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UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnología

2024

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

Comparing cryptic sponge communities in the Main Hawaiian Islands and the Northwestern Hawaiian Islands

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Assinatura

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Assinatura

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Abstract

In the context of climate change and increasing anthropogenic pressures, coral reefs face growing threats, making the need for comprehensive biodiversity assessments critical. Cryptic sponges play a fundamental role in nutrient cycling and dissolved organic carbon (DOC) processing via the sponge loop, contributing to the stability and resilience of coral reef ecosystems. However, despite their ecological importance, they have been historically overlooked in monitoring programs and biodiversity assessments due to their small and flat morphologies and hidden nature. In this context, recent research has revealed the remarkable diversity and abundance of cryptic sponges in Hawaiʻi, yet these findings remain geographically limited to Kāneʻohe Bay in Oʻahu. This study is the first to assess cryptic sponge diversity and community composition across such a broad geographical scale (over 2,500 km) in the Hawaiian Archipelago, and to compare them between its two main regions: the Main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI). Beyond its regional significance, it also represents the first global assessment of cryptic sponge diversity across such an extensive area using innovative, non-invasive methods, including Autonomous Reef Monitoring Structures (ARMS) and metabarcoding. Our findings showed a dominance of Porifera, along with Rhodophyta in cryptobenthic spaces across Hawaiian reefs, and a total of 125 Operational Taxonomic Units (OTUs) of cryptic sponges were identified, highlighting their high abundance and diversity throughout the archipelago. Moreover, significant differences in community composition were observed between the MHI and the NWHI, with MHI exhibiting significantly higher alpha diversity. We hypothesize that nutrient and DOC runoffs from rivers and groundwater discharges in the populated, agriculturally modified landscapes of the MHI, coupled with the greater habitat diversity due to their high island topography, contribute to these patterns. This contrasts with the low atolls of the NWHI, which lack freshwater inputs from rivers and groundwater and exhibit reduced habitat diversity due to their flat topography. These findings provide critical insights into the factors shaping cryptic sponge distribution and underscore the need for region-specific management and conservation strategies. Finally, these results are expected to enhance understanding of reef ecosystem dynamics and guide future research on the spatial distribution of cryptic reef organisms.

Resumo

No contexto das alterações climáticas e das crescentes pressões antropogénicas, os recifes de coral enfrentam ameaças crescentes, como o branqueamento, doenças e surtos de predadores. Estas ameaças são particularmente alarmantes, considerando que os recifes de coral estão entre os ecossistemas marinhos mais diversos e produtivos do planeta. Isso destaca a necessidade de avaliações mais abrangentes da biodiversidade e uma compreensão mais profunda da dinâmica dos recifes de coral para permitir uma correcta implementação de programas eficazes de restauração e conservação. Neste contexto, as esponjas crípticas desempenham um papel fundamental nesses ecossistemas por meio do "sponge loop" (ciclo das esponjas), um processo no qual as mesmas convertem o carbono orgânico dissolvido (DOC) em carbono orgânico particulado (POC). Ao filtrar e absorver DOC, as esponjas produzem detritos através da rápida renovação celular, que são então consumidos por detritívoros, sustentando organismos maiores dos recifes e canalizando nutrientes para a cadeia trófica do recife.

Apesar da sua importância ecológica, as esponjas crípticas têm sido historicamente negligenciadas nos programas de monitorização de recifes de coral. Embora as esponjas em geral enfrentem desafios significativos em estudos científicos, as esponjas crípticas apresentam um conjunto único de dificuldades. As suas formas de crescimento discretas e incrustantes e a dificultade da sua identificação taxonómica fazem com que estas sejam facilmente ignoradas em levantamentos ecológicos, resultando na sua frequente ausência em avaliações de biodiversidade. Além disso, as esponjas crípticas habitam recantos escondidos ou de difícil acesso nos recifes, tornando-se menos propensas a serem amostradas e estudadas em comparação com espécies de esponjas mais visíveis. Estima-se que mais de metade de todas as espécies de esponjas, incluindo muitas esponjas crípticas, permaneçam desconhecidas. Estes vieses de amostragem são agravados pelo foco da pesquisa em habitats mais acessíveis, enquanto cavidades menores dos recifes, especialmente em regiões isoladas do Indo-Pacífico, permanecem pouco exploradas. Como resultado, as esponjas crípticas continuam criticamente sub-representadas na literatura científica, contribuindo para uma lacuna significativa de conhecimento e uma compreensão incompleta da biodiversidade dos recifes de coral. De facto, apenas estudos recentes revelaram a notável diversidade e abundância de esponjas crípticas numa das regiões mais remotas do mundo: o Arquipélago Havaiano, embora essas descobertas tenham sido geograficamente limitadas à Baía de Kāne‘ohe, em O‘ahu.

Este estudo é o primeiro a avaliar a diversidade de esponjas crípticas e a composição das comunidades em todo o Arquipélago Havaiano numa ampla escala geográfica (mais de 2.500 km), comparando as ilhas principais, situadas no sudeste (“Main Hawaiian Islands”, MHI), com as ilhas do noroeste (Northwestern Hawaiian Islands”, NWHI). Além disso, representa a primeira avaliação global da diversidade de esponjas crípticas numa área tão extensa, utilizando métodos inovadores e não invasivos, incluindo as Estruturas de Monitoramento Autónomo de Recifes (ARMS) e técnicas de metabarcoding, com amplificação do gene nuclear 28S rRNA, que oferece uma resolução

superior na avaliação da diversidade de esponjas comparativamente ao gene mitocondrial citocromo oxidase sub-unidade I (COI).

As MHI, intensamente povoadas e localizadas no sudeste do arquipélago, são caracterizadas por picos vulcânicos altos e elevações íngremes, que contribuem para costas acidentadas moldadas pela erosão. A elevação destas ilhas gera o "Island Mass Effect" (efeito de massa insular), criando diferenças ambientais distintas entre os lados barlavento e sotavento. Os lados a barlavento estão expostos aos ventos predominantes e correntes oceânicas resultando em maior precipitação e influxo de nutrientes, enquanto que os lados a sotavento são mais abrigados. Essa combinação de variação topográfica, habitats diversificados e inúmeras fontes de água doce, como rios, riachos e aquíferos, leva ao escoamento de nutrientes e descargas de DOC nas águas circundantes, particularmente devido ao uso agrícola e urbano do solo. Em contraste, as NWHI, compostas principalmente por atóis pouco profundos e de geología rasa e montes submarinos, não possuem picos vulcânicos significativos e exibem menos diferenças climáticas entre os lados barlavento e sotavento, e, como tal, uma menor diversidade de habitats. Além disso, as NWHI estão sujeitas a um influxo menor de nutrientes terrestres e entradas de DOC devido às fontes limitadas de água doce e ao impacto humano mínimo, oferecendo condições mais pristinas.

O nosso estudo identificou 125 unidades taxonómicas operacionais (OTUs) de esponjas em todo o Arquipélago Havaiano, corroborando estudos recentes efectuados na da Baía de Kāneʻohe, que destacaram uma abundância e diversidade de esponjas inesperadamente altas. Tradicionalmente, pensava-se que a diversidade de esponjas era maior em regiões continentais, como as Caraíbas, em comparação com as ilhas oceânicas, mais oligotróficas. No entanto, os nossos resultados desafiam essa noção, revelando uma diversidade e abundância de esponjas muito significativa no Arquipélago Havaiano. Tanto nas MHI como nas NWHI, Rhodophyta e Porifera foram os filos mais abundantes em habitats crípticos, demonstrando sua dominância em ecossistemas criptobentónicos.

Dentro do filo Porifera, a classe Demospongiae foi a mais abundante, representando mais de 95% da diversidade de esponjas em ambas as regiões. No entanto, as abundâncias relativas de outras classes como Calcarea e Homoscleromorpha, variaram ligeiramente entre as MHI e as NWHI, sugerindo diferenças regionais na composição das comunidades de esponjas. Diferenças na abundância de ordens dentro de Demospongiae também foram identificadas. Notavelmente, a ordem Poecilosclerida foi dominante em ambas as regiões, mas a ordem Haplosclerida foi mais proeminente nas NWHI, enquanto a ordem Tetractinellida foi mais comum nas MHI.

Este estudo indicou diferenças significativas entre as MHI e as NWHI na composição das comunidades, com as MHI a exibir uma diversidade alfa significativamente maior. Propomos que o escoamento de nutrientes e DOC de rios e descargas de aquíferos nas paisagens agrícolas das MHI, combinados com a maior diversidade de habitats devido à sua topografia, contribuem para a maior diversidade desta região. Em contraste, a menor disponibilidade de nutrientes e a reduzida diversidade de habitats nas NWHI explicam a

menor diversidade de esponjas observadas nesta área. Apesar destas diferenças geográficas e ambientais, houve uma sobreposição notável de espécies de esponjas entre as MHI e as NWHI, refletindo a possível persistência de linhagens antigas e adaptação. Estas descobertas fornecem insights críticos sobre os fatores que moldam a distribuição das esponjas crípticas e destacam a necessidade de estratégias de manutenção e conservação específicas para cada região. Estes resultados permitem melhorar a nossa compreensão da dinâmica dos recifes e guiar futuras pesquisas sobre a distribuição espacial de organismos crípticos nestes ecosistemas.

Keywords

Cryptic sponges; ARMS; Metabarcoding; MHI; NWHI; Alpha diversity; Community composition

Thesis acknowledgement

I would like to express my deepest gratitude to several individuals who have been instrumental in making this thesis possible. First and foremost, I owe immense thanks to my supervisor, Regina Lopes da Cunha, for granting me this incredible opportunity and for assisting with all the necessary and overwhelming paperwork to make my time in Hawai‘i a reality. A special thank you to Rob Toonen, my supervisor at HIMB, who welcomed me in his lab and without whom none of this would have been possible. Rob’s support and quick solutions to every challenge made the journey so efficient and enjoyable.

My heartfelt thanks also go to Kat and Mindy, the ToBo lab technicians whose kindness and patience were key in making my lab experience so positive and without whom my lab work would have taken so much longer. I am also extremely grateful to Molly Timmers, who provided insightful guidance and helped me overcome several hurdles along the way.

I also extend my thanks to the entire ToBo lab crew for creating such a welcoming and inspiring work environment. Special mention goes to Emily (and her husband Bryan), Cati and Carlos for their warmth and for showing me the beauty of Hawai‘i.

A special mention goes to Gabby and Jan. Gabby was by my side all the way and taught me everything I learnt in the lab. Her hard work and expertise in many sectors were absolutely essential to this study. Gabby handled all the bioinformatics done in this study, and her dedication and passion have been so inspiring to me. I also can’t thank Jan enough — his deep knowledge of sponges and constant readiness to help made a huge difference. He’s one of the kindest people I’ve met, always there when I needed anything, and that really had a big impact on both my work and me personally.

A huge thank you to Joao, my fellow student and adventure partner, for being by my side throughout this journey. Thank you to my friends Iñigo and Ines for accompanying me during this process, even from a distance and for being a constant source of support. Finally, I would like to thank my mom, dad and sister who always have my back and for always being there for me.

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List of abbreviations, acronyms and symbols

ARMS - Autonomous Reef Monitoring Structures
COI - Cytochrome C Oxidase subunit I
DOC - Dissolved Organic Carbon
HLCC - Hawaiian Lee Counter Current
HS - High Sensitivity
HTS - High-Throughput Sequencing
LSU - Large Subunit
MHI - Main Hawaiian Islands
MPAs - Marine Protected Areas
Mya - Million years ago
NHRC - North Hawaiian Ridge Current
NMDS - Non-metric multidimensional scaling
NWHI - Northwestern Hawaiian Islands
OTU - Operational Taxonomic Units
PCR - Polymerase Chain Reaction
PMNM - Papahānaumokuākea Marine National Monument
POC - Particulate Organic Carbon
SCC - Subtropical Counter Current
UDI - Ubique Dual Index
zOTUs - zero-width operational taxonomic units
28S rRNA - structural ribosomal RNA (rRNA) for the large subunit (LSU) gene of eukaryotic cytoplasmic ribosomes

Chapter 1 – GENERAL INTRODUCTION

1.1.Threats to coral reef ecosystems

Coral reefs are the most diverse marine ecosystems per unit area and are experiencing significant biodiversity loss due to a combination of natural and human-induced stressors, including coral bleaching, diseases, and predator outbreaks, all exacerbated by climate change (Hoegh-Guldberg et al., 2007; Carpenter et al., 2008). Moreover, direct human activities, such as pollution from deforestation, overfishing, and unsustainable coastal development, further impact coral reef ecosystems through nutrient runoff and physical damage (Hodgson, 1999; Goldberg & Wilkinson, 2004). This is extremely alarming, since coral reefs are among the most productive and biologically diverse ecosystems on Earth. Their intricate three-dimensional structure is crucial as it supports a wide range of species and provides essential habitat complexity, which is vital for maintaining ecological processes and overall reef health (Graham & Nash, 2013). Additionally, coral reefs offer a suite of crucial services, including coastal protection by reducing wave energy and erosion, providing food resources through their fisheries that support millions of people, and facilitating nutrient cycling that enhances water quality and marine productivity (Moberg & Folke, 1999). Preserving their complex structures and ecological functions is therefore essential in maintaining the health of marine environments and the wide array of services they provide.

1.2.Porifera

1.2.1 Porifera: A historically understudied and challenging phylum

The taxonomy of sponges has long been challenging and often regarded as unattractive for study due to their obscure and variable nature. Historically, they were seen as a 'comparatively limited and isolated' group, with their lack of fixed shape making their taxonomic classification difficult (Johnston, 1842). Despite significant advances in the mid-19th century, which led to the documentation of most of the approximately 8500 known species and the establishment of a classification framework, sponges remain under-studied and not fully understood (Morrow & Cárdenas, 2015). A notable advancement in sponge’s taxonomy was the publication of the Systema Porifera (SP) in 2002, which represented a comprehensive and collaborative effort to revise sponge systematics, providing a valuable updated framework for classification (Hooper & Van Soest, 2002). However, the SP relied heavily on morphological characteristics, which are often subject to homoplasy and may not accurately reflect evolutionary relationships. Despite its initial positive impact, the SP classification has not been substantially updated in response to subsequent molecular phylogenetic research that has revealed numerous polyphyletic groups and new clades. This lack of revision has led to a widening gap between the phylogenetic understanding and the classification framework, creating biases and inconsistencies that hinder a complete understanding of marine ecosystems and evolutionary processes (Morrow & Cárdenas, 2015). While the phylogeny and systematics of sponges remain partially unresolved, with debates continuing over the branching patterns of their main clades and their relationships to other non-bilaterian animals (Cárdenas et al., 2012; Wörheide et al., 2012), significant progress has been made in recent years. New insights have gradually illuminated various aspects of this phylum, enhancing our understanding of sponge biology.

