WORLD MARITIME UNIVERSITY

USE OF ENVIRONMENTAL DNA TO EXAMINE SEAGRASS ECOSYSTEM FUNCTIONALITY

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A dissertation submitted to the World Maritime University in partial fulfilment of the requirements for the award of the degree of Master of Science in Maritime Affairs

2024

Declaration

I certify that all the material in this dissertation that is not my own work has been identified, and that no material is included for which a degree has previously been conferred on me.

The contents of this dissertation reflect my own personal views, and are not necessarily endorsed by the University.

(Signature): .

(Date): **23 September 2024**

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Acknowledgements

I would like to express my appreciation to my donor Dr. Yohei Sasakawa, Chairman of Nippon Foundation, for awarding me the Sasakawa Fellowship and to the World Maritime University (WMU) for the opportunity to pursue my MSc degree in Sweden. I would also like to give my thanks to the wonderful team at the NGO Reef Conservation in Mauritius, who collected the water samples for the eDNA analysis and have supported all logistics for this study. I would also like to thank the Board of Reef Conservation for supporting my studies over this fourteen-month period. A special thank you to my family who have encouraged me, my girls who have cheered me on and are my inspiration for all that I do. And finally, I would like to express my warmest thanks to my supervisor Professor Johan Hollander who has patiently taught me throughout this process, listened as I worked through the concepts and encouraged me to keep going. Thank you all.

Abstract

Title of Dissertation: **Use of Environmental DNA to Examine Seagrass Ecosystem Functionality**

Degree: **Master of Science**

The dissertation is a study of seagrass biodiversity and functioning using eDNA technology.
Climate change together with anthropogenetic processes are today putting enormous stress on coasts and oceans. These impacts on the marine environment include ocean acidification, ocean warming, habitat loss, eutrophication of near shore waters, overfishing and several other stressors that are placing great pressure on biodiversity and ecosystem function, which is eroding ecosystem goods and services. In the Western Indian Ocean, we are today observing such losses of key habitats, with high records of coral bleaching, loss of mangrove forests, and large areas of seagrass meadows disappearing because of poor or non-existent management programs. Mauritius is not an exception, with seagrasses disappearing on a similar rate compared to other areas around the world, with a potential significant decline of marine biodiversity. This is of particular concern based on the many goods and services seagrasses provide to coastal inhabitants of Mauritius, and in particular the high number of economically important fish species supporting the local coastal inhabitants. It is therefore critical that our understanding of the presence and nature of these species is enhanced. To this end, a new revolutionary technique which is fast and relatively cost effective, has recently been developed to examine environmental DNA (eDNA) in marine environments to better capture the presence of species and ecosystem biodiversity. In this study, I used the eDNA metabarcoding technique to examine two lagoons, north and south around Mauritius Island, and to examine seagrass associated species, which was used to obtain information about the seagrass ecosystem function and potential ecosystem services. With eDNA metabarcoding, I was able to discover not only 26 completely new species, 14 genera and two families not before identified for Mauritius – but one species never recorded in the Western Indian Ocean. The study also identified a large number of economically important fish species, exploited elsewhere, but not recognised as a resource of protein in Mauritius. This study not only demonstrates that eDNA has the great potential of being the next-generation tool to monitor biodiversity and study ecosystem functioning – but to provide reliable data which will form the base for good management practices and appropriate and effective policy conservation actions.

KEYWORDS: Biodiversity, Biological Traits, Ecosystem Function, Ecosystem Services, eDNA, Ecosystem Services, Functional Redundancy, Seagrasses

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List of Abbreviations

eDNA	Environmental DNA
ES	Ecosystem Services
GBF	Kunming-Montreal Global Biodiversity Framework
GBIF	Global Biodiversity Information Facility
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
MOI	Mauritius Oceanography Institute
OTU	Operational Taxonomic Unit
WORMS	World Register of Marine Species

Chapter 1 Introduction

Ecosystem loss and degradation is a significant global issue affecting terrestrial, coastal and marine ecosystems resulting from both natural phenomena and anthropogenic activities on different scales (Bodo et al., 2021; CBD, 2020; Dunic et al., 2021; Newton et al., 2020; Scanes, 2018).

Humans, although part of biodiversity, have been significantly changing terrestrial and marine ecosystems by removing, fragmenting and degrading habitats through direct and indirect activities (Bryan-Brown et al., 2020; FAO, 2020; IPBES, 2019). Direct anthropogenic impacts stem from land use changes and resource needs which include; deforestation, agriculture, urbanisation, coastal development, aquaculture, unsustainable fishing practices and unregulated tourism (Bodo et al., 2021; FAO, 2020; IPBES, 2019; IPCC, 2019). Whereas indirect impacts introduce pollutants and invasive species into habitats reducing the functioning of the ecosystem to support biodiversity. In addition, the impacts of climate change, can cause the demise of key stone species such as hard corals, resulting in the loss of coral reef ecosystems (IPCC, 2019; Rogers et al., 2023). Global estimates suggest that approximately 75% of terrestrial ecosystems have been significantly altered by deforestation, urbanisation and agricultural land use (IPBES, 2019). The same holds true for the three main coastal marine habitats where it is estimated that about 20% of the world's mangroves (FAO, 2023), at least 14% of global coral reefs (Souter et al., 2021) and over 29% of seagrass meadows (Dunic et al., 2021) have disappeared globally with localised variation often much higher due to site specific challenges and climate conditions.

Tropical coastal marine ecosystems often form a mosaic of habitats within the same seascape (Berkström et al., 2013, Henderson et al., 2017) and are often interlinked through physio-chemical processes, nutrient cycling, and shared biodiversity (Duffy, 2006). Compared to mangroves and coral reefs, seagrass ecosystems although widespread and highly productive (Hemminga and Duarte, 2000) are generally less considered despite the beneficial services they provide and their role in coastal protection, fisheries, climate mitigation, and as a critical carbon sink (Gullström et al., 2002; Gullström et al., 2018; Thomson et al., 2019).

Seagrasses are submerged marine flowering plants, that can be found globally in shallow coastal waters, and create extensive meadows or ecosystems along coastlines in both temperate and tropical regions (Hemminga and Duarte, 2000). Like other coastal marine ecosystems, seagrasses can be degraded due to direct and indirect anthropogenic impacts, with the most negative being overfishing, nutrient inputs from land-based sources, causing eutrophication, and physical damage due to shallow coastal fishers, as well as boat anchorage and removal for tourism purposes (Daby, 2003; Duffy, 2006; Short and Wyllie-Echeverria, 1996; Unsworth et al., 2019; Waycott et al., 2009). The loss and degradation of seagrass ecosystems will have cascading effects on its associated biodiversity, its functionality and the provision of its ecosystem services.

Similar to other foundation ecosystem species such as reef building corals, different seagrass species become established where physical and chemical parameters, such as protected coastal areas, substrate grain size and nutrients, persist for their survival (Boström et al., 2006, Koch and Gust, 1999), with the extent of their depth range dependent on light attenuation (Beck et al., 2017; Gallegos and Kenworthy, 1996). Seagrasses are known as autogenic ecosystem engineers (Bouma et al., 2009) or primary ecosystem engineers (Gutiérrez et al., 2011) as the structure of the plant itself; its roots, stems and canopy of leaves, modifies the substrate and the physical environment, creating positive feedback loops as they become established, which supports the spread of the meadow (Bouma et al., 2009; Gutiérrez et al., 2011).

The presence of seagrass species also provides a large set of ecosystem services. The canopy height, leaves and density of the seagrass shoots, helps to disperse wave energy that reduces water flow (Paul et al., 2012). Their leaves furthermore create small eddies which traps particles and supports the deposition of suspended sediments and organic matter onto the substrate, thereby reducing water turbidity and improving the environmental conditions for their own growth and survival (Bos et al., 2007; Potouroglou et al., 2017, Gutiérrez et al., 2011). The roots and for some species, dense rhizome mats help to bind, compact and stabilise sediments, building up the substrate over time and help prevent erosion during storms (Gutiérrez et al., 2011). The rooting system of seagrasses also plays an important role in the below ground storage and long-term burial of organic carbon derived from the meadow itself and other marine and land-based sources (Dahl et al., 2023; Gullström et al., 2018; Johannessen, 2022).

As seagrass meadows become established the complex structure of the meadow does not only benefit themselves, but also creates stable conditions, and numerous niche habitats and surfaces for the colonisation of associated infauna, epifauna, sessile and epibenthic organisms (Bouma et al., 2009; Gutiérrez et al., 2011). Associated biota can be found on the plants, between the shoots, under the canopy, as well as on and in the substrate (Gutiérrez et al., 2011). The structural complexity of the meadow provides refuge and hiding places for organisms from predators which makes seagrasses a vital nursery habitat for the recruitment of individuals and juvenile life stages of different organisms including coral reef and pelagic fish species that do not utilise seagrasses as adults (Heck et al., 2003; Unsworth et al., 2022). Seagrasses as primary producers contribute to the high productivity of the ecosystem and are grazed upon by both vertebrate and invertebrate biota (Duffy, 2006). The plants provide structure for epiphytic autotrophs as well as sessile invertebrates that not only form part of the rich biodiversity but add to the complex tropic interactions and food webs associated with seagrass ecosystems (Duffy, 2006; Heck et al., 2003).

Seagrasses, therefore, as a primary engineering species, facilitates biodiversity which includes secondary engineering species or allogenic engineers (Bouma et al., 2009; Gutiérrez et al., 2011). These species help to further change the environment due to their life strategy adaptations or biological traits of habitat utilisation, movement in and over substrate, and feeding strategies (Bouma et al., 2009; Gutiérrez et al., 2011; Moreira-Saporiti et al., 2023). Fauna that live in the substrate or burry into the sediments when in balance, also provide positive feedback loops that facilitate sediment settling and stabilisation and include benthic burrowing invertebrates such as polychaetes, mollusks, and crustaceans (Bouma et al., 2009; Gutiérrez et al., 2011). Such secondary infauna engineers create holes and burrows, some build tubes, aerate and mix sediments, and cycle nutrients thereby supporting the functioning of seagrass ecosystems, further increasing their complexity which attracts more biodiversity (Bouma et al., 2009; Gutiérrez et al., 2011). However, when there is imbalance these same burrowing actions together with the sediment sifting feeding strategies of benthic organisms can change the general function of a seagrass ecosystem (Bouma et al., 2009; Gutiérrez et al., 2011), with the risk of eroding its many ecosystem services.

The number of different species in an area or species richness and the composition of those species that make up the biodiversity, together with their relative abundance are important measures of ecosystem biodiversity (Swingland, 2001) and are used to indicate the health or functionality of an ecosystem. However, an index of biodiversity does not give a complete picture to describe an ecosystem as healthy or functioning, as organisms from different taxonomic groups can perform similar or different functions in the system depending on their biological traits and life strategies (Díaz & Cabido, 2001; Strong et al., 2015). When many taxonomic groups in an ecosystem perform the same functions, this is referred to as functional redundancy (Duffy, 2006; Monteiro, 2016). The response of an ecosystem to disturbances, or loss of species, and the time it takes to recover from such events, or the ability of other species to replace the role of the lost species, depends in part on the functional redundancy of its biological community (Kindeberg, 2024; Micheli & Halpern, 2005).

Closely linked to seagrass ecosystem functions are the ecosystem goods and services (ES) they provide, which are vital to the wellbeing of coastal communities, national economies, and more recently, to country commitments to mitigating climate change and biodiversity loss, wherever seagrasses exist (Lima et al., 2023; Liquete et al., 2013). These ES include sediment and shoreline stabilisation as well as coastal protection (Potouroglou et al., 2017), mitigation of climate change through carbon sequestration (Gullström et al., 2018; Johannessen, 2022), and the provision of vital ecological niches that facilitate habitat, foraging, nursery and protection functions for marine organisms (Heck et al., 2003; Nagelkerken et al., 2000). Seagrass ecosystems are biodiverse and highly productive coastal marine areas that support commercial fisheries globally (Cullen-Unsworth et al., 2014). They have also been proven to be critically important for artisanal fisheries in the Western Indian Ocean, including in Mauritius, where fish and invertebrate species are exploited by coastal and island communities for local market consumption, subsistence use, and to a lesser extent, for sport fishing (Chitará-Nhandimo et al., 2022; Gullström et al., 2002; Herinirina et al., 2023; Nordlund et al., 2018; Unsworth et al., 2018).

Given the many benefits seagrasses offer, monitoring biodiversity is crucial for understanding which species exist in an ecosystem, as well as tracking changes in species occurrence, abundance and distribution over time. A relatively new monitoring method since the 2000's to assess biodiversity associated with terrestrial, aquatic and marine ecosystems is environmental DNA (eDNA) (Seymour et al., 2019; Wang, 2020). eDNA comes from the DNA expelled by organisms due to shedding of tissue, skin, hair, excrement and decay, and is extracted from the environment. (Thomsen and Willerslev, 2015). Using eDNA approaches requires that DNA is extracted, amplified, and quantified from the samples which are then analysed. eDNA provides a non-intrusive, quick, simple sampling methodology that can be easily repeated, and is relatively inexpensive (Seymour et al., 2019). Depending on the eDNA approach used, either a community-wide method known as eDNA metabarcoding or a species-specific targeted approach, the methodology can provide reliable results for species richness, evenness, and community composition (Petit-Marty et al., 2023; Seymour, 2019).

