

3. Predatory fish demonstrated 'station holding' behaviour under both low-intensity white light and high-intensity white light, but mostly in the latter condition. They actively maintaining their position within the illuminated area. This choice involves increased metabolic expenditure and is therefore only preferred in environments when there is a clear net energy gain.

4. The presence of species at a lower trophic level, like *Apogon imberbis*, a nocturnal fish that forages during the night and takes refuge during the day, declined as light intensity increased.

5. Other species such as *Sepia officinalis* and *Hermodice carunculata* also displayed a preference for darker environments.

6. In samples utilizing red light, a significantly higher number of fish were observed in the 'harbour' habitat, characterized by existing ALAN, compared to the 'reef' habitat without ALAN. This observation suggests a potential attraction of fish to the presence of the press light disturbance in the 'harbour' environment.

A clear connection was established between artificial light and the local abundance of both predatory and prey fish in the vicinity of such aforementioned light. The shift in fish community composition around areas influenced by varying light conditions, such as the restaurant and quay region, underscores the impact of ecological light pollution. Similarly, the area with no existing artificial light at night (ALAN), characterized solely by pulse disturbance, further contributes to understanding the effects on fish populations in terms of structure and abundance.

Given that fish at a higher trophic were attracted to the artificial light, this situation raises concerns about the susceptibility of predatory fish to overfishing in such conditions. Notably, the study locations are also a favoured spot for recreational and subsistence fishers who may target a specific species that is attracted to light. This targeted fishing, if not carefully managed, could potentially render a species vulnerable to overexploitation, emphasising the importance of addressing such concerns when introducing an element relating to ALAN at a given location. The utilisation of artificial lighting sources, such as metal halide and incandescent lamps, to attract and enhance fish catch is a widespread practice in global fisheries. This method is considered advanced and efficient and hence poses challenges such as declining fish stocks, coral reef deterioration, and the escalating ratio of fishing input to low catch output and operational expenses. Despite its effectiveness, this fishing aggregating approach has been identified as environmentally unfriendly, leading to issues such as the aforementioned as well as capture of immature stocks, overfishing, a high rate of bycatch and discard, and the emission of greenhouse gases. Consequently, there is a pressing need for the development of an eco-friendly light fishing technology and the implementation of sustainable fishing regulations in the immediate future (Solomon & Ahmed, 2016)

6.2 Limitations

The study's findings may be considered distinctive due to the specific abiotic characteristics of the two chosen sites. The selection of these sites was intentional, considering the contrast between the isolation and distance from external light sources at one site, and the presence of ALAN at the other. While the results underscore the effects of an overlooked disturbance, caution is warranted when applying these findings to other coastal systems that may harbour diverse anthropogenic influences. Interactions between increased light and other anthropogenic disturbances, such as underwater noise, may elicit responses from fish assemblages beyond the study's scope. Furthermore, variations in responses may occur with different press disturbances, particularly since the categorisation of a press disturbance is quite broad without strict predefined standards. The duration in time for a disturbance set in place to be categorised as such is therefore unclear. Moreover, the duration of light exposure required for fish communities to habituate is also not specified as it is likely to be species-specific. This thereby makes the distinction between a press and pulse disturbance more challenging to quantify with regards to standardisation for future studies. These considerations emphasise the need for circumspection in extending the study's conclusions to diverse coastal ecosystems.

Furthermore, the manual counting and identification of fish species in each video sample proved to be exceptionally time-consuming. Consequently, conducting additional samples or replicating the study on a larger scale would have posed significant challenges, given the limited time frame of this study.

6.3 Recommendations

6.3.1 Recommendations for ALAN mitigation

As expanding illuminated zones encroach upon natural darkness, elevated levels of ALAN may result in significant modifications to marine ecosystems. Investigating the impact of ALAN on the ecology of marine organisms enhances the capacity to integrate protective measures into forthcoming developments, to define appropriate thresholds, and to optimise existing lighting strategies to mitigate impacts. Prospective advancements in coastal areas should therefore consider the potential impacts of artificial night lighting on fish communities. Where feasible, efforts should also be made to reduce the light emanating from infrastructure.

In the context of this study, while safety considerations may force the Gozo Channel ferry to maintain its current level of illumination, it is recommended that a more considerate and thoughtful approach is taken for artificial lighting at the Porto restaurant. The excessive lighting fixtures installed at this establishment are poorly planned, thus providing an opportunity for a more effective design or adjustment to minimise their environmental impact. Furthermore, similar considerations should extend to charter boats, where the use of excessively bright floodlights often results in unnecessary spillage. Mindful planning and adjustments in the lighting configurations of charter boats can contribute to a more environmentally conscious approach. Similarly, streetlights can be optimised to direct light purposefully and reduce its excessive spillage, thereby contributing to a more considerate and ecologically-responsible coastal environment. The current illumination levels should thereby be checked against European standards. These standards offer recommendations regarding the minimum illuminance necessary to ensure safe working conditions within a designated area. The required minimum illuminance is contingent upon the primary purpose of the area and is intended as a target value that should not be surpassed (Crymble, 2020).