1.2.2 Earliest branching phylum in the Metazoan

Sponges (Phylum Porifera) are among the earliest living metazoans, with a fossil record extending back to the Late Precambrian, approximately 650 million years ago (Müller, 1998). Sponges are often termed "living fossils" due to their ancient origins and remarkable evolutionary stability, and their minimal morphological change over hundreds of millions of years underscores their significance in understanding early animal evolution (Müller, 1998). To this day, the continuous study of sponges and their fossil record remains crucial for resolving questions about their phylogenetic relationships and the early history of metazoans. Traditionally, sponges (phylum Porifera) were considered monophyletic, meaning they were thought to have descended from a single common ancestor (Reitner & Mehl, 1996; Pick et al., 2010). However, recent molecular phylogenetic studies have suggested that sponges might be paraphyletic, with some classes (Calcarea or Homoscleromorpha) more closely related to non-sponge animals than to other sponges (Borchiellini et al., 2001; Medina et al., 2001; Sperling et al., 2009; Renard et al., 2013). This debate remains ongoing as new molecular and morphological data continue to challenge and refine our understanding of sponge phylogeny (Wörheide et al., 2012) (Figure 1.1).

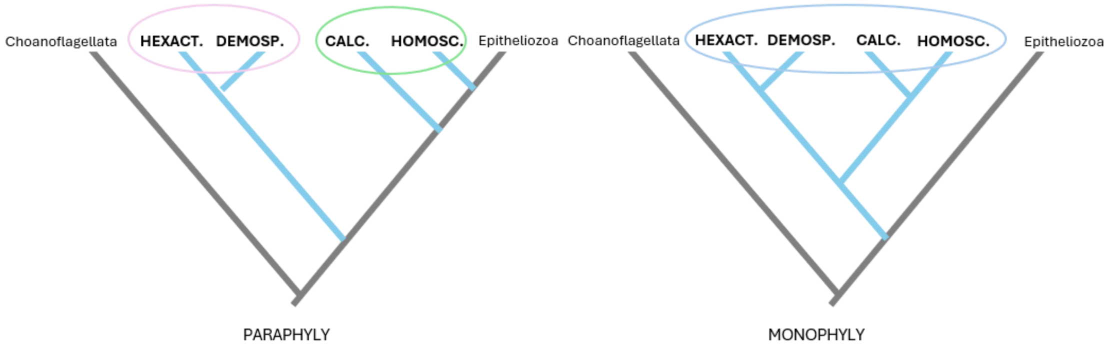
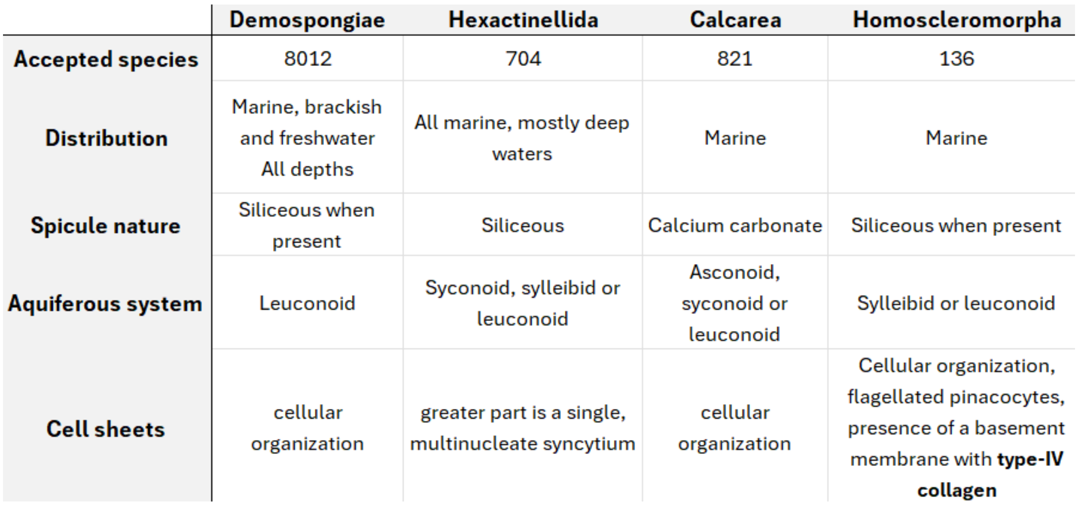


Figure 1.1. *Contrasting hypothesis for higher-level relationships of phylum Porifera. Left: sponge paraphyly. Right: sponge monophyly. Modified and simplified from Wörheide et al., 2012.*

1.2.3 Classification of Porifera

Today, 4 classes are recognized for phylum Porifera: Demospongiae, Hexactinellida, Calcarea (Hooper & Van Soest, 2002) and Homoscleromorpha (Gazave et al., 2012) (Table 1.1.). Below is a general description of the four classes. However, it is important to note that current sponge classification is still evolving as new molecular data emerge. Despite significant progress in understanding sponge phylogeny, more work is needed to fully grasp the relationships and evolutionary history of this diverse group (Wörheide et al., 2012).



	Demospongiae	Hexactinellida	Calcarea	Homoscleromorpha
Accepted species	8012	704	821	136
Distribution	Marine, brackish and freshwater All depths	All marine, mostly deep waters	Marine	Marine
Spicule nature	Siliceous when present	Siliceous	Calcium carbonate	Siliceous when present
Aquiferous system	Leuconoid	Syconoid, sylleibid or leuconoid	Asconoid, syconoid or leuconoid	Sylleibid or leuconoid
Cell sheets	cellular organization	greater part is a single, multinucleate syncytium	cellular organization	Cellular organization, flagellated pinacocytes, presence of a basement membrane with **type-IV collagen**

Table 1.1. *Main characteristics of the four classes inside of Porifera. Modified from Renard et al., 2013 and updated with De Voogd et al., 2024: World Porifera Database, http://www.marinespecies.org/porifera/*

a. Demospongiae

Demospongiae is the largest class within Porifera, encompassing about 81% of all living sponges with nearly 7,000 species, and more than 50 new species described each year (De Voogd et al., 2024). This group is incredibly diverse, consisting of 22 orders (Morrow & Cárdenas, 2015) and inhabiting a wide range of environments, from warm intertidal zones to cold abyssal depths, including all known freshwater sponges (Renard et al., 2013). Demosponges are primarily characterized by monaxonic, tetraxonic, and/or polyaxonic (never triaxonic) silica spicules and/or skeletons composed of spongin fibers and occasionally possess a calcareous basal skeleton (Hooper & Van Soest, 2002; Wörheide et al., 2012**)**. Moreover, some species of Demosponges lack a mineral skeleton altogether (Hooper & Van Soest, 2002). They exhibit various forms, from encrusting sheets to upright branching stalks, and have a leuconoid grade of organization, which refers to a complex internal canal system that maximizes water flow for efficient feeding and respiration. Due to their predominantly soft body structures, demosponges are underrepresented in the fossil record, making their true historical diversity difficult to assess (Hooper & Van Soest, 2002; De Voogd et al., 2024).

b. Hexactinellida

Hexactinellida, or glass sponges, are distinguished mainly by their unique 6-rayed silica spicules. They are the oldest sponges documented and possess distinctive characteristics, such as tissues with fused nuclei and an inability to contract (Reitner & Mehl, 1996; Hooper & Van Soest, 2002; Wörheide et al., 2012). Additionally, they possess a specialized system for rapidly transmitting electrical signals, enabling swift responses to environmental changes (Reiswig & Mackie, 1983; Hooper & Van Soest, 2002).

Currently, there are 5 recognized orders and around 600 species of hexactinellids, representing roughly 7% of all known sponge species (Wörheide et al., 2012). However, this number likely underrepresents their actual diversity, as many deep-sea regions remain unexplored and numerous specimens are yet to be studied (Reiswig, 2002).

c. Calcarea

Calcareous sponges are divided into two subclasses (Calcinea and Calcaronea) and 5 orders (Wörheide et al., 2012). They are the only sponges with skeletons made of calcium carbonate spicules, which are secreted into the extracellular space, unlike the intracellular formation of siliceous spicules seen in other sponge classes (Renard et al., 2013; Wörheide et al., 2012). There are currently about 675 recognized species of Calcarea, all of which are exclusively marine. They are predominantly found in shallow, near-shore environments, with only a few species documented in the deep sea (De Voogd et al., 2024). While most calcareous sponges have skeletons composed solely of free spicules, some species have a rigid basal skeleton formed by fused or cemented spicules.

d. Homoscleromorpha

Homoscleromorpha is a small but distinct group of marine sponges, with 2 families and fewer than 100 described species (Wörheide et al., 2012). This class is notable for being the only Porifera having a basement membrane that includes type-IV collagen, a feature traditionally associated with true epithelia in more complex animals (Boute et al., 1996). This characteristic has led to hypotheses suggesting that Homoscleromorpha may be closely related to Eumetazoa, which include more complex multicellular animals. The small size and unique features of Homoscleromorphs, such as the presence of tetractinal spicules and sometimes an absent skeleton, distinguish them from other sponges (Renard et al., 2013). Historically, they were placed within the subclass Tetractinellida of Demospongiae due to similarities in spicule types (Levi, 1956). However, recent molecular studies have shown that Homoscleromorpha forms a separate clade from Demospongiae and is most closely related to Calcarea, leading to the proposal that they should be elevated to their own class (Gazave et al., 2012). This new classification assumes Poriferan monophyly, and reflects their unique morphology and developmental features, such as the presence of a true epithelium, which is not found in Demospongiae or other sponge groups (Gazave et al., 2012).

1.2.4 Ecology of Porifera

a. Feeding, habitat, and reproductive strategies

Sponges are the most primitive sessile metazoans characterized by a unique feeding system where water is drawn through tiny pores, filtered by specialized cells called choanocytes, and expelled through larger openings (Bergquist, 1978). While most sponges are filter feeders, carnivorous species have also been found to exist in nutrient poor deep environments, feeding on small crustaceans (Vacelet & Boury-Esnault, 1996). Sponges inhabit a wide range of aquatic environments, from marine to freshwater habitats, and display diverse morphologies, including encrusting sheets and complex, large structures with various canal systems (De Voogd et al., 2024). Their reproductive strategies are both sexual and asexual, with many species being hermaphroditic and capable of producing both eggs and sperm. These diverse reproductive methods confer notable resistance to environmental changes, allowing sponges to temporarily colonize and effectively adapt to fluctuating conditions (Renard et al., 2013).

b. Ecological, functional and economical roles

Sponges play critical roles in marine ecosystems and are essential components within coral reefs. They are integral to nutrient cycling through their associations with microbial symbionts, which facilitate processes such as nitrification (Corredor et al., 1988; Diaz & Rützler, 2001; Southwell et al., 2008) and contribute greatly to primary productivity (Bell, 2008; Erwin and Thacker, 2008; Thomas et al., 2016). Moreover, their high abundance and biomass often surpass those of other reef epibenthic organisms, allowing them to engage significantly in space competition and bioerosion, which in turn impacts reef structure and function (Diaz & Rützler, 2001; Wulff, 2001). Sponges play a key role in water filtration by continuously pumping substantial volumes of water through their bodies. This process effectively removes suspended particles and microorganisms, contributing to the regulation of water quality (Wulff, 2001; Renard et al., 2013). In addition to this, sponges possess crucial interactions with substrates and other marine organisms. They function as both food sources (Hill, 1998; Pawlik, 2011; Lesser & Slattery, 2013) and habitat providers (Diaz & Rützler, 2001; Rützler, 2004; Fiore & Jutte, 2010; Pawlik, 2011), supporting a diverse array of marine life and contributing to the overall biodiversity of coral reef ecosystems. Economically, their diverse forms and colours enhance reef aesthetics and therefore tourism, while their bioactive compounds hold pharmaceutical potential (Monks et al., 2002; Amina & Musayeib, 2018). Additionally, sponges with soft, high-quality fibers are popular as bathing sponges and decorative materials, adding to their commercial value (Verdenal & Verdenal, 1987).

c. Challenges in sponge biodiversity assessments

Despite their substantial ecological contributions to coral reef ecosystems, sponges are often underrepresented in coral reef biodiversity surveys compared to other taxa (Hooper & Levi, 1994; Wörheide et al., 2008; Van Soest et al., 2012; Bell et al., 2014). This is mainly due to sponge's limited number of distinct morphological features, coupled with a high degree of variability and an increased likelihood of convergent evolution (Van Soest et al., 2012). More specifically, our current knowledge of the biogeographic and taxonomic distribution of sponge diversity reflects collection biases. While extensive sponge studies and collections have been conducted in accessible regions of the world (Van Soest et al., 2012) such as the Mediterranean (e.g.: Pansini & Longo, 2003; Bertolino et al., 2017; Santín et al., 2018), Northeast Atlantic (e.g.: Hogg et al., 2010; Hestetun et al., 2015), and Caribbean (e.g.: Wilkinson, 1987; Maldonado et al., 2017); few studies have begun to explore sponge diversity on isolated Indo-Pacific islands (Bell, 2008; Hall et al., 2013; Galitz et al., 2023). Moreover, there is a lack of extensive field guides covering particular and large geographical regions and specialists in Porifera taxonomy are scarce, hindering efforts to accurately catalogue sponge diversity (Diaz & Rützler, 2001; Wulff, 2001; Rützler, 2004). These factors collectively contribute to the ongoing taxonomic uncertainty and the difficulty encountered by researchers and non-specialists alike in identifying sponge species and thus in studying accurately their abundance, diversity and distribution around the world. Given their significant contributions to ecosystem functioning and the growing recognition of their diverse roles, it is crucial to fully integrate sponges into coral reef assessments and monitoring programs to achieve a comprehensive understanding of reef biodiversity and health (Diaz & Rützler, 2001; Wulff, 2001; Bell, 2008).