The use of eDNA to monitor marine biodiversity has been growing since its application in 2012 (Thomsen and Willerslev, 2015). Studies show that eDNA metabarcoding approaches can accurately identify species richness and composition at higher taxonomic levels across various marine ecosystems when compared to other sampling techniques. Additionally, it can detect cryptic, rare, and invasive species, making eDNA a reliable tool for marine ecosystems and fisheries resource management (He et al., 2022; Holman et al., 2019; Petit-Marty et al., 2023; Thomsen and Willerslev, 2015; Waters et al., 2023).

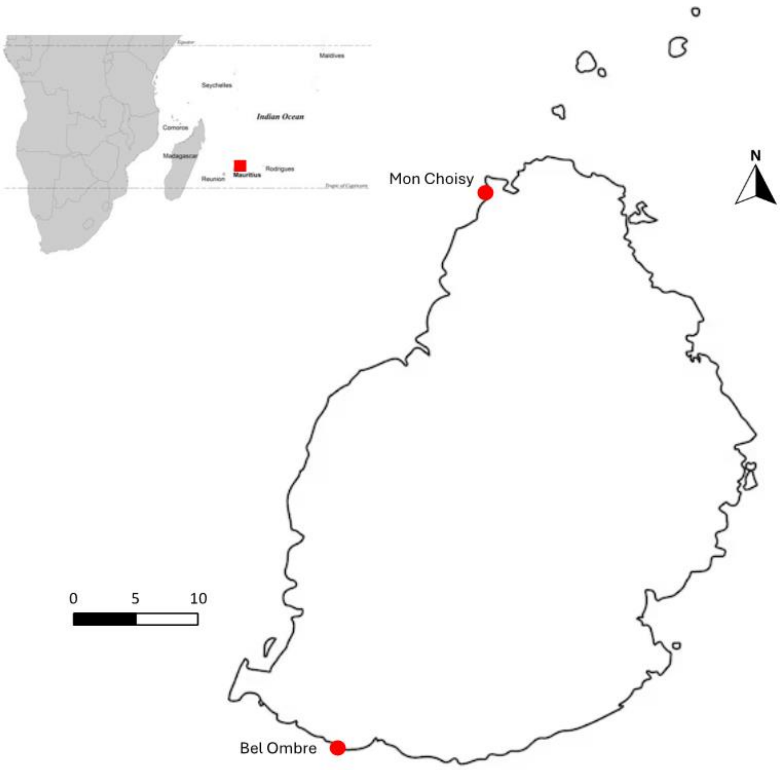
In Mauritius, seagrass ecosystems are understudied with the consequence of inadequate knowledge about the extent, health, biodiversity, and ecosystem services they provide. In this study the eDNA metabarcoding approach will be used to describe the species richness and community composition for fish and invertebrate biodiversity for two seagrass meadows in Mauritius. More specifically, fish and invertebrate community composition and their biodiversity traits will be assessed in the context of seagrass ecosystem functioning and the resulting ecosystem services provided by the seagrass habitats studied. This research will help to close the seagrass biodiversity knowledge gap for Mauritius and provide results that can be used to further research and improve future seagrass management and conservation actions.

Chapter 2 Materials and Methods

2.1 Study Area

Two locations were selected for eDNA sampling along the coast of Mauritius, GPS coordinates for Bel Ombre lagoon S 20.502688, E 57.396894 in the south and GPS coordinates for Mon Choisy lagoon S 20.011074, E 57.554140 in the north of the island (Figure 1), where the seagrass species *Syringodium isoetifolium* dominates both meadows.

Figure 1: *Map of the two study locations in Mauritius, Bel Ombre lagoon South, and Mon Choisy lagoon North*



2.1.1 The Bel Ombre Lagoon

The Bel Ombre lagoon is located in the South of Mauritius. The seagrass meadow is not continuous in the subtidal area along the shore but instead forms two large meadows of approximately 450 m and 250 m in length that begin at <0.5 m and extend outwards into the lagoon to depths of <2 m. Three species of seagrasses can be found in the lagoon dominated by *Syringodium isoetifolium* which extends towards the reef area*,* where *Halophila stipulacea* dominates the inner shore region, and Halodule spp. resides in mid region of the lagoon (Reef Conservation, 2022). Furthermore, a mangrove estuary ecosystem at the mouth of the river St. Martin flows into the lagoon. Within the lagoon, areas of patch reef are in close proximity to the seagrass meadows, and two deeper channels in the reef barrier of approximately 10 m in depth creates passages between the open ocean and the lagoon.

2.1.2 The Mon Choisy Lagoon

The Mon Choisy lagoon is situated in the Northwest of the island and is adjacent to a popular local and touristic sandy beach where swimming, water sports and fishing are daily occurrences. Two species of seagrasses were reported for Mon Choisy lagoon dominated by the species *Syringodium isoetifolium* (Daby, 2003)*.* The seagrass meadow is extensive and continuous and covers an area of approximately 1.2 km in length and lies to the eastern part of the lagoon. The meadow starts at <0.5 m extending towards the reef crest to depths of <1.5 m to 2 m deep. Smaller sparser seagrass patches are found near the center and towards the eastern part of the bay. (Reef Conservation, 2022).

2.2 eDNA Sampling Design

In Bel Ombre, five stations were chosen for collecting sea water for eDNA in the seagrass meadows, and adjacent habitats. These were named and described as (1) BO Add a mixed habitat of algae and sparce seagrass, (2) BO Res a potential restoration area with bare sand (3) BO Cha a deeper natural channel in the reef, (4) BO Don a dense seagrass meadow and 5) BO Con a lagoon patch reef.

In the Mon Choisy lagoon however, three stations were chosen for sampling eDNA in the seagrass meadow and adjacent habitats. The stations are as follows: (1) MC Add a lagoon patch reef (2) MC Con an area of the lagoon with smaller seagrass patches closer to coral reef patches and (3) MC) Don a large intact seagrass meadow.

2.3 eDNA Water Sample Collection

The Reef Conservation team collected aquatic water samples in April 2023 (Summer) and August 2023 (Winter) from Bel Ombre and Mon Choisy lagoons to evaluate fish biodiversity and to capture potential seasonal variations in fish biodiversity assemblages. Additionally, the same water samples collected during the summer season were used to assess invertebrate biodiversity richness and assemblages at both sites.

Sampling was conducted by the team, at both locations using a boat to access the stations. The boat was washed and decontaminated before each sampling day. All materials were sterilised in preparation of sampling and gloves and protective gear were used during the handling of all materials as well as during the onsite water filtering process. At each station in Bel Ombre and Mon Choisy, water sampling was carried out in triplicate, where 3000 ml of surface water was collected from the boat for each of the three replicas. A sealed Maxi Aquatic eDNA sample kit supplied by Nature Metrics Ltd was used for each replica on site. The water was pumped, by using a peristaltic pump, through an enclosed 0.8 µm PES filter, that reduced the risk of DNA contamination. Preservation solution was then added to the filters to preserve the DNA. The enclosed filters and sample sheets with relevant data were placed in sterile bags and sealed and stored on ice packs in an ice cooler on site to preserve DNA integrity. A negative control sample was in addition filtered with bottled water for each location. At Reef Conservation's lab the filters were frozen at -20°C on the same day of the sampling, and stored before the shipment of a total of 48 filters to NatureMetrics Ltd in March of 2024 for DNA extraction, metabarcoding, bioinformatics and taxonomic assignment. In total 30 eDNA water sample kits were filtered for Bel Ombre lagoon from five stations for the summer and winter seasons respectively and 18 eDNA water sample kits were filtered for Mon Choisy lagoon from three stations for the same time periods.

2.4 DNA Extraction

DNA was extracted from each sample by the NatureMetrics laboratory using a commercial DNA extraction kit with modifications made to the standard protocol to ensure maximum DNA yield. An extraction blank was included with each batch of samples for quality control to monitor potential contamination and the samples were then purified using a commercial purification kit to eliminate PCR inhibitors (NatureMetrics, n.d.).

2.4.1 Metabarcoding

Following extraction, the purified DNA for each sample was increased through PCR amplification using the Fish (12S) primer that primarily targets the region of the genetic material for Actinopterygii, and provides a broad coverage for the detection of marine fish, however, the laboratory noted that this primer does not detect elasmobranchs. The marine water eukaryotes (18S) primer was also used to detect invertebrate species. To increase the detection of target and rare species, the laboratory carried out the PCR amplification in 12 replicates per sample. A PCR blank was included with all reactions to control for contamination. The success of the amplified DNA was tested using gel electrophoresis. PCR replicates for each sample were pooled, purified and sequencing adapters were added to distinguish and identify the different DNA sequences in each sample. Library preparation used dual-indexed tags, and quality control was carried out before the libraries were purified, quantified and normalised. The final library was sequenced with a depth of 100,000 sequences per sample using the Illumina MiSeq platform (NatureMetrics, n.d.).

2.4.3 Taxonomic Assignment

Nature Metrics used a custom bioinformatics processing pipeline on the sequenced data, where steps included quality filtering, operational taxonomic unit (OTU) clustering and the use of quality control thresholds to reduce the incidence of false positives. Nature Metrics Laboratory made taxonomic assignments based on sequence searches against relevant reference databases including NCBI Nucleotide (Gene Bank, www.ncbi.nlm.nih.gov/genbank), the Barcode of Life Database (BOLD, https://v3.boldsystems.org/), SILVA (SILVA, https://www.arb-silva.de/) and the NatureMetrics Database of Life. To ensure reliability in taxonomy assignment across the databases the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) was also used by the laboratory. The results from all databases were compared and integrated, and taxa were assigned to the lowest taxonomic level with consistent matches. DNA sequences that were unidentified or identified as contaminants such as human DNA were excluded from the final dataset before being received (NatureMetrics, n.d.)

2.4.4 Taxonomic Results

The taxonomic dataset was received by Reef Conservation from NatureMetrics Laboratory as an excel file. The eDNA results include the total number of samples submitted, the count of samples that met quality control standards and were reported, the overall number of unique Operational Taxonomic Units (OTUs) identified across all the samples at their lowest assigned taxonomic level.

As described by NatureMetrics (n.d), an OTU refers to a DNA sequence identified in a sample that is comparable to a species. However, the term “OTU” is used instead of “species” because eDNA results can sometimes not clearly identify a species. An OTU can have multiple DNA sequences that are very similar due to minor variations in the target DNA among individuals (genetic diversity) within the same species, or slight changes, can occur during sequencing. When this is detected in the process, similar DNA sequences were grouped together and reported as a single OTU and identified as a single species. In addition, if the analysis identified the same OTU to more than one species, or the OTU did not match accurately with a reference DNA sequence, but matched with multiple reference sequences from different species, the results for the OTU were reported at the lowest common taxonomic level.

2.5 Taxonomic Data Preparation and Manipulation

2.5.1 Reference Databases

The taxonomic datasets received from NatureMetrics for fish and invertebrate species biodiversity, was compared with the Mauritius Oceanography Institute (MOI) marine species taxonomic database for the Republic of Mauritius (MOI, https://moi.govmu.org/marinedb/) which is available at genus and species level. The fish and invertebrate biodiversity lists were further cross checked against the open-source databases (FishBase https://fishbase.se/search.php) and the World Register of Marine Species (WORMS, https://www.marinespecies.org/) and distribution occurrence records were further checked to verify that the species geographical range included the Western Indian Ocean region and Mauritius.

2.5.2 Fish Community Characterisation

The compiled fish biodiversity list was compared with the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species to determine the threat status assignment for each species from the nine categories assessed for species globally: Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW), Extinct (EX) and Not Evaluated (NE) (IUCN, https://www.iucnredlist.org/).

The possible occurrence of invasive species was also checked using the open-source Global Register of Introduced and Invasive Species (GRIIS) record database for Mauritius (GRIIS https://www.gbif.org/dataset/69233277-0946-4931-8115-3b247a81a051).

In addition, the FishBase database was also used to determine both the global and local assignment of economic importance of the detected fish species as no official list of commercial coastal fish species was accessible for this study. The categories adopted and used from the global assessment include: commercial fisheries (CF), minor, artisanal or bait fisheries (MABF), game fisheries (GF), and aquarium trade private and public (AT). Classified species were then compared to the list of commercial fish species for Mauritius to evaluate the potential local economic value of each study location.

The data was organised for the whole dataset to report on the overall output of the eDNA results for both locations. Output information included overall species richness (total OTU counts) and the number of OTUs (sensu species) assigned to each taxonomic level. The data was then organised for each sample station to report on the biodiversity found at each station where information on taxonomic levels to family, genus and species, common name, presence or absence in the MOI and FishBase databases, the IUCN threat status category for each species, invasive species identification and economic value of the species were recorded.

2.5.3 Biological Traits Selection for Seagrass Ecosystem Functioning

Fish and invertebrate species were further screened and characterised based on where they lived and how they moved in the ecosystem. If their life strategies have a consistent impact on the sediments (such as bioturbating species) the species was labelled as a “bioturbator” whereby they either support sediment stabilisation, disturb the sediments, create habitat for other organisms or cycle nutrients. These impacts can have either positive or negative feedback loops on the functioning of a seagrass ecosystem (Gutiérrez, et al., 2011). In addition, trophic levels which describe consumer positions of the organisms were characterised and used as a key trait based on the impact that species and community assemblages can have on the functioning of seagrass ecosystems (Duffy, 2006). As an example, the presence or absence of key grazing species together with the community composition can have either positive or negative impacts on the functioning and condition of seagrass ecosystems.