Optimal practices for ecologically-responsible lighting should ideally be integrated into the initial stages of the planning process. By embracing sound outdoor lighting design principles, it becomes feasible to mitigate the impacts of light pollution. Some of the best practices listed in the guidelines compiled by Crymble (2020) done in collaboration with the Light Pollution Awareness Group (LPAG) and Nature Trust Malta included:

- Using full cut-off fixtures
    - Full cut-off fixtures ensure that light is precisely directed to its intended areas. This fixture type is the only one that achieves an Upward Light Output Ratio (ULOR) of 0%.

- Keeping the intensity low
    - The least number of lights and the lowest intensity necessary should be employed to achieve suitable illumination for the designated area.

- Directing light only to where it is needed
    - All luminaires must be oriented in a downward direction. Any light spillage onto private properties, ecologically sensitive areas, or directed upward is deemed unacceptable.

- Using lighting schedules if possible
    - Outdoor lighting ought to be switched off when not in use. Employing dimming or completely turning off lights proves effective in mitigating both light pollution and energy expenditures.

- Minimising blue light
    - Whenever feasible, outdoor lamps with a Correlated Colour Temperature (CCT) below 3000K should be opted for.

- Reducing reflected light
    - Utilising non-reflective, dark-coloured surfaces for roads and infrastructure may help to reduce reflected light.

By adhering to the aforementioned best practices, over-illumination (defined as the presence of lighting exceeding what is necessary) at the Ċirkewwa Quay can be reduced, thereby minimising its impact on the marine community in that area.

6.3.2 Recommendations for future studies

1. As an initial investigation into the impact of ALAN, the approach of this study has revealed significant trends in community assemblage when subjected to a pulse light disturbance, both with and without the addition of a press light disturbance. Subsequent research in this domain would be advantageous to ascertain the persistence of the observed differences in community assemblages between light conditions over an extended period. As discussed in Section **6.2 Limitations**, owing to time constraints in sampling, only 18 nocturnal samples were taken, and these were limited to the summer season. A more comprehensive examination could be achieved through year-round multi-seasonal surveys to discern changes in community composition over time. Given the variable nature of biological effects from fluctuations in oceanographic parameters, future attempts to analyse nocturnal fish communities would gain from more frequent and long-term sampling across diverse water depths, seasons, and environmental conditions.

   Furthermore, this thesis did not centre on any particular fish species but instead addressed a broader community assemblage. For future studies, targeting a specific species, especially one that is vulnerable, threatened, or less understood, opens the possibility of employing variations in bait type, among other factors. Exploring unbaited study approaches could also be considered. If a different community is to be investigated, altering the depth placement of the BRUV may yield noteworthy differences in the recorded species and results due to a different light penetration at such a depth.

2. An exciting opportunity also lies in leveraging machine learning and Artificial Intelligence (AI) applications for the analysis of the extensive video data collected such as was done in the Fish4Knowledge project. The aforementioned project focused on information abstraction and storage methods, machine and human vocabularies for fish description, flexible process architectures, and specialised user query interfaces. Through a combination of computer vision, database storage, workflow optimisation, and

human-computer interaction, manual labour in data extraction was significantly reduced in previous studies (University of Edinburgh, 2011).

The video samples gathered in this study could serve as a valuable baseline database for establishing a similar initiative for Mediterranean fish and other marine species, addressing the geographical gap left by the Fish4Knowledge Project that is based in Taiwan, and the Spot the Jellyfish campaign that is for cnidarians. Applying machine learning (e.g., image analysis) and training AI tools to aid in fish identification could streamline and enhance future studies. Successful applications of AI tools in initiatives like the Spot the Jellyfish campaign demonstrate their potential (Spot the Jellyfish campaign, 2023). Despite the challenges posed by the more complex analytical nature of video samples in comparison to static images, achieving this goal is feasible with the requisite expertise and contributions.

3. The examination of video samples also revealed the presence of various planktonic organisms, drifters, and microorganisms, distinctly visible in the videos as currents brought them close to the camera. While this study did not encompass the analysis and identification of these species, it presents a compelling avenue for future research. Exploring these planktonic organisms in Maltese waters could contribute significantly to the relevant body of knowledge, considering the limited existing knowledge. Some organisms, although large enough to be seen swimming and engaging in movement, remained unidentified due to a lack of knowledge. With the appropriate expertise, there is the potential to document and to create a database of such observations, serving as a valuable resource for future studies or even as a snapshot study of such organisms during the sampling period.

Figure 6.1 *showing a planktonic organism in high-light intensity in the reef habitat*

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Appendix

List of Observed Species

List of Observed Species

Chordata (Fish)
Apogon imberbis
Atherina sp.
Boops boops
Chromis chromis
Coris julis
Diplodus annularis
Diplodus sargus
Diplodus vulgaris
Epinephelus marginatus
Mullus surmuletus
Muraena helena
Oblada melanura
Sarpa salpa
Seriola sp.
Serranus scriba
Sphyraena viridensis
Symphodus roissali
Trachurus trachurus

Mollusca
Buccinum undatum
Sepia officinalis

Cnidaria
Cotylorhiza tuberculata

Annelida
Hermodice carunculata