1.2.5 Cryptobenthic sponges

a. Cryptic habitats

Cryptic habitats, including the crevices and cavities within coral reefs, play a crucial role in the ecosystem by serving as major sinks for dissolved organic carbon (DOC) on Caribbean and Indo-Pacific reefs (De Goeij & van Duyl, 2007). These hidden spaces represent a significant portion of the reef structure: estimates suggest they account for 75-90% of the total reef volume and provide 50% of the reef surface area (Kornder et al., 2021), their complex topography offering up to eight times more additional substrate for colonization than the projected reef surface area (Scheffers et al., 2004). Within these cryptic habitats, sponges are particularly dominant and diverse (Richter & Wunsch, 1999; Richter et al., 2001; Rützler, 2004). Cryptic sponges are distributed among the 4 classes inside of Porifera. They have been found to be predominant in cryptobenthic spaces in the Caribbean (Jackson and Winston, 1982), representing 35% of the total benthic reef biomass (Kornder et al., 2021). Moreover, they constitute up to 60% of the cryptofauna of Red Sea coral reefs, covering up to 50% of the inner cavity walls and outweighing the biomass of epibenthic reef filter feeders by two orders of magnitude (Richter et al., 2001; Wunsch et al., 2003). Thus, cryptic sponges are vital to reef ecosystems as they dominate these hidden habitats, significantly contributing to the reef's structural complexity and overall biomass.

b. Sponge loop

In addition to the huge array of roles mentioned previously, sponges play a distinctive role among metazoans by transforming forms of dissolved organic carbon (DOC) that are otherwise not readily usable by other organisms (Yahel et al., 2003; Van Duyl et al., 2008; De Goeij et al., 2008; 2013; Rix et al., 2016; 2017). While the role of emergent sponges in DOC recycling remains poorly understood (Pawlik, 2011; McMurray et al., 2018), a significant breakthrough was achieved with the discovery of the "sponge loop" in cryptic sponges (De Goeij et al., 2013). Coral reefs are known for their high productivity and biodiversity, which seems paradoxical since they thrive in oligotrophic seas. The sponge loop helps explain this paradox by illustrating how sponges contribute to nutrient cycling in these systems. Sponges play a pivotal role by filtering and absorbing algal and coral-derived DOC from the water (Rix et al., 2016; 2017; 2018). They convert this DOC into particulate organic carbon (POC) through a process of rapid cell shedding and turnover (De Goeij et al., 2013; Alexander et al., 2014; 2015). The expelled detritus, rich in organic matter, is then consumed by small detritivores, such as crustaceans and polychaetes, which are, in turn, preyed upon by larger reef fauna. This transfer of energy and nutrients through the sponge loop mirrors the microbial loop found in open ocean systems, where microbes convert DOC into forms that support higher trophic levels. However, in the sponge loop, carbon turnover occurs at a faster rate, enhancing the efficiency of nutrient cycling within reef ecosystems (Figure 1.2). Thus, it effectively channels the DOC into higher trophic levels, supporting the reef's complex food web (Rützler, 2004; De Goeij & Van Duyl, 2007; Bell, 2008, Rix et al., 2018). The importance of the sponge loop lies in its contribution to the reef's nutrient dynamics, demonstrating how sponges help sustain high productivity and biodiversity in otherwise oligotrophic waters. By efficiently recycling nutrients and providing a food source for other organisms, sponges play a key role in maintaining the ecological balance and resilience of coral reef ecosystems.

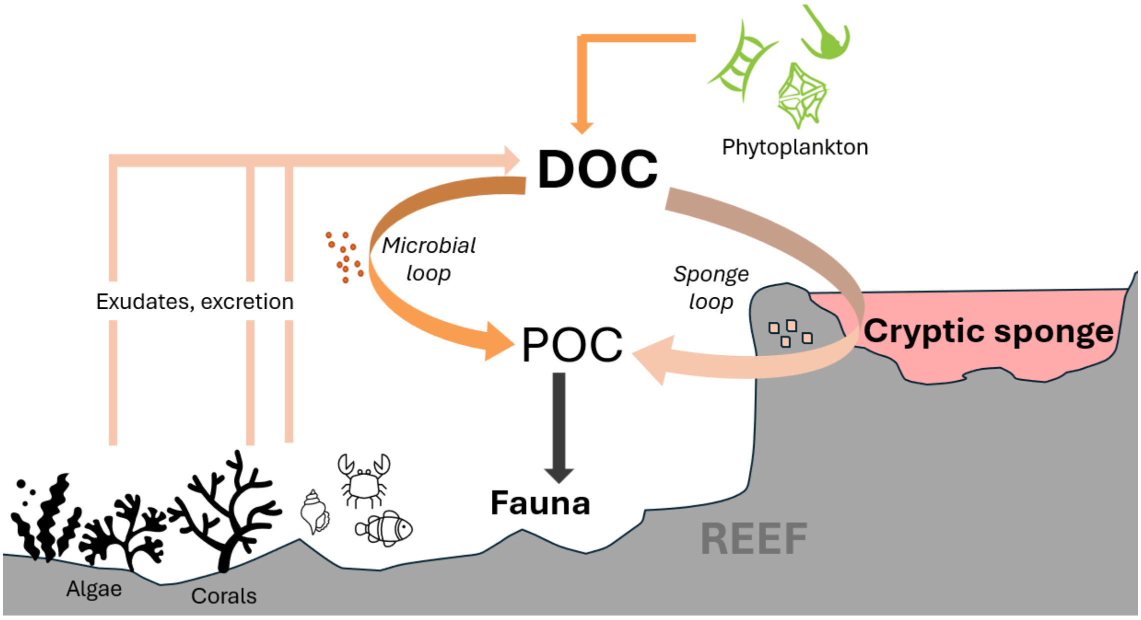


Figure 1.2. *Simplified scheme of organic carbon recycling and transfers on coral reefs ecosystems. Modified from De Goeij et al., 2013. Pink arrows represent the sponge loop while the orange arrows represents the microbial loop.*

c. Additional barriers in studying cryptic sponges

While sponges as a whole face significant challenges in scientific studies, cryptic sponges are particularly overlooked due to their unique set of challenges in detection and identification. Their often inconspicuous, encrusting growth forms and low taxonomic resolution makes them easy to miss in ecological surveys, leading to a notable absence in many biodiversity assessments (Wunsch et al., 2003; Van Soest et al., 2012; Pearman et al., 2016). Additionally, because they inhabit hidden or hard-to-reach reef habitats, they are less likely to be collected and studied compared to more visible sponge species (Wunsch & Richter, 1998; Vicente et al., 2022). As a result, it is estimated that more than half of all sponge species, including many cryptic sponges, are still undiscovered (Appeltans et al., 2012). All these factors contribute to a critical knowledge gap concerning cryptic sponges (Van Soest et al., 2012; Pearman et al., 2016; Vicente et al., 2022), leading to an incomplete picture of coral reef biodiversity (Pearman et al., 2016; Wunsch et al., 2003).

Collection biases further widen this knowledge gap. Research on cryptic sponge diversity has predominantly focused on more accessible habitats like marine caves and tunnels in opposition to smaller, inaccessible reef cavities (Wunsch & Richter, 1998). Moreover, studies on cryptobenthic sponges are relatively high on easily reachable geographical regions such as the Caribbean (Rützler et al., 2014), Mediterranean (Muricy et al., 1996; Gerovasileiou & Voultsiadou, 2012; Guido et al., 2019) and Red Sea (Richter et al., 2001; Wunsch et al., 2003; Pearman et al., 2016), whereas remote and isolated Indo-Pacific islands remain largely unstudied (Vicente et al., 2022).

Given that cryptobenthic communities constitute an immensely significant portion of coral reef ecosystems and are crucial for understanding ecosystem dynamics, it is essential to prioritize the study of cryptic sponges in future research to fully understand their ecological roles, diversity, and responses to environmental changes. Such studies are vital for informed ecosystem management programs and the development of effective marine protection areas.

1.3.Development of new methods

1.3.1 Autonomous Reef Monitoring Structures

As explained before, cryptic species are understudied mainly due to the lack of appropriate tools for their exploration. Autonomous Reef Monitoring Structures (ARMS) have emerged as a pivotal tool in overcoming these challenges by providing a standardized method to assess hidden biodiversity in coral reef ecosystems. ARMS were initially developed as "artificial reef monitoring structures" designed to replicate the structural complexity of natural reefs and to attract diverse marine organisms, including cryptic species. These structures were composed of various substrates, such as concrete, furnace pads, scrub pads, PVC pipes, and coral rubble, mimicking the microhabitats found in natural reefs (Zimmerman & Martin, 2004). Over time, these artificial structures evolved into more standardized and reproducible structures: ARMS. These are inexpensive, non-destructive structures that imitate reef crevices (Figure 1.3) and are capable of being deployed, retrieved, and analysed effectively, thus allowing the settlement and subsequent sampling of coral reef-associated invertebrates (Brainard et al., 2009). Standardized ARMS unit are composed of nine grey PVC plates, measuring 22.5 cm x 22.5 cm x 0.63 cm. These plates are stacked in alternating sequences of four open layers and four semi-closed layers, which provides a range of microhabitats for marine organisms to colonize. The stack is secured to a larger base plate, which measures 45 cm x 35 cm x 1.27 cm, ensuring the unit remains stable when fastened to the reef bottom or ocean floor. The plates and base are securely attached to the substrate, allowing the ARMS to stay in place while collecting data on the surrounding biodiversity over time. The dimensions of each plate and the number of layers can be adjusted to suit the specific objectives of a study (e.g.: mesocosm ARMS in Timmers et al., 2022).

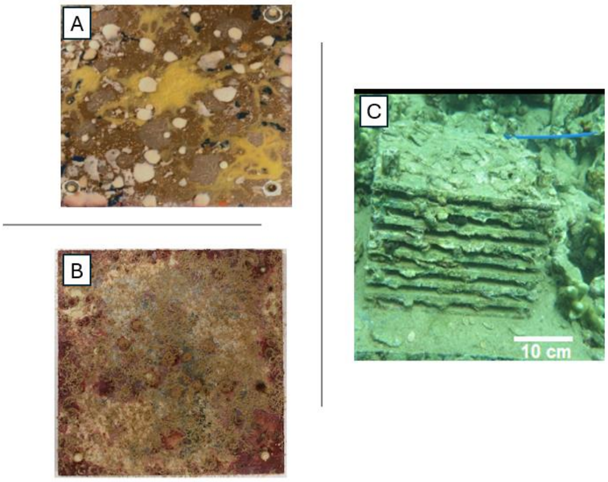


Figure 1.3. *(A) ARMS plate showing cryptobenthic community dominated by sponges; from Vicente et al., 2022. (B)ARMS plate showing the settlement of cryptobenthic organisms; from Timmers et al., 2022 (C)*

example of ARMS deployment at the Hawaiʻi Institute of Marine Biology in Kāneʻohe Bay, Oʻahu; from Vicente et al., 2022.

Recent studies have demonstrated the effectiveness of ARMS in capturing a wide range of cryptic marine species and in uncovering a greater diversity than traditional field surveys (Pearman et al., 2016; Vicente et al., 2022; Vicente et al., 2022). For instance, ARMS have been shown to provide access to 52% of the cryptobenthic specific diversity that would otherwise be unattainable (Vicente et al., 2022). In addition, ARMS were found to capture an unprecedented number of cryptic sponge species, sometimes even doubling the diversity previously reported in certain regions, such as in Kāneʻohe Bay in the Hawaiian archipelago (Vicente et al., 2022).

Therefore, the use of ARMS is crucial for assessing cryptic sponge diversity and understanding the broader ecological dynamics of coral reef ecosystems. Their standardized, cost-effective design allows for consistent monitoring at local, regional, and global scales, once again providing essential data for ecosystem management plans. By enhancing our ability to study cryptic species and their roles within coral reefs, ARMS play an indispensable role in advancing our knowledge of reef biodiversity and resilience, especially in the face of environmental changes (Brainard et al., 2009; Pearman et al., 2016; Timmers et al., 2022).

1.3.2 Metabarcoding

Over the past few decades, metabarcoding has also helped address knowledge gaps in biodiversity studies by overcoming the significant time, financial resources, and taxonomic expertise required by traditional survey methods (Yu et al., 2012; Ji et al., 2013). This technique involves mass collection and homogenization of specimens without the need of subsampling individuals. After homogenization, DNA is extracted and is followed by PCR amplification, high-throughput sequencing (HTS) of barcode genes, and bioinformatic analysis to identify taxa and measure biodiversity efficiently across large spatial scales (Yu et al., 2012; Ji et al., 2013; Cristescu, 2014). Therefore, metabarcoding offers a rapid, cost-effective, and precise technique for biodiversity assessments. In fact, many studies have demonstrated the effectiveness of metabarcoding in assessing the composition and/or diversity patterns of complex cryptic communities

(Leray & Knowlton, 2015; Pearman et al., 2016; Timmers et al., 2022; Vicente et al., 2022).

The cytochrome c oxidase subunit I (COI) gene is the most widely utilized marker in biodiversity studies due to its demonstrated efficacy in amplifying genetic material across a diverse array of taxa (Hebert et al., 2003; Leray et al., 2013). Concerning sponges, COI has proven effective in detecting sponge richness within complex natural communities, though with notable limitations (Vargas et al., 2012). Specifically, primers designed to amplify the COI Folmer region (mlCOIintF and jgHCO2198) struggle to amplify DNA from sponges in the class Calcarea (Timmers et al., 2022), due to high mitochondrial substitution rates (Voigt et al., 2012; Lavrov et al., 2012) and has limited accuracy in estimating the diversity of sponges at species and order level in Demospongiae due to the slow evolutionary rates found in this class (Timmers et al., 2022).

The structural ribosomal RNA (rRNA) for the large subunit (LSU) gene of eukaryotic cytoplasmic ribosomes (28S rRNA) has emerged as a more effective marker than the COI gene for metabarcoding in the phylum Porifera (Voigt & Wörheide, 2016; Martineau et al., 2024). Studies have shown that 28S sequences offer higher variability, enabling more precise discrimination of operational taxonomic units (OTUs) and revealing genetic differences undetected by COI (Voigt & Wörheide, 2016). This marker also provides a more resolved phylogeny for sponges, including Calcarea (Erpenbeck et al., 2016) and has demonstrated superior performance in detecting and quantifying sponge species, achieving higher detection rates and greater taxonomic coverage across species-rich cryptic natural communities (Martineau et al., 2024). These attributes make the 28S gene a powerful tool for sponge molecular taxonomy and biodiversity studies.

As mentioned before, it is well known that ARMS are significantly more effective in assessing cryptic biodiversity and composition as opposed to other methods such as SCUBA field surveys and endoscopic camera explorations. Moreover, a study by Pearman et al. (2016) in the Red Sea demonstrated that after the recovery of ARMS, cryptic community assessments were more sensitive when using metabarcoding techniques in comparison to visual censuses of ARMS by taxonomists. In fact, in this study, metabarcoding showed a significant increase in the estimates of species diversity compared to photo analysis of the ARMS plates. Therefore, combining traditional reef surveying with more advanced and sensitive methods such as ARMS and metabarcoding in future studies will help increase our understanding of reef communities and dynamics, providing critical insights for informed decision-making in coral reef management programs (Leray & Knowlton, 2015; Pearman et al., 2016; Timmers et al., 2022; Vicente et al., 2022). However, taxonomists remain indispensable for identifying new taxa and creating a baseline of all species subsampled individually for in-depth taxonomic assessments. Without a well curated database, the metabarcoding efforts would be significantly limited in their effectiveness (Fautin et al., 2010; Fisher et al., 2015). This underlines the need for developing expert taxonomists to ensure the accuracy and reliability of these advanced technologies in assessing biodiversity.