The biological traits for each species were categorised using the following categories below:

- Habitat preference of associated fauna within the seagrass meadow were characterised as:
    - infauna (in) invertebrate species that burrow and live in the substrate.
    - epifauna (epi) invertebrate species that live on the surface substrate.
    - sessile (se) species that are attached to a substate and do not move.
    - planktonic (plk) species that live suspended in the water column with limited mobility.
    - benthic (ben) fish species that lives on or in holes in the substrate or bury themselves under the substrate to hide or ambush prey.
    - benthopelagic (bep) species that lives in the mid waters of the habitat but feed on benthic organisms.
    - epipelagic (epp) species that live and feed in the upper or near surface waters of the habitat.
    - mesopelagic (mp) species that live at 200m to 1000m in the water column below the epipelagic zone.
    - bathypelagic (ba) species that live below the mesopelagic zone.

- Feeding habits and trophic level of associated fauna:
    - carnivore (c) species that are secondary or tertiary consumers.
    - omnivore (o) species that feed on both plant and animal materials, secondary consumers.
    - herbivore (h) species that only feed on plants and algae, primary consumers.
    - detritivore (d) species that feed on detritus.
    - sediment sifters (ss) species that disturb the sediments by gulping or moving as part of their feeding strategy.
    - planktivore (plv) species that feed on zooplankton secondary consumers and phytoplankton primary consumers.
    - filter feeders (f)

- suspension feeders (s) that feed on plankton and small organic matter from the water can be primary or secondary consumers depending on if they feed on phytoplankton or zooplankton.

2.6 Analysis

2.6.1 Species Richness

The basic indicator of species richness (SR) was collected, and defined as the number of species in an identified area (Brown et al., 2016), and all unique OTUs were assigned 0 or 1 to represent presence or absence. Total SR was measured to compare diversity between Bel Ombre and Mon Choisy, and within each station.

SR for the fish dataset was calculated as follows; (1) overall SR represented by total OTUs. (2) SR at family, genus and species taxonomic levels pooled locations, and for Mon Choisy and Bel Ombre independently, (3) pooled and within location SR for seasons summer and winter, and (4) overall SR for each sampling station within a location disaggregated into seasons, summer and winter.

For the invertebrate dataset, data included both, target and non-target OUT counts. Although the genetic primer to target invertebrates should only record invertebrates, additional taxonomic species such as a few fish species and primary producers was included. For that reason, I here split the dataset, where the full dataset includes all species recorded (target and non-target), while the target dataset has been cleaned and only includes invertebrates. Accordingly, SR for the full dataset was calculated to give (1) overall (pooled locations) SR of target and non-target OTU Counts.

Separately, SR for the target dataset was calculated to give (1) SR of the target invertebrate taxa only. (2) SR at all taxonomic levels for the whole sample of target invertebrate taxa. (3) SR for each location Mon Choisy and Bel Ombre at all taxonomic levels, and (4) SR for each sampling station within a location.

2.6.2 Statistical tests

In order to test the variation between and within locations, the R statistical package (R Core Team, 2024) was used to perform univariant ANOVA analysis (alpha < 0.05), to examine the relationship between locations, (north and south), stations (within locations) and seasons (summer and winter), on the dependent variable, species richness. Where necessary, a Tukey's HSD post hoc test was performed to obtain pairwise comparisons when factors had more than two levels. The assumptions about constancy of variance and normality of errors were checked and met (Crawley, 2007).

2.6.3 Relative Abundance

To visualise the community composition for fish and invertebrate species at each station, the relative abundance was calculated for each species from the OTU count reads as a proportion of the total OTU reads. The mean for each species for the three replicas were calculated. Results for species within a family were then totalled to obtain the relative abundance at family level for each station. Community composition for each station was then illustrated as bar graphs.

2.6.4 Biological Traits Assessment

All fish taxa were assessed for biological relevant traits which were assigned where applicable with a 1 given to demonstrate that the species possessed or exhibited the trait, and 0 where the trait was not present. Each fish OTU was analysed at the taxonomic level of family if a lower taxonomic level was not assigned.

This procedure was also applied to the invertebrate dataset. Similarly, invertebrate OTUs were assigned to the lowest taxonomic level, preferable species level. However, when this was not possible, it was assigned to the level of family. Note however, the invertebrate data set only assigned 86.89% of the OTUs to family level.

Chapter 3 Results

3.1 eDNA Species Detection

Readings were acquired for 43 out of the 48 water samples analysed for fish and from 20 out of the 21 water samples analysed for invertebrates from the two locations Bel Ombre and Mon Choisy. No readings were accordingly obtained for five samples where although three out of four passed all quality control tests, no species were detected (BO Add sample 2 winter, MC Add sample 3 summer and MC Con sample 3 winter). One sample did not pass the quality control tests (BO Add sample 1 summer) and no amplifiable DNA was detected on the fifth sample (BO Add sample 3 winter).

3.2 New Records of Fish Species for Mauritius

The efficiency of eDNA and the strength of the technique was evident since as many as two families, 14 genera and 26 new species were detected by the eDNA methodology that are not only not recorded in the Mauritius MOI database, but have never been recorded in the global database FishBase checklist of marine species for Mauritius (Table 1).

Table 1

New Species Detected in Mauritius at Lowest Taxonomic Level from eDNA Metabarcoding Analysis

Family	Genus	Species	Common Name	Possible Occurrence
		Gymnothorax fuscomaculatus	Brown-Spotted Moray	Yes
		Uropterygius alboguttatus	Freckleface Reef-Eel	Yes
		Synodus dermatogenys	Sand Lizardfish	Yes
		Amblygaster leiogaster	Smoothbelly Sardinella	Yes
	Benthosema		Lanternfish	Yes
	Bolinichthys	*Bolinichthys indicus*	Lanternfish	Yes
	Ceratoscopelus	*Ceratoscopelus warmingii*	Warming's Lanternfish	Yes
	Diaphus	*Diaphus brachycephalus*	Short-Headed Lanternfish	Yes
		Diaphus effulgens	Headlight Fish	Yes
	Lampadena		Lanternfish	Yes
	Lampanyctus		Lanternfish	Yes
	Triphoturus	*Triphoturus nigrescens*	Highseas Lampfish	Yes
		Naso annulatus	Whitemargin Unicornfish	Yes
	Foa		Cardinalfish	Yes
		Ostorhinchus fleurieu	Flower Cardinalfish	Yes
		Aspidontus dussumieri	Lance Blenny	Yes
	Amblycirrhitus		Hawkfishes	Yes 1 species
		Amblygobius phalaena	Sleeper Banded Goby	No
	Asterropteryx	*Asterropteryx semipunctata*	Starry Goby	Yes
		Cabillus lacertops	Lizard Cabillus	Yes
		Eviota guttata	Spotted Dwarfgoby	Low probability
		Coris gaimard	Yellowtail Wrasse	No
	Cymolutes	*Cymolutes praetextatus*	Knife Razorfish	Yes
		Thalassoma lutescens	Yellow-Brown Wrasse	Yes
		Pomacentrus trilineatus	Threeline Damsel	Low probability
		Chlorurus microrhinos	Blunt-Head Parrotfish	No
		Scarus prasiognathos	Singapore Parrotfish	No
Scombrolabracidae	*Scombrolabrax*	*Scombrolabrax heterolepis*	Longfin Escolar	Yes
Xenisthmidae	*Xenisthmus*		Collared Wrigglers	Yes 1 species
		Sebastapistes tinkhami	Darkspotted Scorpionfish	Yes
		Cyclothone pseudopallida	Slender Bristlemouth	Yes
	Vinciguerria	*Vinciguerria nimbaria*	Oceanic Lightfish	Yes

The family Scombrolabracidae which contains only one species, *Scombrolabrax heterolepis,* was detected down to species level and demonstrates an occurrence record for the Western Indian Ocean (WIO) region. It is a bathypelagic deep-sea species that migrates vertically to feed at night (FishBase, 2024) and was not expected to be detected in shallower waters between 5–10 m. Additionally, seven new genera and five new species of lanternfish belonging to the family Myctophidae, has also been detected, but only in the Bel Ombre lagoon. The specific station from where these species are reported came from water samples in the reef channel, BO Cha, that is linked to the open ocean.

Moreover, the family Xenisthmidae and genus *Xenisthmus,* which was detected here for the first time in Mauritius, includes only one species of a burrowing goby that could occur for Mauritius, which is *Xenisthmus africanus*. Due to its very small size, which is less than 5 cm in length, and its cryptic burrowing nature, it is not surprising this species has remained undetected until now.

However, three species that were recorded are possibly identified by different species names for the region. These are *Coris gaimard* (Yellowtail Wrasse) which could be *Coris cuvieri*, *Chlorurus microrhinos* (Blunt-Head Parrotfish) with a known species complex in the region *Chlorurus strongylocephalus* and *Scarus prasiognathos* (Singapore Parrotfish) which is possibly replaced by *Scarus falcipinnis*. Further surveys and investigation are needed to determine if these are new species detected in Mauritius, or the same species represented by different names.

Regarding the new taxa discovered for Mauritius at species level, one out of the 26 detected species, remarkably does not occur in the distribution range records for the WIO region, nor the Mascarene Islands including Mauritius. Still, the species *Amblygobius phalaena* (Sleeper Banded Goby or Whitebarred Goby), was detected in the Bel Ombre Location at three stations BO Add, BO Res and BO Don. The expected distribution range for this species is the Pacific Ocean, southern Australia and Micronesia.

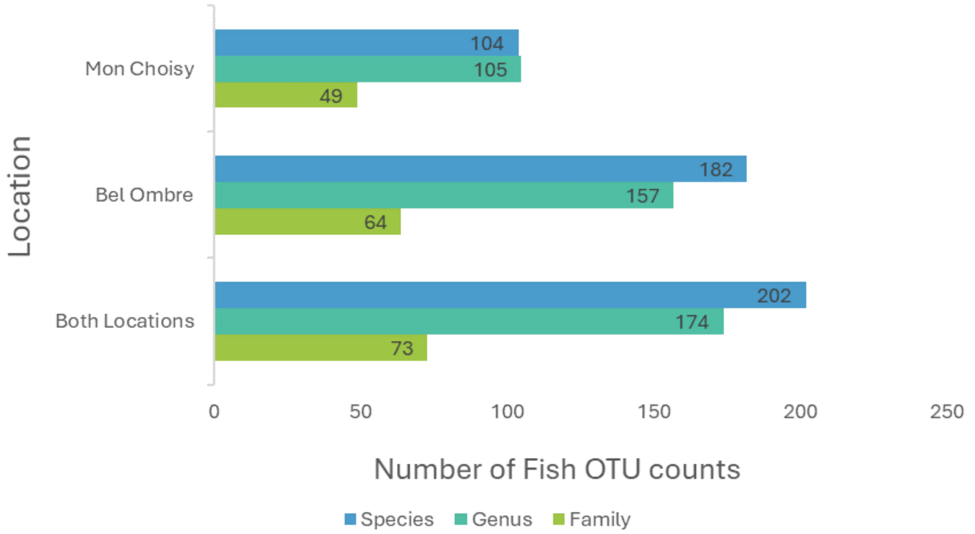
3.3 Assessment of Fish and Invertebrate Diversity

3.3.1 Fish Species Richness

Out of the 43 samples analysed for marine fish, 367 Operational Taxonomic Units (OTUs) were detected where each OTU is equivalent to a fish taxa. The OTUs were classified to the lowest taxonomic level possible where 100% were classified at family level, 88% at genus level and 55% at species level.

Figure 2

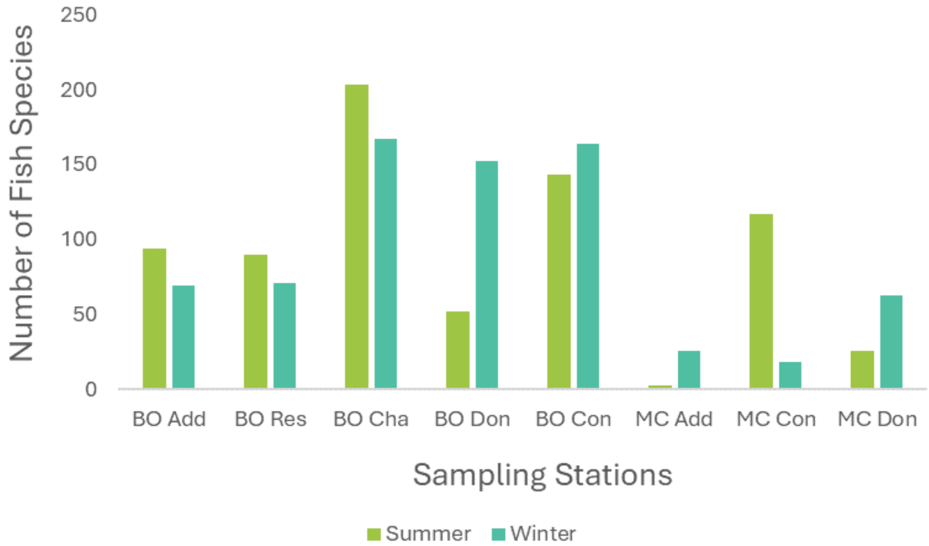
Species Richness of Fish OTU Taxa at Family, Genus and Species Level for, Both Locations, Bel Ombre and Mon Choisy



The overall species richness for both locations recorded 73 families, 174 genera and 202 species of fish. Between the locations, the southern Bel Ombre lagoon demonstrate a higher species richness with an additional 15 families, 52 genera and 78 species detected, compared to the northern Mon Choisy lagoon. Notably, Bel Ombre had similar species richness values to the combined results for both locations compared to Mon Choisy across all taxonomic levels (Figure 2).

Figure 3

Number of Fish Species Detected for each Sampling Station During Summer and Winter Periods at Bel Ombre and Mon Choisy Locations

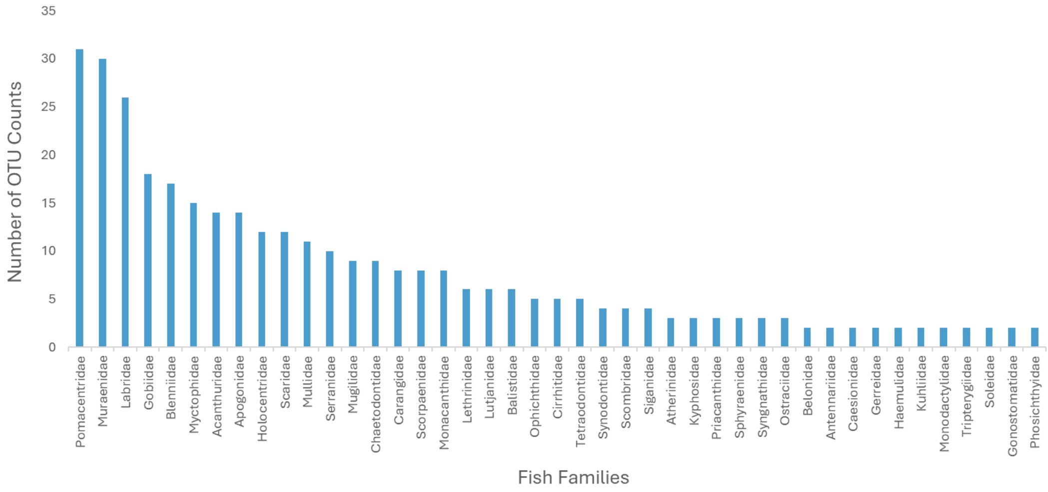


Seasonal differences in fish species presence were evident at the two locations, with variations in the number of species detected between the locations and within lagoons themselves (Figure 3). Three out of the five stations in Bel Ombre and one station in Mon Choisy had higher species richness in the summer compared to the winter, with higher species richness standing out particularly in the reef channel station BO Cha in Bel Ombre for both seasons.

Specifically, from the samples collected in the channel station, 203 fish species were detected in the summer and 167 fish species were recorded in the winter. Station BO Con which is a lagoon patch reef had the second highest detection counts of 143 species in the summer and 164 in the winter. With the exception of station MC Add in Mon Choisy, the seagrass meadow stations BO Don and MC Don at both locations, had the lowest species counts for the summer with 52 and 26 species detected respectively.

Figure 4

Taxonomic Diversity at Family Level for OTU Counts greater than one



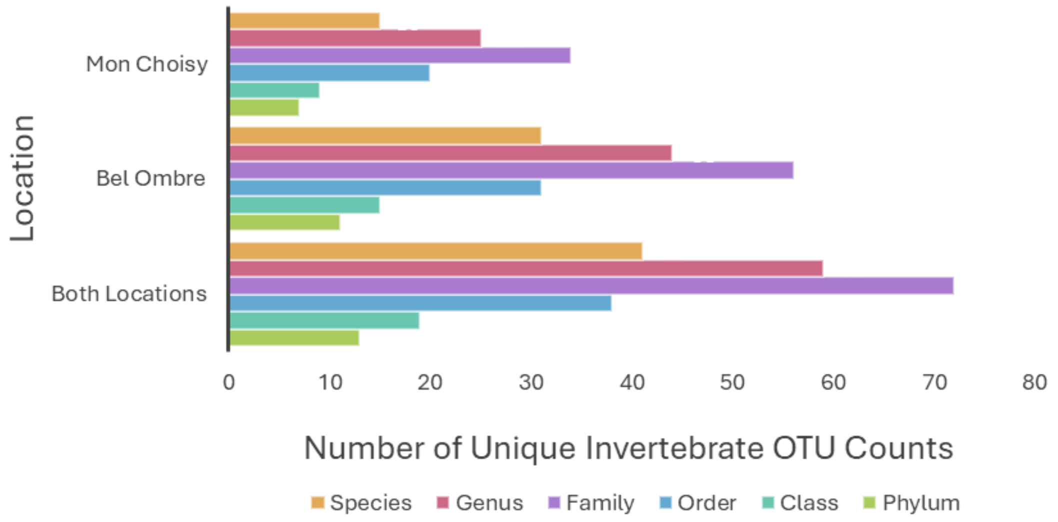
Moreover, of the 73 fish families recorded in the dataset, 43 families had more than one OTU count, where an OTU count represents a single taxon at either family, genus or species level. 12 of these families had high OTU counts of at least ten unique taxonomic assignments, these were; Pomacentridae (Damselfish) (31), Muraenidae (Moray Eels) (30), Labridae (Wrasses) (26), Gobiidae (Gobies) (18), Blenniidae (Blennies) (17), Myctophidae (Lanternfish) (15), Acanthuridae (Surgeonfish, Tang and Unicornfish) (14), Apogonidae (Cardinalfish) (14), Holocentridae (Squirrelfish) (12), Scaridae (Parrotfish) (12), Mullidae (Goatfish) (11) and Serranidae (Groupers) (10) (Figure 4). For 30 out of the 73 families (40%), only one unique OTU was detected. Two of these families, Scombrolabracidae and Xenisthmidae, are new records for Mauritius.

3.3.2 Invertebrate Species Richness

Out of the 20 samples analysed for marine invertebrates, 270 Operational Taxonomic Units (OTUs) were detected, of which 122 were target taxa invertebrates, while 148 were non-target taxa including vertebrates, macro and micro algae and fungi. The invertebrate OTUs (targeted taxa) were classified, with 100% assigned to phylum, 97.54% to class, 93.44% to order, 86.89% to family, 56.56% to genus, and 33.61% to species level unlike the fish dataset where all taxa were identified to at least family level.

Figure 5

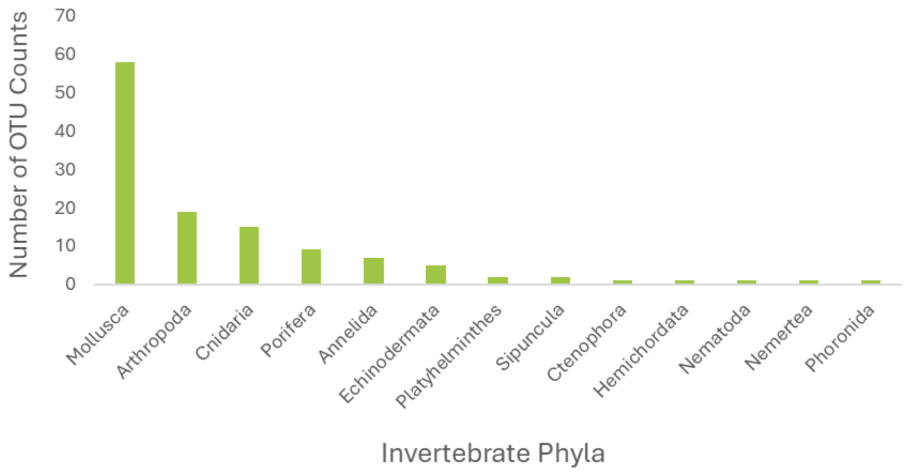
Species Richness of Target Invertebrate Taxa Identified at all Taxonomic Levels



The total OTU counts of target invertebrates, pooled for both survey locations, and at each location for all taxonomic levels from phyla to species, is shown in Figure 5. The overall species richness recorded 72 families, 59 genera and 41 invertebrates named at species level.

Figure 6

Taxonomic Diversity at Phylum Level for Target Invertebrates



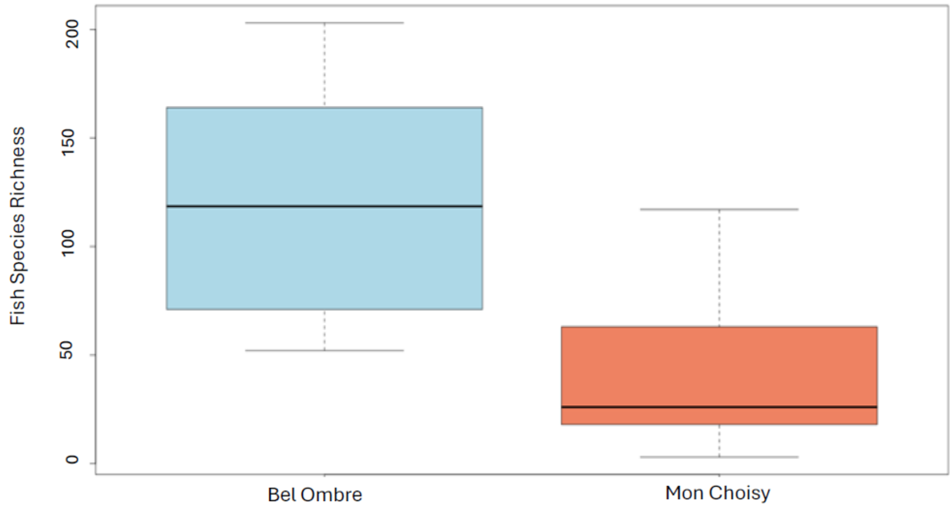
For the target invertebrates of the 13 phyla detected (Figure 6) Mollusca (58 OTUs) was the most abundant phylum with 95% of the OTUs assigned to the class Gastropoda and 5% to Bivalvia. Other abundant phyla included Arthropoda (19 OTUs) with 58% made up of Hexanauplia (Copepods) and only one swimming crab *Thalamita admete* from the class Malacostraca. This was followed by Cnidaria (15 OTUs), where all OTUs were assigned to order, which included 40% Scleractinia (Hard corals), 20% Actiniaria (Sea anemones), 20% Alcyonacea (Soft corals), and 20% Anthoathecata and Leptothecata (Hydrozoans). For the phylum Porifera (9 OTUs) 100% of these OTUs were assigned at class and order level, and 89% at family level. While the phylum Annelida (7 OTUs) only included polychaetes with 100% assignment to family level. Finally, the phylum Echinodermata (5 OTUs) included the classes Echinoidea (3 OTUs), Holothuroidea (1 OTU) and Ophiuroidea (1 OTU). Phyla with only one or two OTU assignments included, Hemichordata (1 OUT), Nematoda (1 OTU), Nemertea (1 OTU), Phoronida (1 OTU), Platyhelminthes (2 OTUs) and Sipuncula (2 OTU) with all detected to family level.

3.3.3 Species Richness, Taxa and Effect of Location

To determine if locations, seasons and sample stations had an effect on the species richness recorded in the dataset the following tests were conducted.

Figure 7

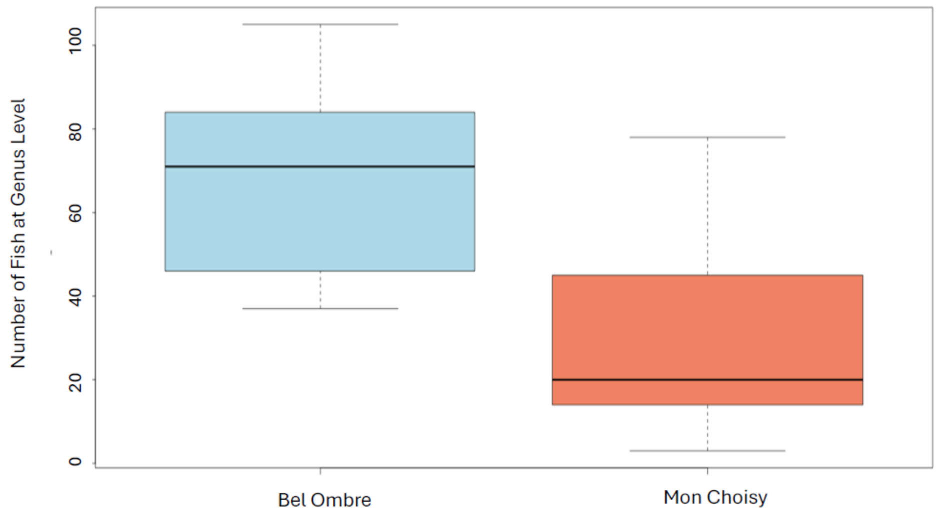
Differences in Fish Species Richness between the two Locations Bel Ombre and Mon Choisy



When the variation in fish species richness between the two locations Bel Ombre in the south and Mon Choisy in the north was tested, the one-way ANOVA showed that location had an effect on species richness and was significantly different ($F_{1, 13}$ = 7.602, p = 0.016) (Figure 7).