1.4.Region of study

1.4.1 Hawaiian Archipelago

The Hawaiian Archipelago is a linear chain of volcanic islands located in the North Pacific Ocean (Figure 1.4), between approximately 19° and 28° N and 154° and 178° W, and is the most remote island chain on Earth. It stretches over 2 500 kilometres from the island of Hawaiʻi in the southeast to Kure Atoll (Figure 1.5), the northernmost atoll in the world. Formed by volcanic activity over a stationary hotspot beneath the Pacific Plate, the Hawaiian Islands are part of the 6 126 km-long Hawaiian-Emperor seamount chain, the longest in the world. As the Pacific Plate moves northwest at a rate of around 10 cm per year (Moore, 1987), new islands continue to form, with the island of Hawaiʻi being the youngest, which is still volcanically active.

Figure 1.4. *Satellite image showing remoteness and location of the Hawaiian Archipelago (red). Taken and modified from Google Maps (https://www.google.com/maps).*

The archipelago’s isolation has contributed to exceptionally high levels of marine endemism and some of the most unique ecosystems on Earth (Friedlander et al., 2004; Friedlander & Brown, 2019). It’s coral reefs constitute 8% of the total coral reef habitat within U.S. territorial waters, increasing to 14% when considering deeper areas down to 183 meters (Friedlander & Brown, 2019). Hawai‘i’s nearshore marine ecosystems are subject to large ocean swells and strong trade winds, which vary by season and location (Gove et al., 2013), significantly shaping the ecological communities along the coast. Moreover, ocean currents such as the North Hawaiian Ridge Current (NHRC), the Hawaiian Lee Counter Current (HLCC) and the Subtropical Countercurrent (SCC) also influence the region, further contributing to its distinct biodiversity (Toonen et al., 2011) (Figure 1.5).

1.4.2 MHI and NWHI

The Hawaiian Archipelago is divided into two distinct regions separated by around 400 km: the Main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI) (Figure 1.5). The MHI, located in the southeast, are characterized by their populated, high volcanic islands and fringing, patch and barrier reefs (Fletcher et al., 2008). They include both active and younger lava flows (around 0.6 mya) in Hawai‘i Island, and older islands like Ni‘ihau (around 5.6 mya). In contrast, the NWHI to the northwest consist of mostly uninhabited atolls, banks, and seamounts (Fletcher et al., 2008), representing the older, more eroded portion of the archipelago with ages reaching up to 29.8 mya (Neall & Trewick., 2008). These regions share a common geological origin but differ significantly in their ecological and geomorphological characteristics.

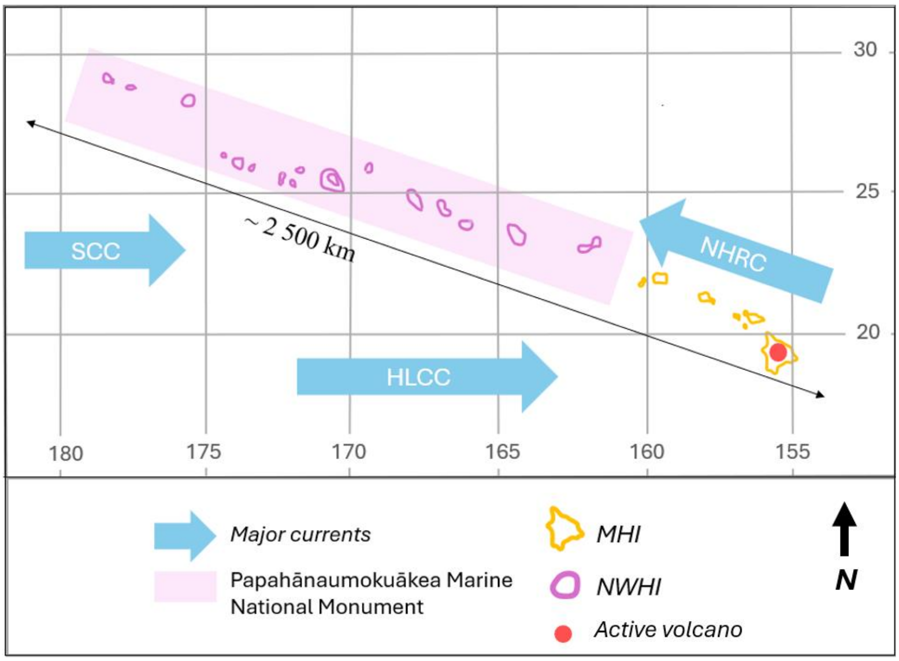


Figure 1.5. *Hawaiian Archipelago and its main characteristics. Modified from Toonen et al., 2011.*

a. MHI

The MHI consist of eight islands which are, from south to north: Hawai‘i, Maui, Kaho’olawe, Lāna’i, Moloka’i, O’ahu, Kaua’i and Ni’ihau. They are notable for their high volcanic peaks, such as Mauna Kea (4205 m) and Mauna Loa on Hawai‘i Island (4169 m), and Haleakalā (3055 m) on Maui, and are characterized by their deep valleys and rugged coastlines, shaped by erosional processes from rain, wind, and sea high wave energy (Lau & Mink, 2006). Furthermore, due to the “Island Mass Effect”, these high islands influence local oceanography by altering wind patterns and forming eddies and in turn upwelling (Gove et al., 2016). This causes deep water to rise, bringing nutrient-rich waters to the surface around their coastal waters (Seki et al., 2001; Gove et al., 2016; Friedlander & Brown., 2019; De Falco et al., 2022). The MHI’s high elevation is also responsible for distinct climatic differences between their windward and leeward sides: the windward sides receive more rainfall and have lush vegetation, while the leeward sides are drier and warmer (Fletcher et al., 2008; Friedlander & Brown., 2019). This topographical influence, combined with human-induced habitat alterations, results in a diverse range of coastal habitats, including natural ones like coral reefs, sandy beaches, and tide pools, as well as human-modified environments such as mangrove forests, fishponds, and coastal development areas (Friedlander & Brown, 2019). Moreover, the MHI are home to a population of approximately 1.4 million people, including 953 207 on

O‘ahu alone (Hawaii Population 2024, https://worldpopulationreview.com), and many regions are allocated to urbanization, agricultural expansion, and land modification. This, added to the islands’ high inclinations and landscapes of deep valleys have led to increased runoff and nutrient inputs into surrounding waters, further affecting local marine ecosystems (Friedlander et al., 2004).

b. NWHI

The NWHI consist of a chain of low-lying atolls and small islands that extend over 2000 km from Nihoa and Necker Islands in the south to Midway and Kure Atolls in the north (Lau & Mink, 2006). Unlike the high volcanic islands of the MHI, the NWHI are older and lower in elevation, and their coastlines are primarily shaped by subsidence and erosion processes (Friedlander et al., 2004; Friedlander, 2009). Due to their low-lying nature, the islands do not create significant windward or leeward climatic differences and lack persistent freshwater sources such as estuaries, rivers and groundwater (Friedlander & Brown, 2019). However, seasonal fronts and eddies are important characteristics of the NWHI, and nearshore productivity is highly enhanced by the flushing and associated outflow of the atoll’s lagoonal waters when large ocean swells push water over their barrier reefs (Gove et al., 2016), thus supporting nearshore ecosystems. Unlike the MHI, the NWHI are largely uninhabited, with limited human activity mainly restricted to former military installations at Midway Atoll and French Frigate Shoals (Friedlander et al., 2004). Because they aren’t affected by the “Island Mass Effect” and because they have fewer human-modified areas, habitat diversity in the NWHI is significantly lower than in the MHI (Friedlander & Brown, 2019). Nevertheless, the NWHI support critical habitats for endangered species such as the Hawaiian monk seal and green sea turtles, with over 90% of Hawai‘i’s sea turtles hatching in the NWHI (Friedlander et al., 2004). Despite historical chronic overfishing and overexploitation of resources such as pearl oysters and lobsters, the reefs of the NWHI remain relatively pristine and represent one of the last large-scale apex predator-dominated coral reef ecosystems on the planet (Friedlander & Brown, 2019).

c. Conservation efforts

The Hawaiian Archipelago is characterized by remarkable levels of endemism and biodiversity (Friedlander et al., 2004). In fact, in the NWHI, mesophotic coral reefs

(30–90 m deep) have shown an average endemism of up to 92% at its northernmost end (Kane et al., 2014). In addition to this, the Hawaiian Archipelago is home to numerous endangered and threatened species (Friedlander, 2009; Friedlander & Brown, 2019). Economically, these coral reefs are crucial to the state's economy, supporting an $800 million per year marine tourism industry and contributing nearly $10 billion in overall economic value through tourism, fishing, and recreational activities (Friedlander et al., 2004). These reefs also hold cultural significance for native Hawaiians, especially for subsistence fishing and preserving sociocultural practices (Gregg et al., 2015; Kittinger et al., 2016). All this makes the coral reef ecosystems of the Hawaiian Archipelago globally unique.

However, these reefs face numerous threats, including overfishing, pollution, habitat destruction (accentuated by a growing human population), invasive species, and climate change-induced coral bleaching (Hoegh-Guldberg et al., 2007; Friedlander et al., 2004; Carpenter et al., 2008; Friedlander & Brown, 2019). Therefore, many Hawaiian reefs are protected by local, state, and federal laws in order to conserve its biodiversity, while restoring and preserving its overall ecosystem functions. In the MHI, over 90 marine protected areas (MPAs), including Marine Life Conservation Districts, Fisheries Management Areas, and Fish Replenishment Areas, regulate fishing and other activities (Friedlander et al., 2019). In contrast, the NWHI is protected by the Papahānaumokuākea Marine National Monument (PMNM), one of the largest no-take marine conservation areas in the world, covering 1.5 million km² (https://papahānaumokuākea.gov) (Figure 1.5). This monument is globally significant, preserving a predator-dominated ecosystem rich in endemic and endangered species, and is recognized as a UNESCO World Heritage site for both its natural and cultural value. Moreover, the PMNM's biocultural managing approach integrates Native Hawaiian traditions with modern conservation science (Friedlander, 2009; PMNM, papahanaumokuakea.gov), making it a model for marine conservation worldwide.

1.5.Present study

1.5.1 Cryptic sponges in Hawaiian Archipelago

Sponge diversity in the Hawaiian Archipelago has historically been considered low (De Laubenfels, 1950; Van Soest et al., 2012). This pattern is consistent with the hypothesis by Wilkinson (1987) and Birkeland (1987), who proposed sponges tend to be more abundant in nutrient-rich continental coral reefs compared to oligotrophic oceanic coral reefs, where they are less conspicuous and diverse. This hypothesis suggests that the lower levels of available nutrients in oceanic regions like Hawaiʻi limit the growth and diversity of large, emergent sponges.

However, early studies, including De Laubenfels' (1950), indicated that sponge diversity in the Hawaiian Archipelago could be more significant than initially thought. De Laubenfels documented the presence of numerous invertebrates, including sponges, in the interstices of coral; with around 23 species of sponges in Kāneʻohe Bay (O'ahu). However, these studies were incomplete and involved destructive sampling methods—entire coral masses were broken off and examined—ultimately revealing that sponges, though present, were thin and sparsely distributed. The limitations of these early surveys were compounded by a lack of repeatability, as suitable, non-destructive sampling methods did not exist at the time. In 2007, another study by Kahng & Kelley in the Au'au Channel (Hawaiʻi) also reported low densities of sponges in deeper coral reefs. However, they pointed out that cryptic sponges were likely underrepresented in surveys relying on video recordings because they tend to grow thin and hidden, often making them difficult to detect.

Only in the past few years have studies begun to focus on cryptic sponge abundance and diversity in Hawaiʻi, made possible by advancements in sampling techniques (Timmers et al., 2022; Vicente et al., 2022; Vicente et al., 2022; Martineau et al., 2024). These investigations have uncovered an extraordinary abundance, diversity and dominance of cryptic sponges, far greater than previously known. Vicente et al. (2022) documented 186 sponge OTUs in Kāneʻohe Bay, O'ahu, of which 150 were new records for the Hawaiian Islands and over ¾ were cryptobenthic, revealing the previously overlooked diversity of these organisms. Therefore, these findings suggest that cryptic sponges play a potentially critical role in the marine ecosystems of the Hawaiian Archipelago, contributing significantly to coral reef dynamics and trophic relations.

1.5.2 Relevance of present study and implications for future conservation efforts

Building on the information presented thus far, the following key points summarize the major findings and considerations related to coral reefs and cryptic sponges in the Hawaiian Archipelago.

- Coral reefs in the Hawaiian Archipelago:
    - Represent the most common natural habitat along with associated sand channels and patches (Friedlander & Brown, 2019).
    - Possess significant functional, ecological, and economic value, which underscores their critical conservation importance.
    - Are subjected to numerous threats, including climate change, pollution, and overfishing, all of which jeopardize their long-term health.

- Cryptic sponges:
    - Play crucial roles in nutrient and DOC cycling via the "sponge loop" and contribute to fuelling the overall diversity of reef ecosystems.
    - Are historically understudied and present challenges for research due to their cryptic nature within the reef matrix and encrusting growth
    - Have recently been recognized as highly diverse and dominant components of the benthic communities of Kāne'ohe Bay, O'ahu.

These key points underline the critical importance of incorporating information on cryptic sponge diversity and abundance into coral reef surveys and monitoring programs in the Hawaiian Archipelago. Doing so will enhance our understanding of reef dynamics and functions, which is essential for effective protection and restoration efforts. Furthermore, this approach will improve biodiversity assessments, aiding in the detection of invasive and non-indigenous species and helping to understand the impacts of climate change and anthropogenic pressures on reef ecosystems. Moreover, it is clear that despite being part of the same Archipelago, the MHI and NWHI have different ecological, geomorphological and demographic characteristics and cryptic sponges may exhibit distinct adaptations to factors like water quality, nutrient availability and habitat complexity across these regions.

This study is the first to assess cryptic sponge diversity across a broad spatial scale of 2,500 km, utilizing innovative, non-destructive methods such as ARMS and metabarcoding. While previous research on cryptic sponge communities in the Hawaiian

Archipelago has been limited to Kāne’ohe Bay on O’ahu, this study offers a comprehensive view of these communities throughout the entire archipelago. Using a standardized approach to compare cryptic sponge diversity and composition between the MHI and the NWHI, this research aims to clarify how varying environmental, ecological, and anthropogenic pressures shape their distribution. Additionally, it may provide valuable insights that could inform future studies on the distribution and dynamics of other reef organisms. Finally, the results of this research will offer critical insights into the distinct needs of these regions, aiding in the development of region-specific management and conservation strategies.

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Chapter 2 - MANUSCRIPT

Comparing cryptic sponge communities in the Main Hawaiian Islands and the Northwestern Hawaiian Islands

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Keywords

Cryptic sponges; ARMS; Metabarcoding; MHI; NWHI; Alpha diversity; Community composition

Abstract

In the context of climate change and increasing anthropogenic pressures, coral reefs face growing threats, making the need for comprehensive biodiversity assessments critical. Cryptic sponges play a fundamental role in nutrient cycling and dissolved organic carbon (DOC) processing via the sponge loop, contributing to the stability and resilience of coral reef ecosystems. However, despite their ecological importance, they have been historically overlooked in monitoring programs and biodiversity assessments due to their small and flat morphologies and hidden nature. In this context, recent research has revealed the remarkable diversity and abundance of cryptic sponges in Hawaiʻi, yet these findings remain geographically limited to Kāneʻohe Bay in Oʻahu. This study is the first to assess cryptic sponge diversity and community composition across such a broad geographical scale (over 2,500 km) in the Hawaiian Archipelago, and to compare them between its two main regions: the Main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI). Beyond its regional significance, it also represents the first global assessment of cryptic sponge diversity across such an extensive area using innovative, non-invasive methods, including Autonomous Reef Monitoring Structures (ARMS) and metabarcoding. Our findings showed a dominance of Porifera, along with Rhodophyta in cryptobenthic spaces across Hawaiian reefs, and a total of 125 Operational Taxonomic Units (OTUs) of cryptic sponges were identified, highlighting their high abundance and diversity throughout the archipelago. Moreover, significant differences in community composition were observed between the MHI and the NWHI and MHI exhibited significantly higher alpha diversity. We hypothesize that nutrient and DOC runoffs from rivers and groundwater discharges in the populated, agriculturally modified landscapes of the MHI, coupled with the greater habitat diversity due to their high island topography, contribute to these patterns. This contrasts with the low atolls of the NWHI, which lack freshwater inputs from rivers and groundwater and exhibit reduced habitat diversity due to their flat topography. These findings provide critical insights into the factors shaping cryptic sponge distribution and underscore the need for region-specific management and conservation strategies. Finally, these results are expected to enhance understanding of reef ecosystem dynamics and guide future research on the spatial distribution of cryptic reef organisms.

2.1 Introduction

Coral reefs are the most diverse marine habitat per unit area but are suffering from major biodiversity loss attributed to a combination of both natural and anthropogenic stressors (Hoegh-Guldberg et al., 2007; Carpenter et al., 2008) Understanding diversity patterns is crucial for evaluating ecosystem resilience and developing successful conservation strategies in the face of rapid environmental shifts (Butchart et al., 2010; McCauley et al., 2015). Ecological theories predict that regional species assemblages are mediated by historical and geographical processes (such as oceanic transport, geological and historical evolutionary processes) (reviewed in Gaston, 2000). Local communities are assembled from a regional species pool and are hence shaped by regional processes, but can also be influenced by biological interactions, abiotic factors, and disturbance. With respect to coral reef ecosystems, habitat type seems to best explain the regional variation in taxonomic composition of corals and fishes in the Indo-Pacific (Bellwood & Hughes, 2001, Karlson et al., 2004). However, studies estimating the biogeographical patterns of coral reef diversity at the regional scale have focused on conspicuous surface-dwelling taxa, such as corals and fish (e.g. Glynn & Ault, 2000; Edinger et al., 2000; Bellwood & Hughes, 2001; Cornell et al., 2008), but overlooked important benthic invertebrate groups, such as sponges.

Sponges (phylum Porifera) are fundamental components of coral reef ecosystems due to their numerical dominance and functional importance. They provide essential structural roles by offering microhabitats and settlement substrates for various organisms (Diaz & Rützler, 2001; Rützler, 2004; Fiore & Jutte, 2010; Pawlik, 2011). They allocate energy to either chemical defences against predators or somatic growth, often outcompeting corals in overfished areas (Pawlik et al., 1995; Leong & Pawlik, 2010; Loh et al., 2015). Through microbial symbionts, sponges contribute to primary production and nitrogen cycling (Corredor et al., 1988; Bell, 2008; Southwell et al., 2008; Thomas et al., 2016; Erwin & Thacker, 2008) and are key in spongivorous diets (Hill, 1998; Pawlik, 2011; Lesser & Slattery, 2013). In addition to being efficient filter feeders that consume nano and picoplankton (Gili & Coma, 1998; Turon et al., 1997; Ribes et al., 1999; Kötter, 2003), sponges are unique among metazoans in their ability to metabolize biologically unavailable forms of dissolved organic carbon (DOC) (Yahel et al., 2003; Van Duyl et al., 2008; De Goeij et al., 2008; 2013; Rix et al., 2016; 2017). In fact, they have been shown to remove over 90% of the total organic carbon as DOC and consume it at twice the speed of their bacterioplankton uptake (De Goeij et al., 2008).

The role of emergent sponges in recycling DOC is not yet well understood, although it has been hypothesized that the assimilated DOC could be retained for somatic growth as biomass (McMurray et al., 2018), therefore being accessed by higher trophic levels through predation (Pawlik, 2011). In contrast, sponges in cryptic habitats that are smaller and encrusted in the reef matrix have been found to be part of the "sponge-loop" (De Goeij et al., 2013) where they convert algal and coral-derived DOC into nutritional, particulate organic carbon (POC) (De Goeij et al., 2013; Alexander et al., 2014; 2015). Through this process, they provide resources for detritivores at lower trophic levels through rapid tissue turnover and somatic growth levels close to zero (De Goeij et al., 2013; 2017; Rix et al., 2016; 2017; 2018, Alexander et al., 2014; 2015). Thus, cryptic sponges play a crucial role as ecosystem engineers, effectively distributing nutrients and energy that shape coral reef food webs (Rützler, 2004; De Goeij & Van Duyl, 2007; Bell, 2008, Rix et al., 2018).

Despite their significant ecological roles in coral reef ecosystems, sponges are often underrepresented in biodiversity surveys compared to other taxa (Hooper & Lévi, 1994; Wörheide et al., 2008; Van Soest et al., 2012; Bell et al., 2014), with cryptic sponges being particularly overlooked (Van Soest et al., 2012; Appeltans et al., 2012; Vicente et al., 2022a). This knowledge gap largely reflects collection biases (Van Soest et al., 2012), as sponge research has focused on accessible regions like the Mediterranean, Northeast Atlantic, and Caribbean (Van Soest et al., 2012), while studies on sponge diversity, especially cryptic species, in isolated Indo-Pacific islands remain scarce (Hall et al., 2013; Vicente et al., 2022a; b; Galitz et al., 2023).

Robust estimates of sponge diversity in Indo-Pacific regions are hampered by logistical challenges involved in accessing remote oceanic islands and the associated sponge fauna of the coral reef cryptobiome. Historically, the fauna of sponges within tropical basins were thought to only be dominant closer to continental reefs such as the Caribbean and in reefs adjacent to land in the western Pacific but become less abundant around oceanic islands (Birkeland 1987; Wilkinson 1987). However, recent surveys have found that although emergent sponges in remote Oceania are indeed rare, encrusting sponges are dominant within the cryptobiome of coral reef communities (Vicente et al., 2022a). It is, however, extremely challenging to sample cryptic sponges residing within the reef interior without destroying the surrounding reef. Altogether, collection and sampling challenges limit our ability to survey cryptic sponges on isolated Indo-Pacific islands.

To overcome these sampling obstacles, in recent years, Autonomous Reef Monitoring Structures (ARMS) have been developed. These are inexpensive, non-destructive, and standardized structures that imitate reef crevices and thus allow the settlement and subsequent sampling of coral reef-associated invertebrates (Zimmerman & Martin., 2004; Brainard et al., 2009). ARMS have been shown to capture a greater abundance and diversity of organisms compared to traditional hand-collection methods and can accurately capture the taxonomic richness and community composition of a broad diversity of cryptic eukaryotic fauna on coral reefs (e.g.: Zimmerman & Martin, 2004; Casey et al., 2021; Timmers et al., 2022b; Vicente et al., 2022a).

Despite these advances in field-based sampling tools, estimating the diversity of Porifera on a larger scale remains challenging since sponges are notoriously difficult to identify using traditional taxonomy. This challenge arises from the lack of clear morphological hard structures and ontogenetic plasticity among different groups in the phylum (Rützler, 2004; Rützler et al., 2007; Wörheide et al., 2007; Van Soest et al., 2012). As a result, different sponge species can appear morphologically identical, or conversely, individuals of the same species can have marked different morphologies. This is further complicated in cryptic sponges, which often display small, flat morphologies.

Molecular identification of complex communities via in-bulk amplification of a shared locus (Baird & Hajibabaei, 2012; Hajibabaei et al., 2011; Taberlet et al., 2012; Yu et al., 2012; Ji et al., 2013) termed DNA metabarcoding, has helped address these gaps in sponge biodiversity studies (e.g: Timmers et al., 2022a; b; Martineau et al., 2024). Although traditional taxonomy based on morphology remains crucial for initial specimen identification and describing new taxa (Fautin et al., 2010; Fisher et al., 2015), metabarcoding offers a rapid, cost-effective, and precise technique for biodiversity assessments (Yu et al., 2012; Ji et al., 2013). The cytochrome c oxidase I (COI) and the 28S rRNA gene marker have been used for sponges as a target locus (Vargas et al., 2012; Voigt & Wörheide, 2016; Timmers et al., 2022b; Martineau et al., 2024). However, the 28S marker has proven to perform better in detecting and quantifying sponge species than the COI (Erpenbeck et al., 2016; Voigt & Wörheide, 2016; Martineau et al., 2024).

Thus, ARMS sampling methods, coupled with molecular identification of sponges, provide a powerful approach for surveying the diversity of cryptic sponges. In fact, a recent study exploring the diversity of cryptic sponges on the island of Oʻahu, Hawaiʻi, —an isolated archipelago in the Indo-Pacific—revealed 150 new sponge records, increasing the known sponge fauna by 2.5-fold for the entire archipelago (Vicente et al., 2022a). This study not only demonstrates the effectiveness of ARMS sampling devices in delineating cryptic sponge diversity but also highlights the potentially immense diversity of cryptic sponges in Hawaiʻi, challenging previous hypothesis that sponge abundance decreases with distance from the continental shelf (Birkeland 1987; Wilkinson 1987). While new records and species of cryptic sponges have been described on Hawaiian reefs (Nunley et al., in review), current diversity studies of Porifera are confined to the island of Oʻahu (Timmers et al., 2022a; b; Vicente et al., 2022a; b). As a result, the species richness, composition of cryptic sponge assemblages, and their geographic distribution across the Hawaiian archipelago remain unknown.

The Hawaiian Archipelago is situated in the central Pacific Ocean and extends over 2,500 km from the southeastern island of Hawaiʻi (19º N, 154 º W) to Kure Atoll in the northwest (28º N, 178 º W). It is divided in two regions: the Main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI). The MHI are characterized by high volcanic islands, a large human population and freshwater sources from rivers and groundwater (Fletcher et al., 2008). Moreover, habitat diversity in the MHI is considerably high due to their larger size, varied topography, and the influence of volcanic peaks, which create distinct windward and leeward climates that support a wide range of coastal ecosystems (Friedlander & Brown, 2019). In contrast, the NWHI consist mostly of uninhabited, low-lying atolls without freshwater sources, limited habitat diversity, and minimal human presence (Friedlander et al., 2004; Friedlander & Brown, 2019). While the MHI experience significant human-induced habitat changes and land inputs from river and groundwater runoffs, the NWHI remain largely untouched and support critical habitats for endangered species (Friedlander et al., 2004; Gove et al., 2016).

In this study, we present the first assessment of cryptic sponge diversity across the Hawaiian Archipelago. For this we used non-destructive sampling methods, monitoring sponge species richness over 3-year periods on ARMS deployed at 29 sites across 8 islands along the Hawaiian Archipelago. We assume the biodiversity found on the ARMS is representative of the cryptic communities of Hawaiian reefs, since earlier research suggested that cryptic communities in tropical reefs require a minimum of three years to fully establish (Choi, 1984), which is in accordance with our recruitment periods (Table 1). Furthermore, metabarcoding was performed on these complex ARMS communities, targeting the C2-D2 region of the 28S rRNA gene using newly designed sponge-specific universal primers (Martineau et al., 2024). By comparing cryptic sponge communities between the MHI and the NWHI, we aim to understand how regional factors influence their distribution and diversity. This research is crucial because cryptic sponges play significant roles in nutrient cycling and reef resilience but have been historically understudied. Incorporating data on their diversity and abundance into future coral reef monitoring efforts will support future efforts to identify and protect native species, detect invasive sponge introductions, and monitor community changes due to human impacts. Moreover, it will enhance our understanding of reef dynamics, by facilitating biodiversity assessments, and informing effective conservation strategies tailored to the unique characteristics of each region. Ultimately, our findings will potentially serve as a baseline for future research on cryptobenthic community assessments, thus supporting global diversity assessments in coral reef ecosystems.

2.2 Materials and methods

2.2.1 Sampling

Autonomous Reef Monitoring Structures (ARMS), 3-dimensional tiered standardized sampling units, were deployed by divers at 29 sites across 8 islands along the Hawaiian Archipelago (Figure 2.1, 2.2, Table 2.1). ARMS were composed of 10 grey type 1 PVC plates (23 cm x 23 cm) stacked in an alternating series of open and semi-enclosed layers. ARMS units were affixed to the seafloor at an approximate depth of 40 m. After a three-year soak period, divers encapsulated the ARMS units with a 106 um nitrex-lined crate to retain all organisms that recruited to the units. Once at the surface, ARMS were placed into a container full of seawater, where they were dismantled plate-by-plate. Plates were photographed and scraped clear of all the accumulated biomass that colonized plate surfaces. Scraped biomass was then immediately homogenized in a blender, and 10 grams of the homogenate was subsampled and preserved in DMSO for DNA metabarcoding. All the field processing occurred on NOAA ships during Reef

Assessment and Monitoring Program research cruises conducted by the Pacific Islands Fisheries Science Center.