Figure 8

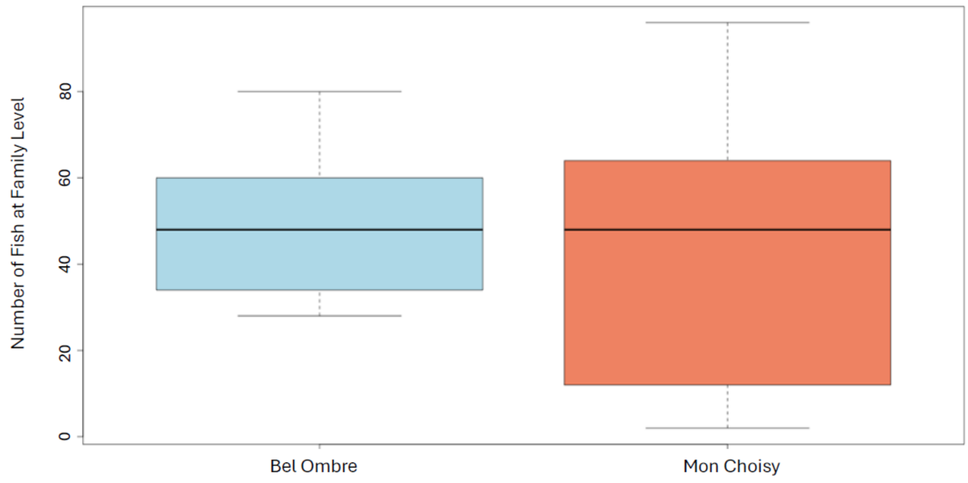
Differences in Number of Fish at Genus Level between Locations Bel Ombre and Mon Choisy



At genus level, the variation between number of fish detected and location was also significant ($F_{1, 13}$ = 7.105, p = 0.019) (Figure 8)

Figure 9

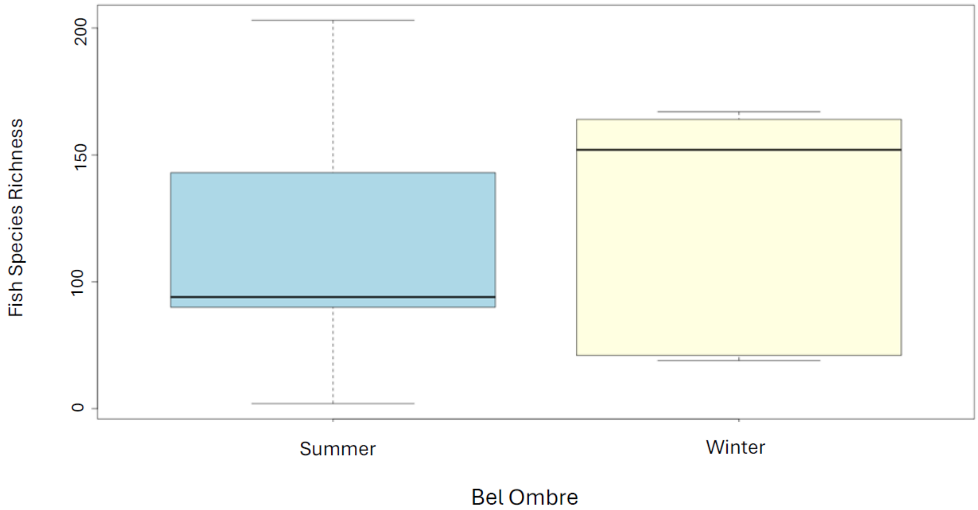
Differences in Number of Fish at Family Level between Locations Bel Ombre and Mon Choisy



However, there was no significance when the number of fish at the family level was tested against location ($F_{1, 13} = 0.170$, $p = 0.687$) (Figure 9).

Figure 10

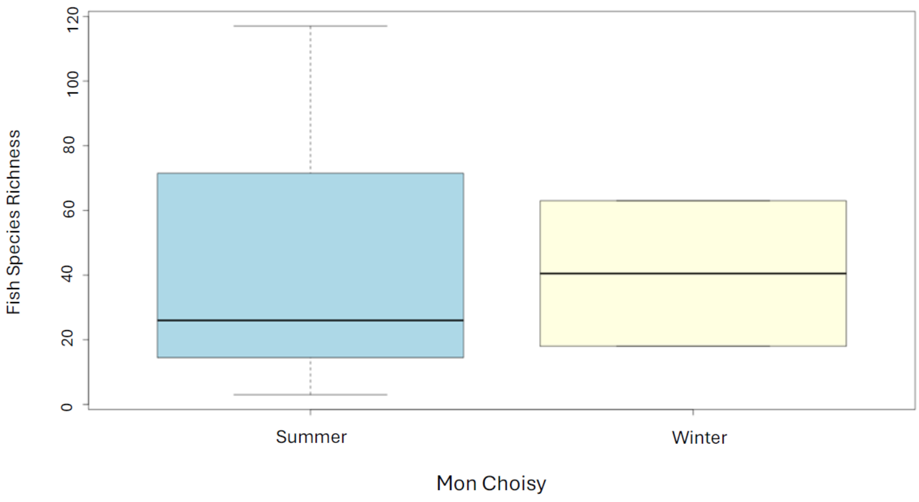
Difference in Seasons Summer and Winter, on Fish Species Richness for Bel Ombre Location



In addition, the test within the location Bel Ombre for the summer and the winter season, examined for species richness, showed no effect ($F_{1, 8} = 0.057$, $p = 0.817$)

Figure 11

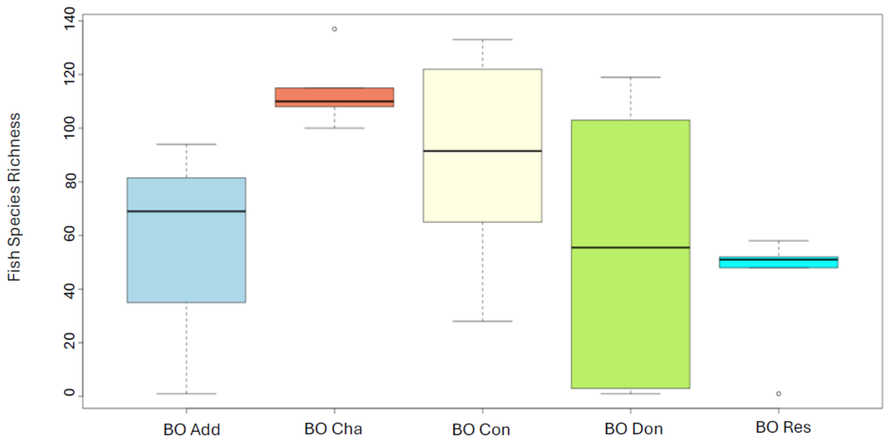
Difference in Seasons Summer and Winter for Fish Species Richness for Mon Choisy Location



Again, when the test within the location Mon Choisy for the summer and the winter season was examined for fish species richness, it also showed no significant effect ($F_{1,\,3} = 0.029$, $p = 0.876$).

Figure 12

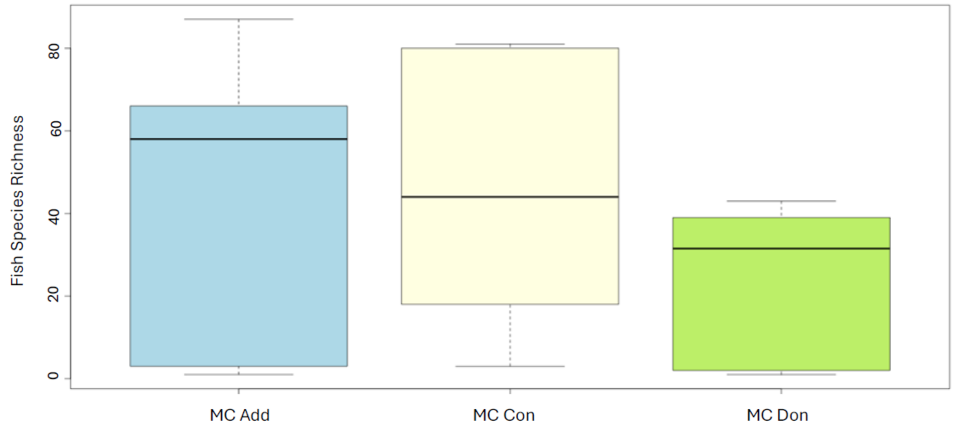
Differences in Fish Species Richness between the Stations in the Bel Ombre Location



Furthermore, a test for the variation of fish species richness within the Bel Ombre location among the different sampling stations, revealed that there was a significant difference between the stations ($F_{4, 22}$ = 3.889, p = 0.016). The following post hoc test (Tukey test) with pairwise test among the stations found significant differences between BO Res vs BO Cha (p = 0.017) as well as between BO Don vs BO Cha (p = 0.067) within the lagoon of Bel Ombre (Figure 12)

Figure 13

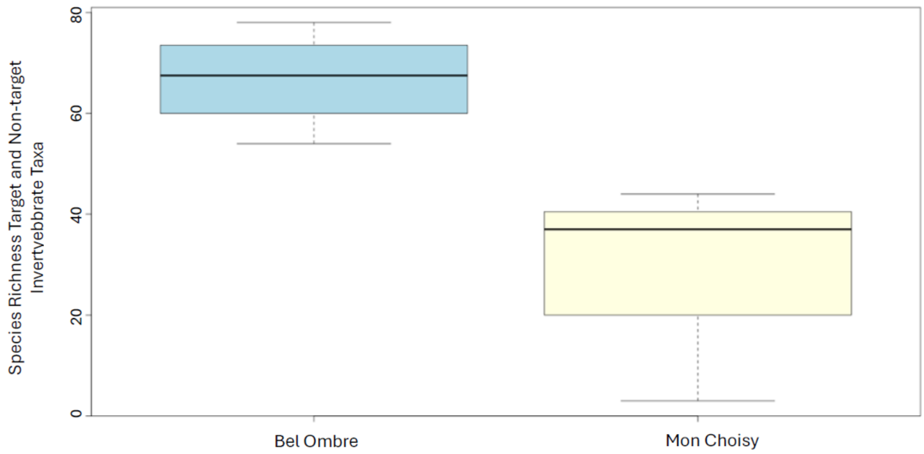
Differences in Fish Species Richness between the Stations within Mon Choisy Location



For the test among stations regarding fish species richness in the lagoon of Mon Choisy (Figure 13), revealed no significant difference ($F_{2, 13}$ = 0.723, p = 0.504).

Figure 14

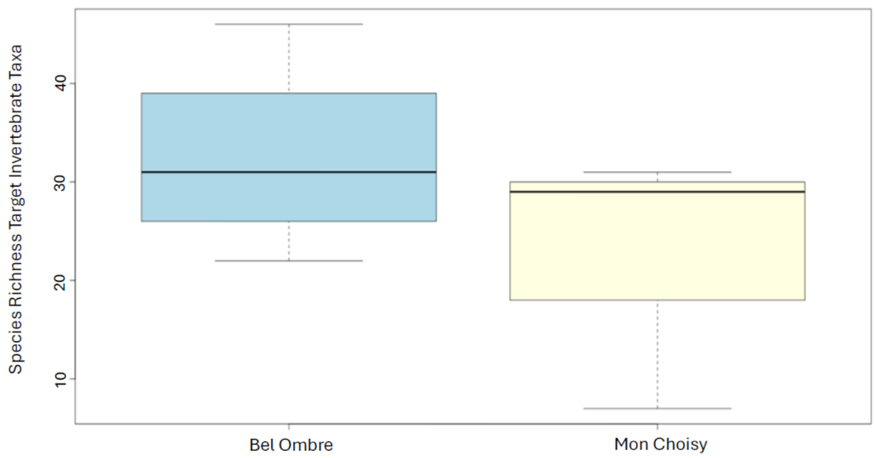
Difference in Species Richness for Target and Non-target Invertebrates between Bel Ombre and Mon Choisy Locations



For the test examining if location had an effect on the combined species richness for target and non-target invertebrates, it revealed that location was significantly different ($F_{1, 5}$ = 10.240, p = 0.024) (Figure 14).

Figure 15

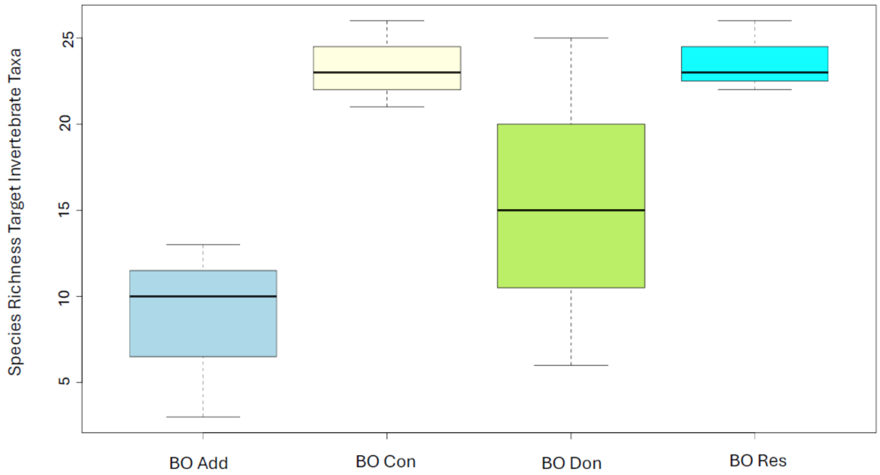
Difference in Species Richness for Target Invertebrates between Bel Ombre and Mon Choisy Locations



However, when the same test was done to determine the effect of location on the target dataset which only included invertebrates, no significant differences could be detected ($F_{1, 5}$ = 1.355, p = 0.297) (Figure 15).