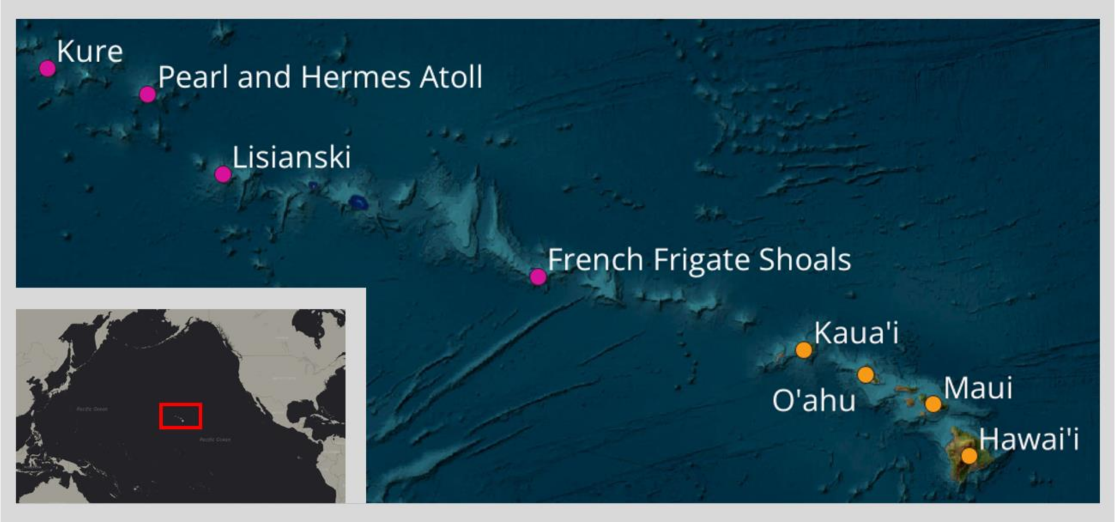


Figure 2.1. *Geographic location of the remote Hawaiian Archipelago and the 8 studied islands: MHI (orange circles) and NWHI (pink circles).*

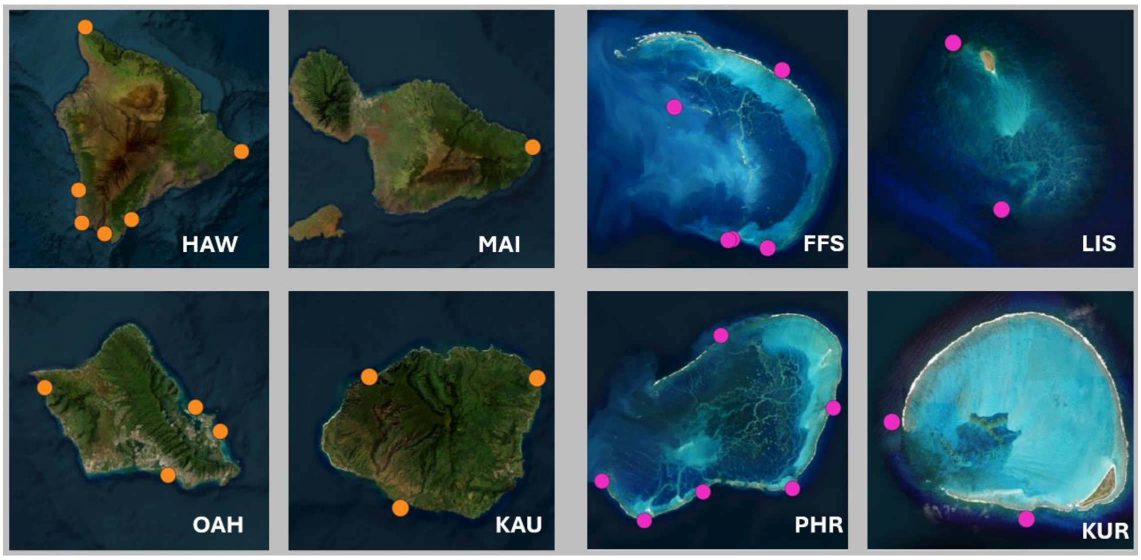


Figure 2.2. *Exact location of ARMS deployment sites for each studied island in MHI (orange circles) and NWHI (pink circles).*

Region	Island	Site	Latitude	Longitude	Depth (m)	Deployment	Recovery	Replicates
MHI	Hawaii	HAW22	18.9686	-155.73073	50	2010	2013	3
		HAW24	19.03822	-155.88255	48	2010	2013	3
		HAW41	20.26824	-155.86038	46	2013	2016	2
		HAW43	19.24436	-155.90028	48	2013	2016	3
		HAW46	19.48638	-154.81766	41	2013	2016	3
		HAW48	19.06071	-155.55237	43	2013	2016	2
	Maui	MAI01	20.76226	-155.97977	38	2010	2013	3
	Oahu	OAH12	21.41213	-157.7098	49	2010	2013	3
		OAH21	21.53412	-158.23441	50	2013	2016	3
		OAH22	21.47971	-157.783	42	2013	2016	3
		OAH23	21.28838	-157.86536	43	2013	2016	3
	Kauai	KAU02	22.16405	-159.2988	45	2010	2013	2
		KAU06	21.88976	-159.60887	40	2010	2013	2
		KAU08	22.16684	-159.68001	41	2010	2013	2
NWHI	French Frigate Shoals	FFS12	23.63835	-166.18005	38	2010	2013	3
		FFS34	23.62792	-166.13538	34	2010	2013	3
		FFS40	23.6369	-166.18527	47	2013	2016	3
		FFS41	23.7924	-166.2538	41	2013	2016	3
		FFS44	23.83515	-166.11682	55	2013	2016	1
	Lisianski	LIS10	25.94462	-173.95361	50	2010	2013	1
		LIS14	26.07842	-173.99701	48	2010	2013	2
	Pearl and Hermes Atoll	PHR26	27.78583	-175.78028	45	2010	2013	2
		PHR42	27.75313	-175.94877	48	2010	2013	1
		PHR50	27.78237	-175.88194	47	2013	2016	3
		PHR51	27.86691	-175.73351	47	2013	2016	3
		PHR52	27.9405	-175.86172	47	2013	2016	3
		PHR54	27.79317	-175.9971	47	2013	2016	3
	Kure	KUR12	28.38231	-178.32448	32	2010	2013	2
		KUR33	28.41677	-178.37843	45	2010	2013	1

Table 2. 1. *Exact coordinates, depth, deployment and recovery years of ARMS for each island and region.*

2.2.2 DNA metabarcoding

Genomic DNA was isolated from environmental samples using the DNeasy PowerMax Soil Kit (Qiagen) following the manufacturer's protocol with modifications from Ransome et al., 2017. ~ 318 pb amplicons of the 28S rRNA gene were generated via polymerase chain reaction (PCR) using the 28S POR 100 FDG and LSU 300RV primers (Martineau et al., 2024; Redmond et al., 2011; Olson (NHM London, Per. Comm)). The PCR reactions were conducted in 20 μL volumes, including 7.8 μL of nanopure H_2O, 10 μL of ImmoMix Red Polymerase (Bioline, NSW, Australia), 0.6 μL of each primer (10μM), and 1 μL of template DNA. A touchdown PCR profile was used with an initial denaturation at 95ºC for 3 min, followed by 46 cycles: 3 cycles of 95ºC for 30 s, 50ºC for 30 s, and 72ºC for 60 s; 3 cycles with decreasing annealing temperature to 48ºC; followed by 40 cycles of decreased annealing temperature of 45ºC and a final extension of 72 °C for 5 minutes (Martineau et al., 2024). The success of PCR amplifications was examined on a 2% agarose gel stained with GelRed (Biotium, Fremont, CA, USA) and validated by a distinct and clear band of ~ 318 bp.

PCR products were then purified with Mag-Bind TotalPure NGS beads (Omega Bio-Tek, Norcross, GA, USA) at a 0.85 X ratio of amplicon concentration, following the manufacturer's protocol. Amplicon concentration was quantified using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). End repair and A-tailing, and adapter ligation were carried out on cleaned PCR products using the Watchmaker DNA Library Prep Kit (Watchmaker Genomics, Boulder, CO, USA). A purification was then conducted in 40 µL reaction volumes (1X bead ratio) using Mag-Bind TotalPure NGS beads (Omega Bio-Tek, Norcross, GA, USA) on automated KingFisher Flex Purification System (Thermo Fisher Scientific, Waltham, MA, USA). Unique indexes were added to each sample using xGen Ubique Dual Index (UDI) combinations (IDT, Coralville, IA, USA). Products were then purified with Mag-Bind TotalPure NGS beads (Omega Bio-Tek, Norcross, GA, USA). Libraries were quantified using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Invitrogen, Invitrogen, Waltham, MA, USA) and visually inspected on a 2% agarose gel. Libraries were combined into two distinct pools: one with samples that showed a clear, bright band on the gel (high-concentration pool, n = 9 samples) and another with samples that showed fainter bands (low-concentration pool, n = 63 samples). In an attempt to equalize the coverage of libraries, we performed paired-end sequencing of the two pools, targeting ¼ of a lane for the high-concentration pool and ¾ of a lane of the low-concentration pool on an Illumina MiSeq (v3 2x300PE) at the Illumina Headquarters (San Diego, CA, USA).

2.2.3 Bioinformatics

Quality filtering, removal of chimeric sequences, denoising, and dereplication were performed on raw demultiplexed reads using the eDNAFlow pipeline (Mousavi-Derazmahalleh et al., 2021). Briefly, raw reads were quality-filtered (minimum Phred score of 20) and merged using AdapterRemoval v2 (Schubert et al., 2016) (minimum overlap length of 12 bp and minimum length of 50 bp). Chimeras were removed using the UCHIME2 algorithm, and sequences were denoised and dereplicated to zero-width operational taxonomic units (zOTUs) with a minimal sequence abundance of 8 using the UNOISE3 algorithm in VSEARCH (Edgar, 2016a; 2016b). The resulting abundance tables of both sequencing runs were merged, and samples were rarefied to an equal sequencing depth of 30,000 reads using the Rarefy command from the GUniFrac package in R (Chen & Chen, 2018). Samples that had fewer than 10,000 were discarded and not considered in the analysis. Singletons were considered spurious sequences and were not retained for downstream analyses.

Taxonomic assignments were performed in two steps as per Martineau et al., 2024. Briefly, the amplicons were first queried against a sponge database constructed from a curated sponge reference library to enable accurate identification of Porifera, a group for which public databases are highly unreliable (BLASTn parameters: E value < 1e-3, minimum query coverage of > 95%, and minimum percent identity of 95%). Taxonomy was assigned based on the match with the highest sequence similarity to queries using the curated local database. We subsequently annotated the sequences that remained taxonomically unassigned using BLASTn matches against the entire GenBank nucleotide database with the same parameters and downstream filtering criteria to identify non-sponge taxa found in the homogenated bulk coral reef communities of the ARMS. We eliminated sequences for which the best match was below 95% identity in downstream analyses.

2.2.4 Data analysis

Data analysis and visualization were performed using R v.4.2.2. The study focused on comparing the MHI and NWHI regions in Hawaiʻi, excluding Maui due to the limited representativeness of a single site in the overall region (Table 1). The Phyloseq v.1.46.0 package was used to generate the relative abundance of phyla across regions, with only the five most abundant phyla displayed for clarity. The relative abundance of classes within the phylum Porifera and orders inside of the class Demospongiae were also analysed. For this, data were filtered to include only Porifera OTUs for the relative abundance of classes, and only Demospongiae OTUs for the relative abundance of orders inside this class. Moreover, visualizations were limited to the seven most abundant orders in Demospongiae to enhance interpretability.

Alpha diversity was assessed using Chao diversity indices, and visualized by means of boxplots generated via the tidyverse v.2.0.0 package and ggplot2 functions. ANOVA assumptions, specifically normality and homogeneity of variance, were not met and thus the Kruskal-Wallis test was used to evaluate significant differences in alpha diversity between regions. For beta diversity analyses, the plot_ordination function from

Phyloseq was used to generate non-metric multidimensional scaling (NMDS) ordination plots based on Bray-Curtis dissimilarity indices for each region. Ellipses indicating 95% confidence intervals around the mean position of samples from each region were added using tidyverse, and outliers were identified with the vegan v.2.6.4 package. Significant differences in beta diversity were tested using the PERMANOVA test within the vegan package. Additionally, shared and unique sponge orders and species between the regions were identified and visualized using Venn diagrams from the systemPipeR v.2.8.0 package.

2.3 Results

2.3.1 Relative abundance in MHI vs NWHI

The 4 most abundant phyla in both the MHI and NWHI were Rhodophyta, Porifera, Mollusca and Pseudomonata. Porifera was the second most abundant phylum in both regions (Figure 2.3). However, around 15% and 40% of the total phyla detected on the ARMS were unclassified for the MHI and NWHI respectively.

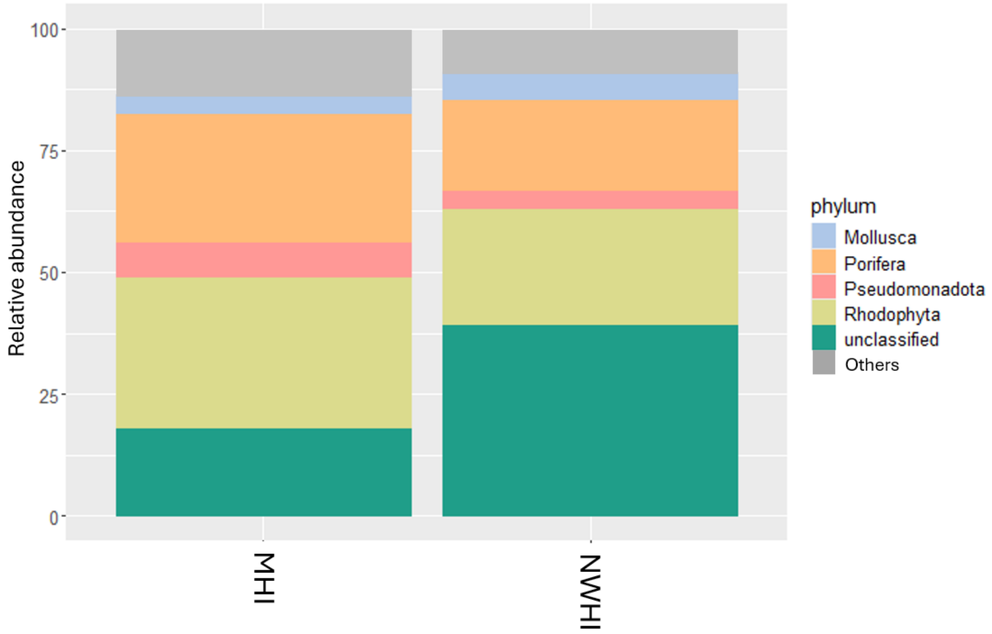
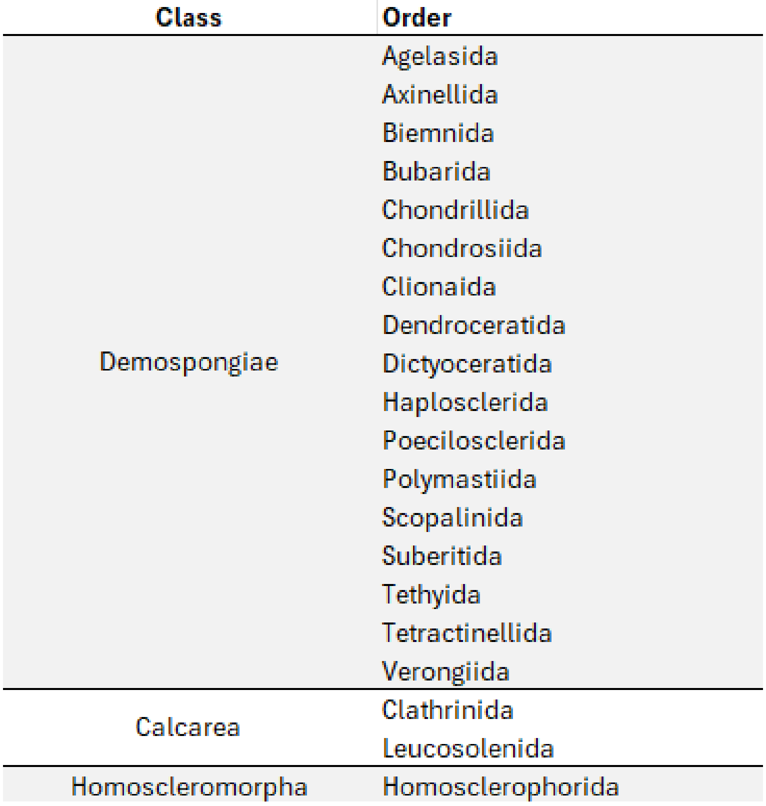


Figure 2.3. *Relative abundance of 5 most abundant phyla found on ARMS for each region.*



Class	Order
Demospongiae	Agelasida
	Axinellida
	Biemnida
	Bubarida
	Chondrillida
	Chondrosiida
	Clionaida
	Dendroceratida
	Dictyoceratida
	Haplosclerida
	Poecilosclerida
	Polymastiida
	Scopalinida
	Suberitida
	Tethyida
	Tetractinellida
	Verongiida
Calcarea	Clathrinida
	Leucosolenida
Homoscleromorpha	Homosclerophorida

Table 2.2. *Classes and orders identified in the Hawaiian Archipelago*

We identified a total of 125 sponge OTUs, 3 sponge classes and 20 sponge orders on the ARMS displayed in the MHI and NWHI (Table 2.2). Within phylum Porifera, the class Demospongiae was clearly dominant, representing more than 95% of the total abundance of sponges in both regions (Figure 2.4 A). In the MHI, the second most abundant class was Homoscleromorpha and the third and final was Calcarea. In contrast, Calcarea was the second most abundant class in the NWHI followed by Homoscleromorpha. The most dominant order among Demospongiae was Poecilosclerida which dominanted in both regions and accounted for almost half and over half of the total abundance in the MHI and NWHI respectively (Figure 2.4 B). While the NWHI were mostly dominated by poecilosclerids (~60 %), orders Haplosclerida (~25%) and Tetractinellida (~5%) also were abundant. The MHI showed similar relative abundances of orders with poecilosclerids (~45%) and tetractinellids (~35%), followed by haplosclerids (>5%) and Dictyoceratida (<5%).

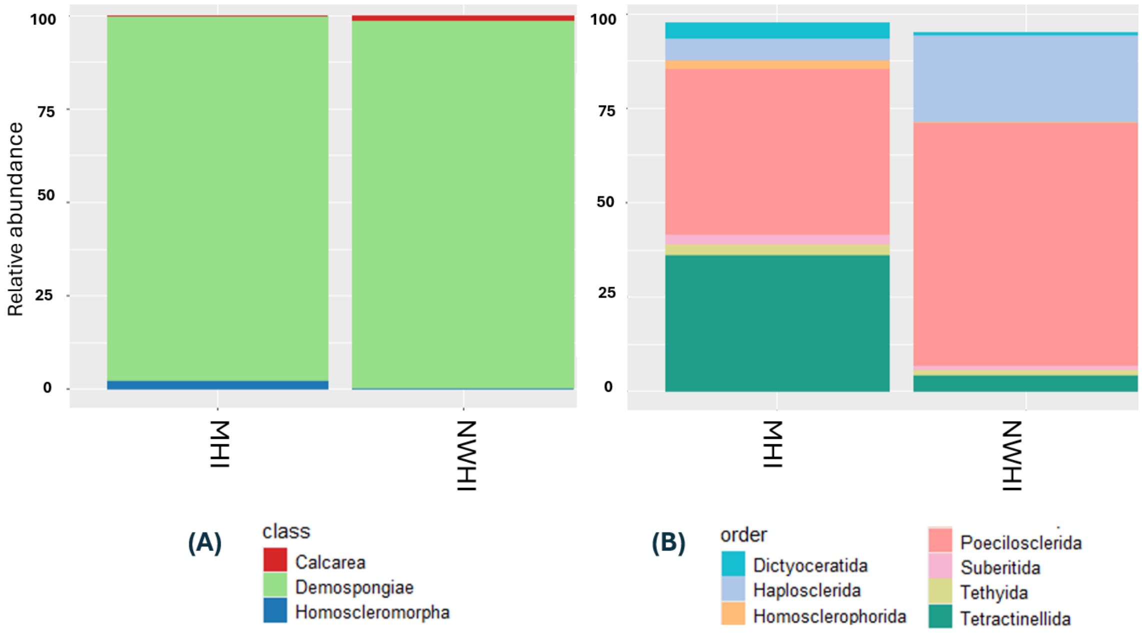


Figure 2. 4. *Relative abundance (A) by class in phylum Porifera for each studied region; (B) of 7 most abundant orders in class Demospongiae for each studied region.*

2.3.2 Porifera alpha and beta diversity in MHI vs NWHI

The Kruskal-Wallis rank sum test revealed statistically significant differences in alpha diversity between the NWHI and MHI regions (chi-square = 7.7021; df = 1; p = 0.0055) (Figure 2.5 A), suggesting that species richness is higher in MHI. In addition, sponge community composition differed significantly by regions (PERMANOVA: F= 3.0381; R^2= 0.04338; p= <0.001) (Figure 2.5 B).

Moreover, Venn diagrams showed the existence of unique orders and species in both regions. The MHI showed higher numbers of both unique orders (4) and species (31) in comparison with NWHI which had 1 unique order and 12 unique species (Figure 2.6). However, the number of shared orders and species was relatively high when comparing both regions (16 orders and 82 species).

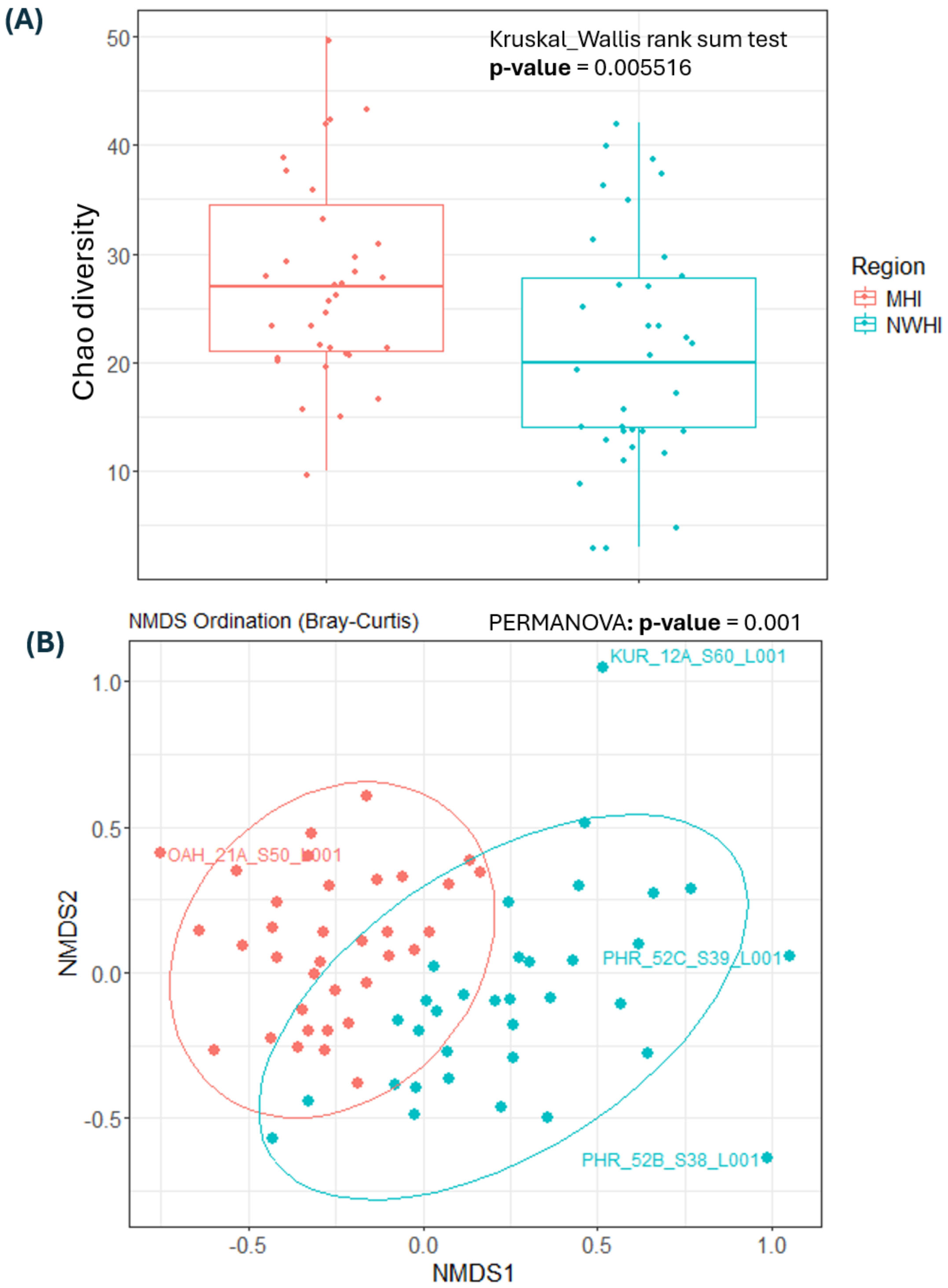


Figure 2.5. *(A) Alpha diversity per studied region: boxplot of chao diversity; (B) Beta diversity of each studied region: community composition using multidimensional scaling of Bray-Curtis distances. Community composition is outlined by blue and red ellipses and outlier sites are indicated. Plots show significant Kruskal-Walis and PERMANOVA results respectively.*

Moreover, Venn diagrams showed the existence of unique orders and species in both regions. The MHI showed higher numbers of both unique orders (4) and species (31) in comparison to NWHI which had 1 unique order and 12 unique species (Figure 2.6).

However, the number of shared orders and species was relatively high when comparing both regions (16 orders and 82 species). All in all, the MHI showed higher order and species diversity when compared to the NWHI.

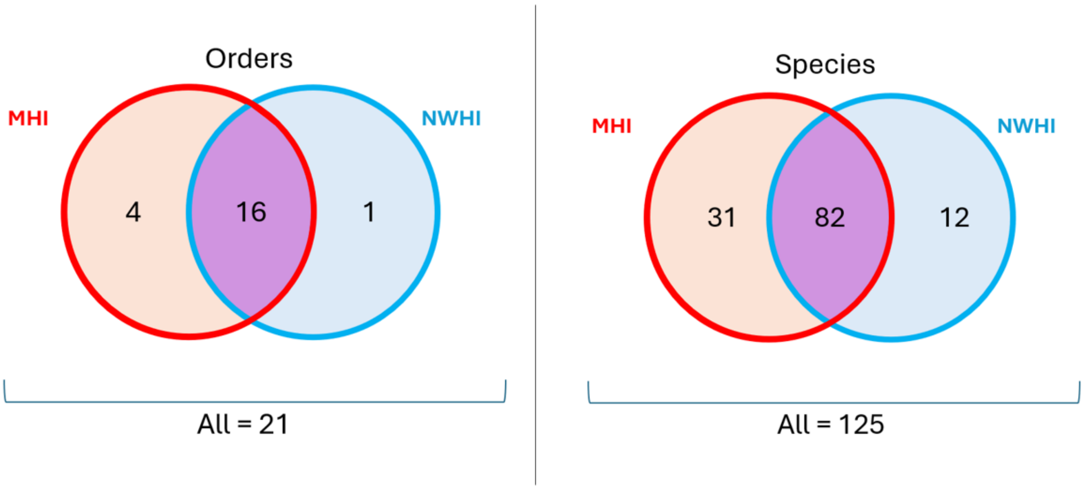


Figure 2.6. *Venn diagrams indicating shared (purple area) and unique orders and species between the two studied regions.*

2.4 Discussion

Coral reef ecosystems are of utmost importance due to their exceptional biodiversity and the crucial ecological functions they provide (Richter et al., 2001; Rützler, 2004; De Goeij & Van Duyl, 2007; Bell, 2008; Knowlton et al., 2010). Despite this, they are increasingly threatened by climate change, which causes rising sea temperatures from greenhouse gasses and ocean acidification from the absorption of CO_2 in seawater (Carpenter et al., 2008; Sunday et al., 2017). Sponges are considered one of the winners under warmer and acidified conditions expected for the end of the century (Bell et al., 2013; 2018; Timmers et al., 2022a). In this context, cryptic sponges are crucial for sustaining biodiversity within coral reef ecosystems, acting as sinks for DOC and converting it into POC (Richter et al. 2001; De Goeij & Van Duyl, 2007; De Goeij et al., 2013; Rix et al., 2018); a role that underpins their significant economic value in supporting food security through fisheries and tourism. However, despite their importance, cryptic sponges that are hidden within complex habitats remain extremely understudied (Van Soest et al., 2012; Appeltans et al., 2012; Vicente et al., 2022a). The present study is the first to comprehensively assess the abundance and diversity of cryptic sponges using Autonomous Reef Monitoring Structures (ARMS) and the first to explore the diversity of cryptic sponges between the Main Hawaiian Islands (MHI) and Northwestern Hawaiian Islands (NWHI) of the Papahānaumokuākea Marine National Monument listed as a World Heritage site. By establishing a baseline for cryptic sponge diversity in these regions, our findings will provide a critical foundation for future research, contribute valuable data essential for a comprehensive understanding of coral reef health and resilience, and inform conservation policies and strategies aimed at protecting these vital yet overlooked components of marine ecosystems.