Figure 16

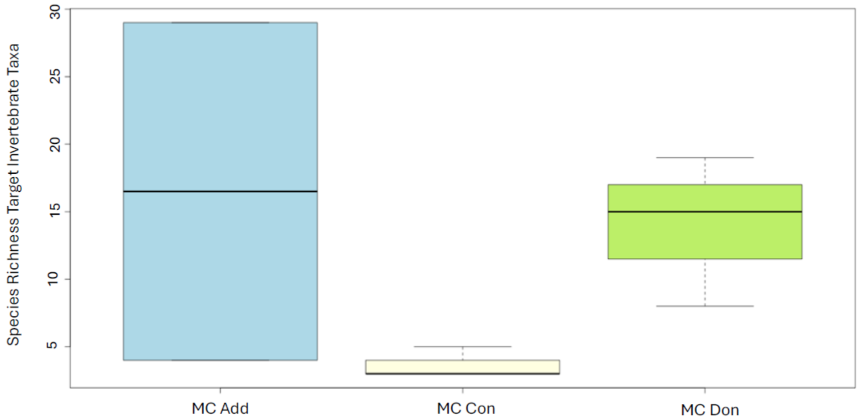
Differences in Target Invertebrate Species Richness between the Stations in the Bel Ombre Location



While analysing potential differences among the sample stations in Bel Ombre for invertebrate species richness using the target dataset, it revealed significant differences among stations and species richness ($F_{3, 8}$ = 4.854, p = 0.033). The Tukey pairwise comparison identified variances that were significantly different between BO Con vs BO Add (p = 0.051) as well as between BO Res vs BO Add (p = 0.046) at Bel Ombre (Figure 16).

Figure 17

Differences in Target Invertebrate Species Richness between the Stations within Mon Choisy Location



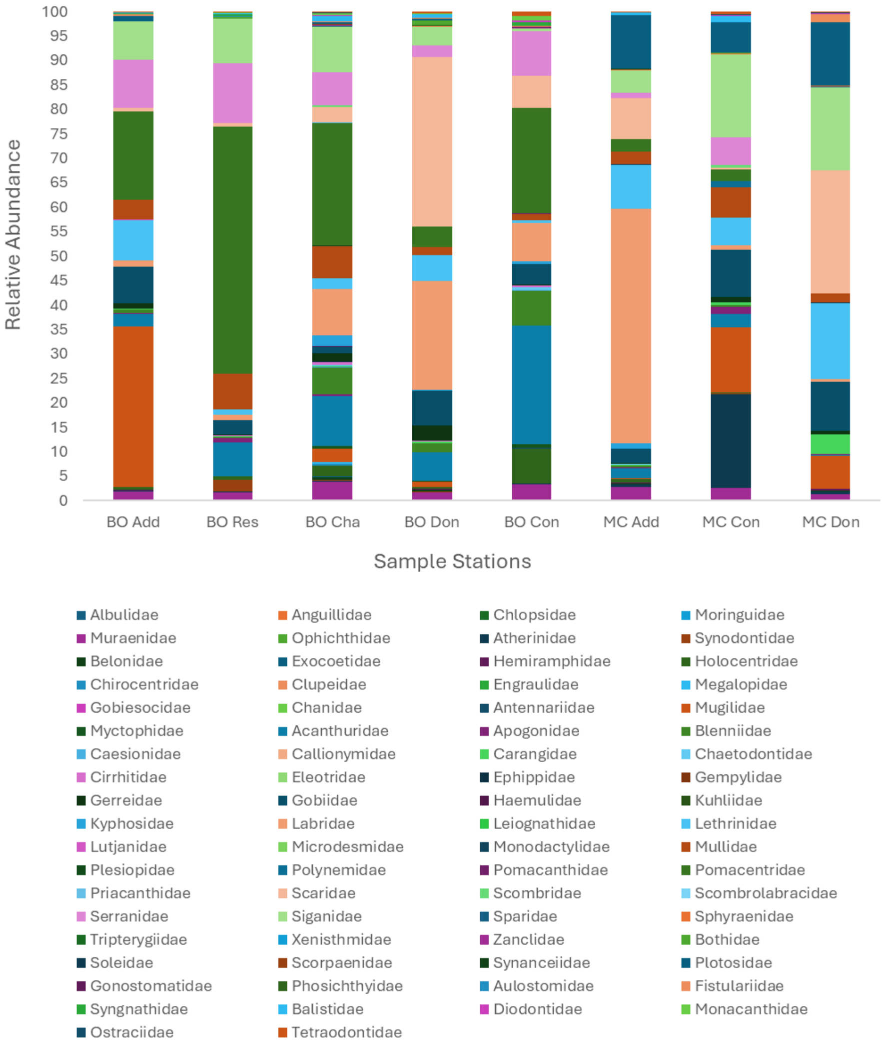
The same test, among sample stations for invertebrate species richness using the target dataset, but for Mon Choisy, disclosed no significant difference ($F_{2,\ 5} = 1.646$, $p = 0.282$) (Figure 17).

3.3.4 Fish Community Composition at Family Level

An analysis of the community composition for all 73 fish families across all eight sampled stations (Figure 18), illustrated large variation in the structure of the fish communities between and within locations.

Figure 18

Community Composition of the Fish Families Detected across the Stations in Bel Ombre and Mon Choisy



For the Bel Ombre lagoon for example, station BO Cha demonstrated the highest number of families (60 families), followed by stations BO Con (50 families), BO Don (40 families), and BO Add a BO Res both included (36 families). For Mon Choisy lagoon, the station encompassing the highest number of families was MC Con (43 families), followed by stations MC Add (41 families), and MC Don (40 families).

Table 2

The Dominant Fish Families at each Sampling Station represented by Percent Relative Abundance. Families with <5% are indicated as present (p) if detected in the station

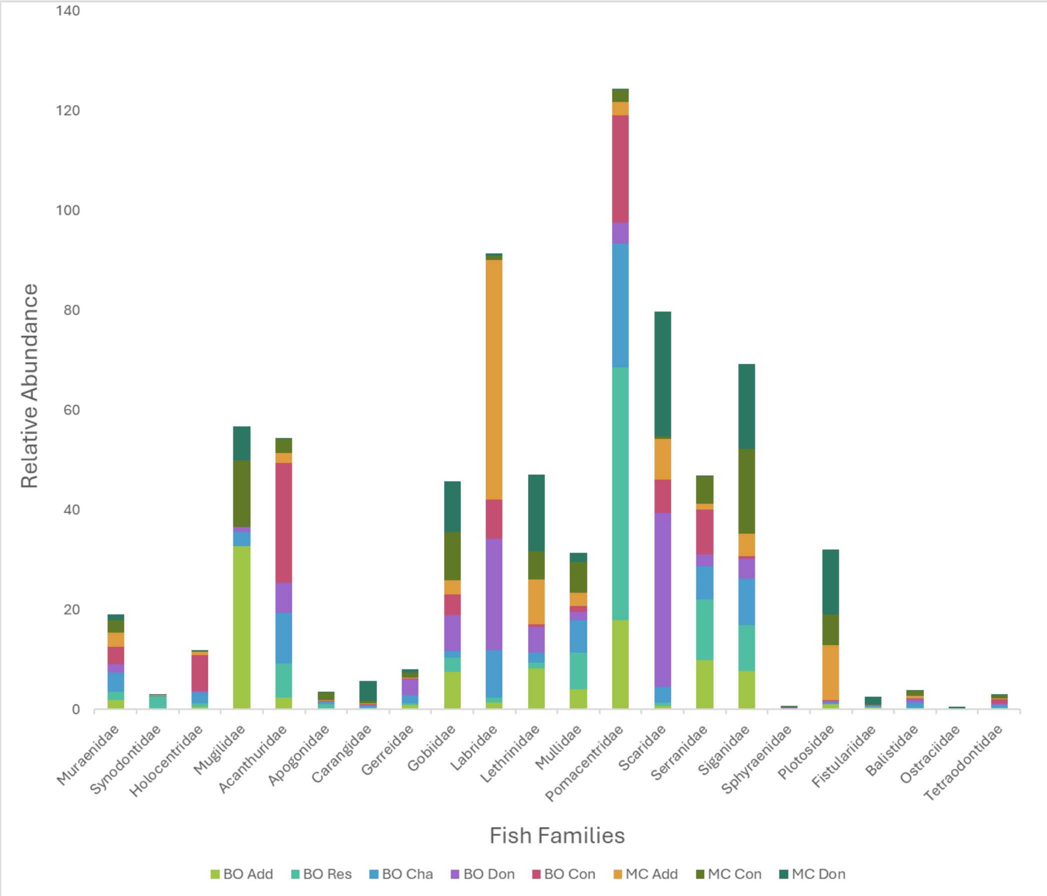
Fish Families	BO Add	BO Res	BO Cha	BO Don	BO Con	MC Add	MC Con	MC Don
Atherinidae (Silversides)	p	p	p	p	p	p	19.31	p
Mugilidae (Mullets)	32.79	p	p	p	p	p	13.29	6.74
Acanthuridae (Surgeonfish, Tang and Unicornfish)	p	6.77	10.24	5.89	24.11	p	p	p
Gobiidae (Gobies)	7.50	p	p	7.16	p	p	9.64	10.12
Labridae (Wrasses)	p	p	9.48	22.37	7.90	47.94	p	p
Lethrinidae (Emperors)	8.24	p	p	5.13	p	8.97	5.63	15.43
Pomacentridae (Damselfish)	17.94	50.61	24.96	p	21.51	p	p	p
Scaridae (Parrotfish)	p	p	p	34.77	6.67	8.32	p	25.10
Serranidae (Seabass and Groupers)	9.88	12.25	6.68	p	9.09	p	5.52	p
Siganidae (Rabbitfishes)	7.79	9.17	9.36	p	p	p	17.07	16.96
Plotosidae (Eeltail catfishes)	p	p	p	p	p	10.99	6.21	12.92

The families with the highest relative abundances for each station within the two locations are shown in (Table 2). Although all fish species represented in Table 2 are present in all the stations, their relative abundance differs across the stations.

A further investigation of the 73 families detected in the fish dataset, revealed that 22 of these families were present and detected in all stations at both locations of Bel Ombre and Mon Choisy as shown in (Figure19).

Figure 19

Composition of the Twenty-two Common Fish Families Detected at All Stations



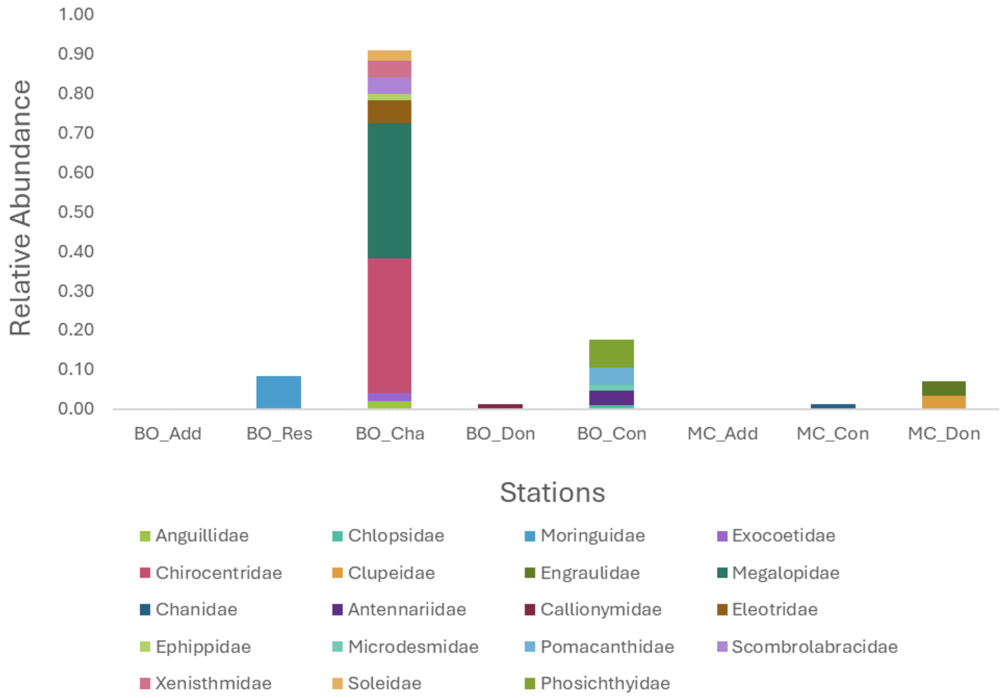
Again, although all the species were present in all stations the relative abundance of those species differs greatly even between the stations within the same location.

3.3.5 Fish Families Unique to Bel Ombre and Mon Choisy

A further analysis, concentrating on the 51 families not detected at all stations, revealed that 19 of these families are unique to or only found at one particular station.