Our study identified 125 sponge OTUs across the Hawaiian Archipelago. This supports recent findings from Kāneʻohe Bay, Oʻahu, revealing unexpectedly high sponge abundance and diversity in the Archipelago (Vicente et al., 2022a). Historically, sponge faunas were believed to be more abundant and diverse near continental reefs, such as in the Caribbean and the "continental" western Pacific, but less abundant around oceanic oligotrophic islands (Birkeland 1987; Wilkinson 1987). Previous research also suggested that sponges were less diverse in the insular central Pacific compared to areas like the Coral Triangle (Van Soest et al. 2012). Our findings 1. challenge the notion that oceanic islands harbour low sponge abundance and diversity 2. highlight the importance of cryptobenthic sponges in Hawaiian reef ecosystems and 3. emphasize the critical role of cryptic habitats in supporting sponge biodiversity.

Rhodophyta and Porifera were identified as the most dominant phyla in both regions, followed by Mollusca and Pseudomonadota, at lower densities. These results support previous findings for the most common phyla found within cryptic habitats in the Red Sea (Richter et al., 2001; Wunsch et al., 2003; Pearman et al., 2016) and in the Eastern Mediterranean (Gerovasileiou & Voultsiadou, 2012; Guido et al., 2019). These studies found sponges (Porifera) and coralline algae (Rhodophyta) to be highly dominant in reef and cave crevices. The higher dominance of Rhodophyta over Porifera also coincides with finding from Wunsch et al. (2003) in the Red Sea, who found that red algae was the only phylum which surpassed sponges in terms of cover. However, a substantial proportion of the phyla detected on the ARMS—approximately 15% for the MHI and 40% for the NWHI—remained unclassified. It is plausible that many of these unclassified phyla are sponges, given their limited representation in current molecular databases. The higher proportion of unclassified phyla observed in the NWHI could also results from the limited exploration of the cryptic biodiversity of these remote islands. Because this field of study is very recent, further studies are needed to fully understand these patterns.

The class Demospongiae was clearly dominant and represented more than 95% of the total abundance in both the MHI and NWHI. This is also in accordance with previous studies of sponge diversity and abundance in natural reefs and in ARMS placed in mesocosms experiments receiving unfiltered seawater from the adjacent reef of Kāne'ohe Bay in O'ahu (Vicente et al., 2022a; b; Timmers et al., 2022b). Calcarea and Homoscleromorpha classes were clearly much less abundant in cryptic communities of the Hawaiian Archipelago and whereas Homoscleromorpha seemed to be more abundant in MHI, Calcarea was more abundant in the NWHI. However, both of these classes were relatively scarce and there is not enough evidence and previous studies to explain why these differences could exist, since sponge cryptic communities and remote Pacific regions are understudied (Van Soest et al., 2012; Appeltans et al., 2012). Nonetheless, it is an initial indication that differences in the composition of cryptic sponge communities between the NWHI and MHI do exist.

Distinct order abundances within demosponges were also indicative of differences in community composition between the two regions. Poecilosclerids were the most dominant order in both regions, coinciding with Vicente et al.'s findings in Kāne'ohe Bay (2022a). However, whereas Vicente and colleagues (2022a) found haplosclerids to be nearly as dominant as poecilosclerids, this was not the case in this study. Haplosclerids were found to be relatively dominant in NWHI (~25%) but not nearly as abundant as Poecilosclerids (~60%). In MHI, haplosclerids were much less dominant in comparison (~5%), and instead tetractinellids seemed to dominate the cryptic communities (~25%). Recent studies have described 5 potentially native tetractinellid species in Kāne'ohe Bay and one introduction from the Caribbean (Nunley et al., in review). Future analyses of our dataset will help determine the distribution of these species between the MHI and NWHI. The similarities between the sponge community composition in Kāne'ohe Bay and the NWHI, despite Kāne'ohe Bay being part of the MHI, suggest that it may be an outlier within the MHI or that significant variability exists in cryptic sponge communities across different islands and even between sites within the same island. This points to the potential for substantial regional differences in sponge community structure, emphasizing the need for localized studies at finer spatial scales to better understand the biogeography and ecology of these understudied and complex communities.

Moreover, significant differences in alpha diversity between the MHI and NWHI were observed, with the MHI showing higher species richness. This was accentuated by the higher order and species diversity found in the MHI (20 orders and 113 species) when compared to the NWHI (17 orders and 94 species) (Figure 2.6). Wilkinson (1987) demonstrated that sponge populations on Caribbean coral reefs and the Great Barrier Reef (GBR) differed significantly in biomass, largely due to varying levels of nutrient input. Caribbean reefs, often associated with islands and receiving substantial land-derived nutrients from runoff, exhibited sponge biomass that was five to six times greater than that of the GBR. Similarly, within the GBR, sponge biomass was highest on reefs adjacent to the mainland where nutrient input was elevated, and it decreased progressively on reefs further from shore in more oligotrophic waters (Wilkinson, 1987; Wilkinson & Cheshire, 1989). This gradient in sponge biomass corresponded with the influence of land-derived nutrients and DOC, which is a primary food source for sponge growth (Reiswig, 1981; McMurray et al., 2018; Hildebrand et al., 2022). While Wilkinson's study focused on biomass, these findings suggest that higher nutrient availability might also support greater sponge diversity due to enhanced food resources. In fact, there was evidence that within the GBR, sponge species diversity was highest on DOC and nutrient rich reefs adjacent to the mainland (Wilkinson, 1987).

Nutrient values in the MHI reefs fluctuate significantly with rainfall due to river and episodic terrestrial runoff and groundwater discharges (Friedlander et al., 2004). Frequent rainfall events during the wet season in the winter increase surface runoffs, raising concentrations of nutrients, DOC and suspended solids in rivers and streams entering coastal waters (Lau & Mink, 2006). Additionally, groundwater discharges further supply DOC and nutrients to coastal waters, enriching fringing reefs and potentially influencing local marine ecosystems. These runoffs are intensified by agriculture due to land modification and the disappearance of wetlands, which once served as natural filters, as well as increased soil erosion (Friedlander et al., 2008). Parallelisms can be drawn between Wilkinson’s findings and ours. It is known that the main threats to coral reefs in the MHI are local and human-induced, such as coastal development, pollution from land, and excessive recreational activities (Friedlander et al., 2008; Rodgers et al., 2010). In this context, the MHI resemble the nutrient-enriched environments of the Caribbean and inshore reefs of the GBR. In the MHI, 376 perennial streams exist (Harrison, 1992), contributing substantial inputs of DOC from terrestrial runoffs and groundwater into coastal waters (Lau & Mink, 2006). We therefore hypothesize that nutrient and DOC inputs into the MHI fringing reefs could explain the higher sponge diversity observed. In contrast, the NWHI atolls lack rivers or groundwater (Friedlander & Brown, 2019) and could be compared to the outer reefs of the GBR, where limited terrestrial influence and oligotrophic conditions prevail, and could explain the reduced sponge diversity compared to the MHI. These comparisons suggest that the differences in sponge diversity between the MHI and NWHI could be driven by variations in nutrient availability and DOC inputs into the neighbouring reefs (Figure 2.7), corresponding with the patterns described by Wilkinson (1987).

Our results also showed significant differences in the beta diversity of cryptic sponges, indicating differences in sponge community composition between the MHI and NWHI. These results are also in accordance with Wilkinson's findings (1987), which reported significant differences in species composition between the two coral reef regions. Therefore, we hypothesize that differences in nutrient and DOC inputs from terrestrial sources (high in the MHI and low to null in the NWHI) could also explain the differences observed in beta diversity between the two regions. However, evidence of higher productivity in the reefs of the MHI compared to the NWHI is needed to determine whether more organic matter is indeed available. Thus, this study highlights the necessity of conducting and expanding productivity assessments across the Hawaiian archipelago in order to better understand the distribution and diversity of marine organisms in Hawaiian coral reef ecosystems. Moreover, there is also a need for data on land derived runoffs, since information on contaminants entering the reefs through stream flow, surface runoff, and groundwater is inadequately documented (Rodgers et al., 2010).

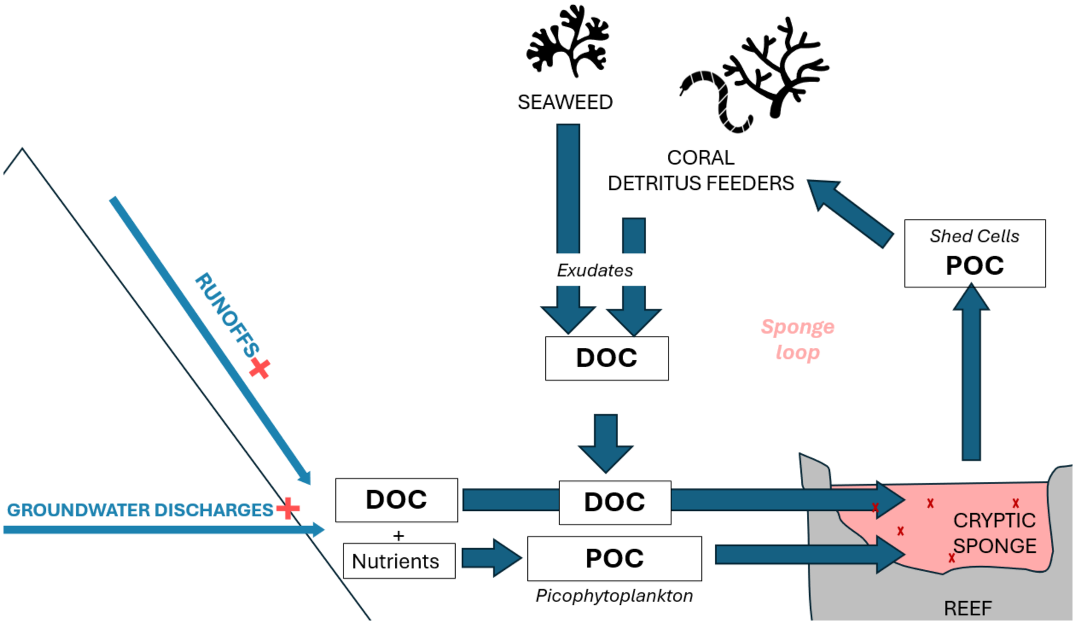


Figure 2. 7. *Simplified scheme showing hypothesis of nutrient and DOC dynamics in MHI with inputs from land runoffs (light blue arrows) adding to DOC pools available for cryptic sponges.*

The higher cryptic sponge diversity observed in the MHI compared to the NWHI could also be attributed to the greater complexity and diversity of habitats found in this region. The MHI's elevated, volcanic islands create pronounced climatic differences between their windward and leeward sides, with the windward side receiving higher rainfall and supporting lush vegetation, while the leeward side is drier and warmer (Fletcher et al., 2008; Friedlander & Brown, 2019). This variation leads to a wide array of coastal habitats, including coral reefs, sandy beaches, and tide pools. Moreover, human-modified environments like mangrove forests and fishponds add to this diversity. In contrast, the NWHI consist of low-lying atolls and small islands, lacking these elevation-driven climatic variations and the associated habitat diversity (Friedlander et al., 2004; Friedlander & Brown, 2019). Additionally, the MHI have unique geological features, such as lava tubes and aquifers, that increase habitat complexity by facilitating the mixing of fresh and seawater, introducing nutrients and sediments into the reef groundwater (Lowe, 2004). Lava tubes, in particular, have been shown to exhibit significant habitat heterogeneity, which supports greater species diversity and may even contribute to speciation (Wilkens et al., 2009). In contrast, the NWHI lack these underground systems. The combination of the MHI's higher elevation, varied topography, and anthropogenic influences creates a more diverse set of habitats and niche spaces that can support greater biodiversity. This environmental complexity, along with increased nutrient inputs from human activity and runoff in the MHI, likely contributes to the higher cryptic sponge diversity found there, compared to the more uniform habitat structures of the NWHI (Friedlander et al., 2004; Gove et al., 2016).

Finally, our study revealed a high degree of shared orders (76%) and species (66%) between the cryptic sponge communities of the MHI and NWHI. This significant overlap in species and orders is intriguing, given the geographical and environmental differences between the MHI and NWHI. However, a closer look at the geological and evolutionary context helps to clarify these findings. The Hawaiian Archipelago is relatively young in geological terms, with its oldest atoll, Kure, estimated to be around 29.8 mya old (Friedlander, 2009) and it's youngest island, Hawai'i, around 0.6 mya old (Friedlander et al., 2008). In contrast, sponges are among the most ancient metazoans, with origins tracing back more than 600 mya to the Cryogenian period (Erwin et al., 2011). Given this deep evolutionary history, sponges have likely developed a wide range of adaptive strategies that have enabled them to survive and thrive across different environmental conditions and geological eras.

The high degree of shared species and orders between the MHI and NWHI could, therefore, reflect the persistence of these ancient sponge lineages that have endured various environmental changes over millions of years. Despite the relatively recent emergence of the Hawaiian Islands, these sponges may have dispersed and adapted rapidly to the available habitats, maintaining their core biological traits while responding to new ecological opportunities. Thus, the cryptic sponge communities of the Hawaiian Archipelago tell a story of ancient survival, evolutionary resilience, and ongoing adaptation in a dynamic and evolving landscape.

In conclusion, this study revealed the dominance of cryptic sponges in the oceanic Hawaiian Archipelago reefs, challenging previous assumptions about their global distribution. Notably, the cryptic sponge community composition significantly differed between the MHI and NWHI, with higher alpha diversity observed in the MHI. This is potentially linked to factors such as increased nutrient and DOC inputs and habitat complexity in the high volcanic MHI. The high degree of shared species and orders between the two regions likely reflects the persistence of ancient sponge lineages that adapted to various environmental changes over millions of years. This underscores their potential role as indicators of past geological processes, revealing biogeographic relationships from millions of years ago. Moreover, this study confirmed the effectiveness of ARMS and metabarcoding using targeted 28S rRNA primers in assessing cryptic sponge biodiversity in complex coral reef ecosystems. Future investigations are needed to assess the global diversity of cryptic reef communities and facilitate the identification of endemic, native, or introduced species. In this context, our research will provide a foundational framework for these studies, enhancing our understanding of reef dynamics and informing effective conservation strategies tailored to the unique characteristics of each region.

2.5 Acknowledgements

This study's development was made possible by the support of the ToBo Lab at the Hawai'i Institute of Marine Biology, which provided the essential resources and conditions for conducting the experiments.

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