Figure 20

Relative Abundance of Unique Fish Families only Detected in one Station in either the Bel Ombre or Mon Choisy Location



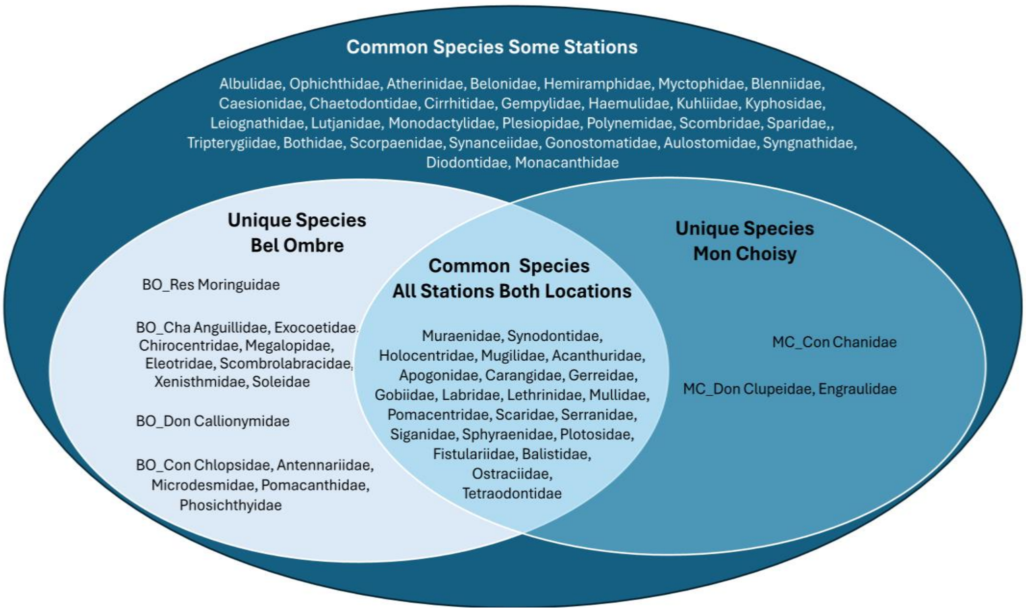
16 of these unique families were identified in Bel Ombre whilst three families were assigned to Mon Choisy. The reef channel station stood out particularly, with the station BO Cha possessing the highest number of unique families with nine identified, whilst at the patch reef station BO Con, five unique families were detected.

Amongst the unique families, a rare species identified in the channel station was *Anguilla marmorata*, also known as the Giant mottled eel, from the family Anguillidae. This is a catadromous family that spends most of its life cycle in freshwaters and river systems, present in the Bel Ombre seascape, and migrates to the ocean to spawn. Moreover, three families of artisanal fisheries value in Mauritius; Exocoetidae (Flying fishes), Chirocentridae (Herrings) and Soleidae (Soles or Flatfishes) were also detected at the same location, indicating that the channel station could be an area of local importance to fishers.

Within the patch reef station BO Con, two cryptic species were detected; *Antennatus coccineus* (Scarlet frogfish) from the family Antennariidae and a species from the genus *Kaupichthys* sp. (False morays) which is known to often hide in the sediment. Interestingly the bioluminescent species *Vinciguerria nimbaria* (Oceanic lightfish) from the family Phosichthyidae was also detected in this lagoonal coral patch reef.

Figure 21

Distribution of Fish Families within each Location Bel Ombre, North and Mon Choisy, South

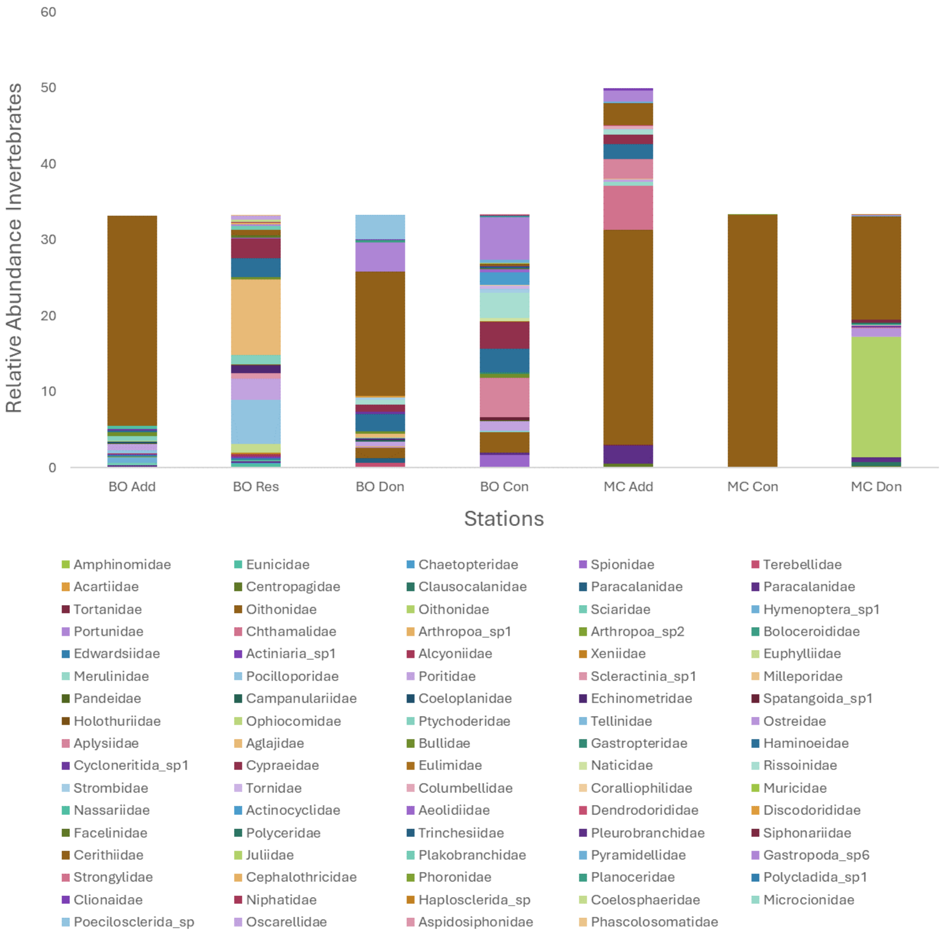


The diagram illustrates the distribution of fish families in the different locations. The analysis revealed that a large portion of the fish families detected in the survey overlapped between the two locations (Figure 21). However, unique fish families were identified at specific stations in each location, where Bel Ombre in the south demonstrate the greatest composition of unique fish families not found in the north.

3.3.6 Invertebrate Community Composition

An analysis of the community composition for all 72 invertebrate families across the seven sampled stations (Figure 22), illustrated large variation in the structure of the invertebrate communities between the stations in Bel Ombre. However, the invertebrate communities at the stations in Mon Choisy show similarity. At most stations one or more families dominate the invertebrate community, except at the stations BO Con and BO Res where the abundance of the families present is more evenly spread. At station MC Con only one family Oithonidae is prevalent.

Figure 22 *Community Composition of the 72 Invertebrate Families Detected across the Sample Stations in Bel Ombre and Mon Choisy Location*



For the Bel Ombre location, station BO Con demonstrated the highest number of invertebrate families (33 families), followed by stations BO Res (31 families), BO Don (22 families), and BO Add (19 families). For Mon Choisy lagoon, the two stations MC Add and MC Don had 23 families, followed by stations MC Con with only seven families represented.

Table 3

Dominant Invertebrate Families at each Sampling Station represented by Percentage Relative Abundance. Families <1% are indicated as present (p) if detected in the station, and by an empty cell if not detected

Phylum	Invertebrate Families	BO Add	BO Res	BO Don	BO Con	MC Add	MC Con	MC Don
Arthropoda	Paracalanidae	p	p	p	p	2.37		p
	Oithonidae			1.36	2.72	28.29	33.24	15.87
	Chthamalidae					5.87		
Cnidaria	Pocilloporidae	p	5.80		p			
	Poritidae	p	2.77	p	1.21	p		p
Echinodermata	Echinometridae		1.04	p				
Hemichordata	Ptychoderidae	p	1.27	p				
Mollusca	Ostreidae							1.07
	Aplysiidae				5.25	2.58	p	p
	Aglajidae		10.01	p				p
	Haminoeidae	p	2.47	2.24	3.15	1.93		
	Cypraeidae		2.66	1.03	3.47	1.24	p	
	Rissoinidae			p	3.31	p		
	Actinocyclidae				1.58			
	Cerithiidae	27.70	p	16.35	p	2.81		13.56
	Gastropoda sp.		p	3.79	5.63	1.4	p	
Porifera	Poecilosclerida sp.			3.27				

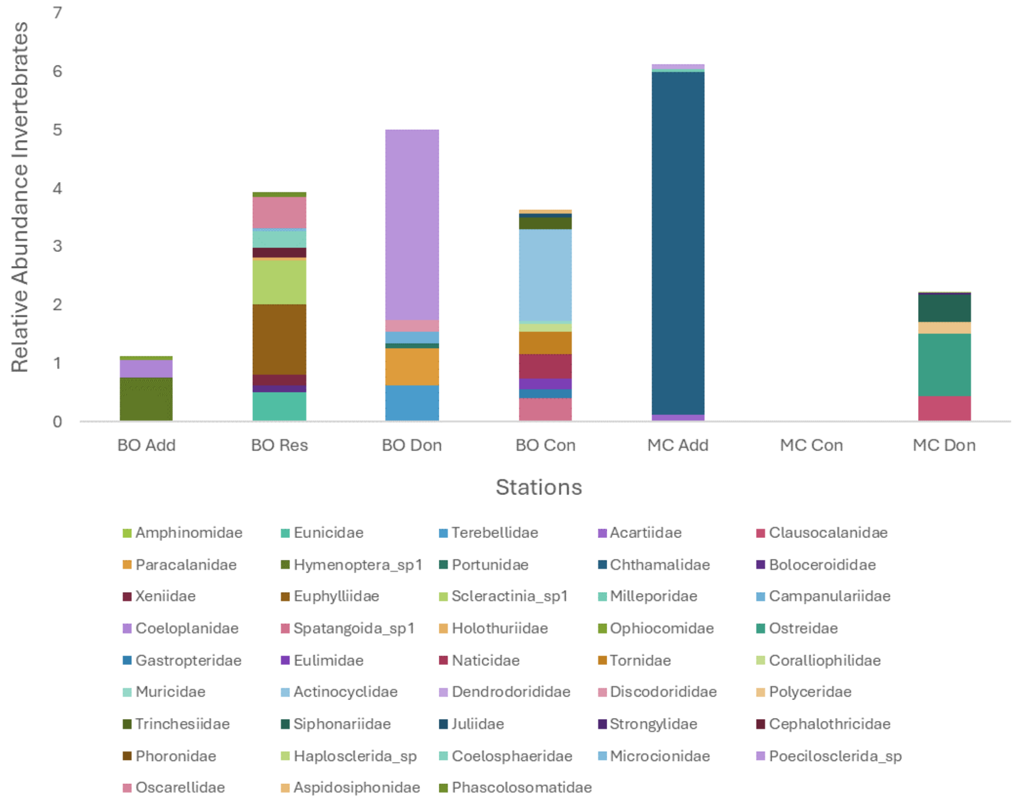
The composition of the dominant invertebrate families at each station is shown in Table 3. Seven families from the phylum Mollusca had high relative abundances with over 3% across stations when present. Other abundant families included Oithonidae and Chthamalidae (Copepods), from the phylum Arthropoda, together with the family Pocilloporidae (Stony corals) from the phylum Cnidaria, and the order Poecilosclerida (Sponges) from the phylum Porifera. None of the invertebrate families detected were represented in all sampling stations.

3.3.7 Invertebrate Families Unique to Bel Ombre and Mon Choisy

For the target invertebrate families, 43 out of the 72 were unique and detected only at one sampling station. Bel Ombre had the most unique families, with 31 families detected only in this lagoon, while 12 unique families were identified at the stations in Mon Choisy.

Figure 23

Relative Abundance of Unique Invertebrate Families only Detected in one Station in either Bel Ombre or Mon Choisy Location



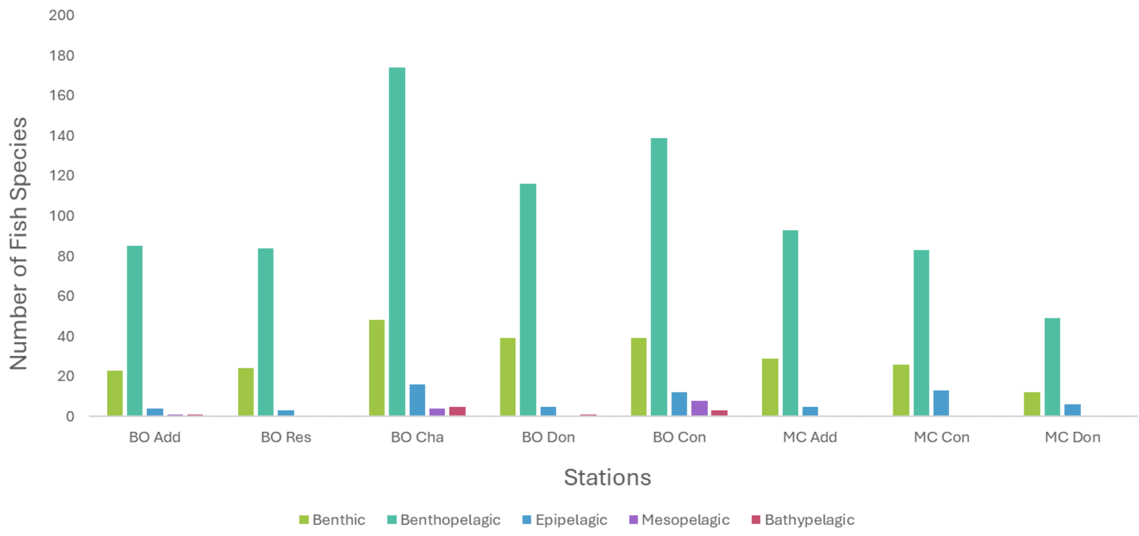
Many of these families had relative abundances of less than 1%. However, the taxa that were more abundant to some extent included the family Chthamalidae (Barnacles) identified at Station MC Add (6%), and the order Poecilosclerida (Sponges) recorded in station BO Don (3%). The families Euphylliidae (Hard corals) at station BO Res, family Ostreidae (Bivalve) at MC Don, and family Actinocyclidae (Nudibranchs) at the station BO Con, all had abundances between 1-2%.

3.3.8 Fish Community Biological Traits

When fish species were categorised into the five habitat preference categories; benthic, benthopelagic, epipelagic, mesopelagic or bathypelagic, the majority of the fish analysed were found to utilise the benthopelagic zone (239), located between the mid waters and benthos, followed by benthic fish species (85).

Figure 24

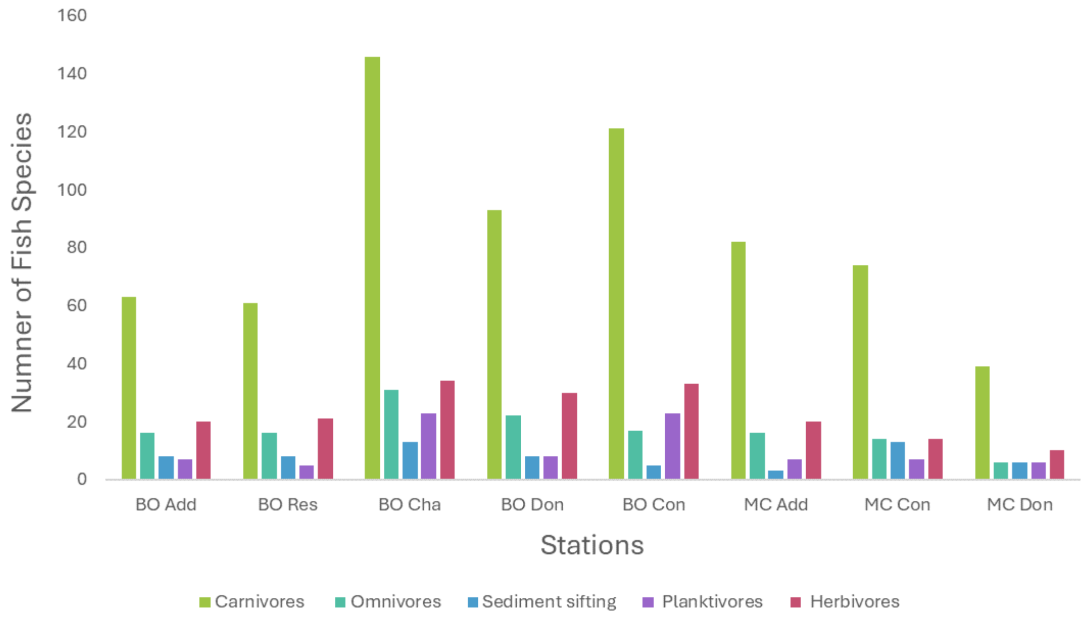
Number of Fish Families and Distribution in the Water Column and Habitat at each Station in Bel Ombre and Mon Choisy



In addition, epipelagic species (26) were detected in all the stations, however mesopelagic (9) and bathypelagic (8) species were only recorded in the stations in the Bel Ombre lagoon (Figure 24). Almost all detections of mesopelagic and bathypelagic species were at the lagoon patch reef station BO Con and the channel station BO Cha with species belong to the families Myctophidae (Lanternfish) and Phosichthyidae (Lightfishes). At the seagrass meadow station BO Don one count of the bathypelagic family Myctophidae was detected and at the mixed habitat station BO Add one count of the family Myctophidae was detected along with the species *Cyclothone pseudopallida* from the family Gonostomatidae.

Figure 25

Number of Fish Species Assigned to the Trophic Level Categories; carnivore, omnivore, planktivore or herbivore and Feeding Behaviour Category; sediment sifting at each Station in Bel Ombre and Mon Choisy



At all stations, the same pattern was evident, where carnivorous fish made up the greatest part of the species composition, between 55-61% of all species detected, followed by herbivores 12-19% and omnivores 9-14%. Planktivorous fish species however were mostly abundant in the coral dominated habitats, such as Bo Cha and BO Con in Bel Ombre, where 23 species were recorded in each station compared to about 5-7 species detected for the other stations. In total, 679 carnivores, 138 omnivores, 182 herbivores, 86 planktivores were detected. However, regarding fish that employ the feeding strategy of sand sifting, these made up between 2-11% of the species composition, dependent on the station where they were detected. 64 fish that sift sediments, were detected when both locations were combined. The lagoon in Bel Ombre had although a higher numbers of carnivores (484), omnivores (102), planktivores (66) and herbivores (138) compared to Mon Choisy which demonstrated lower numbers; carnivores (195), omnivores (36), planktivores (20) and herbivores (44).

Sediment sifting fish belonged to the families Plotosus (Catfishes), Labridae (Novaculichthys taeniourus - Rockmover wrasse), Soleidae (Soles), Mugilidae (Mullets), and Gobiidae (Gobies). With the family Soleidae only being detected in the channel station BO Cha. The stations BO Cha and MC Con had the highest number of sediment sifting fish detected with (13) species at each station. At both stations BO Add and BO Res (8) species were detected each, and at the seagrass stations BO Don and MC Don (8) and (6) species were detected respectively.

Table 4

Common Fish Families to all Stations their Assigned biological traits; benthic; b, benthopelagic; bp, epipelagic; ep, carnivore; c, omnivore; o, herbivore; h, sediment sifter; ss, and their role in the ecosystem

highest number of taxa detected, common families to all stations, top ranked families 1-8 for % average relative abundance.
common families to all stations, top ranked families 1-8 for % average relative abundance.
common families to all stations

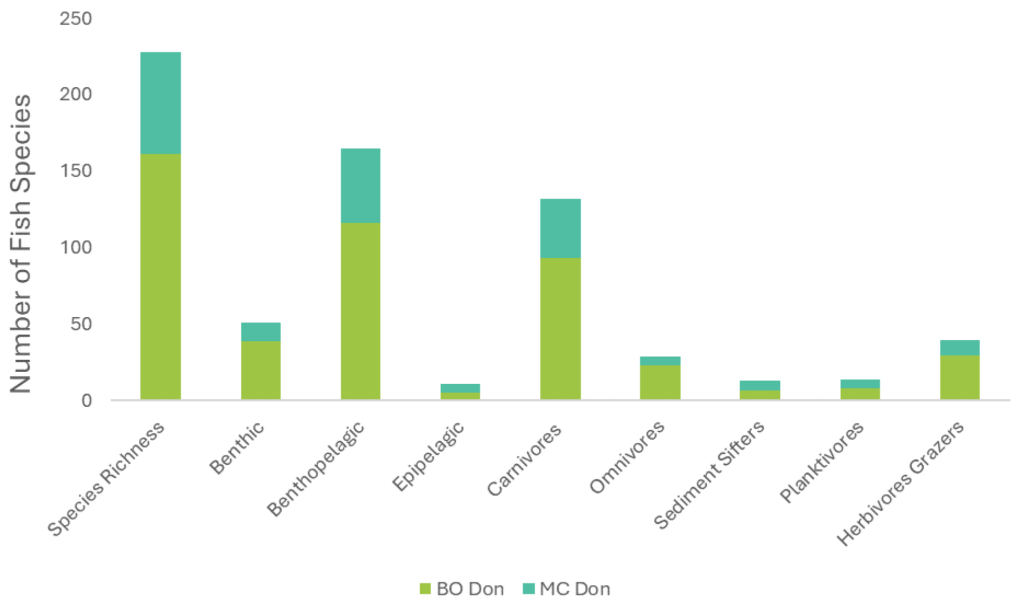
Family	b	bp	ep	c	o	h	ss	Role in Ecosystem
Muraenidae (Moray Eels)	✓			✓				(crustaceans and fish) **helps to maintain benthic community balance.**
Synodontidae (Lizardfishes)	✓			✓				(fish and invertebrates) **helps to maintain benthic community balanc**e.
Holocentridae (Squirrelfish)	✓			✓				(smaller invertebrates) **helps to maintain benthic community balance.**
Mugilidae (Mullets)	✓				✓		✓	**(**detritus, algae and small invertebrates). **Bioturbation** feeding habit of scooping up sand and filtering it through gills - **sediment turnover and nutrient cycling**.
Acanthuridae (Surgeonfish, Tang & Unicornfish)		✓				✓		(algae and detritus) **controls algae growth.**
Apogonidae (Cardinalfish)		✓		✓				(fish and invertebrates) **helps to maintain community balance.**
Carangidae (Jacks)			✓	✓				**Top predator** (smaller fish, crabs, crustaceans and squid) **helps to maintain the balance of populations.**
Gerreidae (Mojarras)	✓			✓				(smaller invertebrates) **helps to maintain benthic community balance.**
Gobiidae (Gobies)	✓				✓		✓	**(**detritus algae and small invertebrates) **Bioturbation** feeding habit of scooping up sand and filtering it through gills - **sediment turnover and nutrient cycling**.
Labridae (Wrasses)	✓			✓			✓	(hard-shelled invertebrates - crustaceans, mollusks and sea urchins) **helps to maintain benthic community balance. One species genus** Novaculichthys - **Bioturbation** - buries itself in the sand **sediment turnover and nutrient cycling**.
Lethrinidae (Emperors)	✓			✓				(hard-shelled invertebrates - crustaceans, mollusks and invertebrates) **helps to maintain benthic community balance.**
Mullidae (Goatfish)	✓			✓				(hard-shelled invertebrates - crustaceans, mollusks also worms) **helps to maintain benthic community balance**
Pomacentridae (Damselfish)		✓			✓	✓		Depending on species **herbivore** (algae), **omnivore** (algae and invertebrates) **helps to maintain benthic community balance** or **planktivore** (primary consumer).
Scaridae (Parrotfish)		✓				✓		(benthic algae) **controls algae growth.**
Serranidae (Groupers)		✓		✓				**Top predator** (smaller fish, crabs and crustaceans) **helps to maintain the balance of populations.**
Siganidae (Rabbitfishes)		✓				✓		(benthic algae and seagrass) **controls algae growth and seagrass.**
Sphyraenidae (Barracudas)		✓	✓	✓				**Top predator** (smaller fish, crabs and crustaceans) **helps to maintain the balance of populations.**
Plotosidae (Eeltail catfishes)	✓			✓			✓	(hard-shelled invertebrates, crustaceans, mollusks and worms) Forager contributes to **bioturbation - sediment turnover and nutrient cycling.**
Fistulariidae (Cornetfishes)		✓		✓				(fish and invertebrates), **helps to maintain community balance.**
Balistidae (Triggerfishes)	✓			✓				hard-shelled invertebrates, crustaceans, mollusks and invertebrates **helps to maintain benthic community balance.**
Ostraciidae (Boxfishes)	✓			✓				(hard-shelled invertebrates, crustaceans, mollusks and invertebrates) **helps to maintain benthic community balance.**
Tetraodontidae (Pufferfishes)	✓				✓			**omnivore** (algae and invertebrates - mollusks, crustaceans, tunicates) **helps to maintain benthic community balance**

3.3.9 Fish Community Biological Traits in Pure Seagrass Stations

As pointed out above, the sampling stations comprised various other habitats not necessarily pure seagrass meadows, and the lagoons are a mosaic of seagrass, coral patches and bare sediment.

Figure 26

Number of Fish Species in the Seagrass Stations BO Don and MC Don, their Distribution in the Water Column and Habitat, and their assigned Trophic Level and Feeding Behaviour



However, when the data only included stations of pure seagrass, Bel Ombre was not only more species rich compared to Mon Choisy with 161 and 67 fish taxa detected respectively, but also had greater numbers of benthic (39) and benthopelagic species (116), compared to Mon Choisy where benthic (12) and benthopelagic (49) species were recorded. The number of epipelagic species were, however, very similar for both locations, with five species recorded for Bel Ombre and six species recorded for Mon Choisy (Figure 26).

Similar trends were further noted when comparing carnivores, herbivores and omnivores between the two locations, where the Bel Ombre seagrass station was more species rich for all three trophic levels with carnivores (93), omnivores (23), and herbivores (30) species detected compared to Mon Choisy which demonstrated lower numbers; carnivores (39), omnivores (6), and herbivores (10). Sediment sifters and planktivores showed similar numbers of species present in both seagrass stations. With detection counts for Bel Ombre seagrasses being; sediment sifters (8) and planktivores (8) and for Mon Choisy seagrasses sediment sifters (6) and planktivores (6). Table 5 below, further describes the main families and genera of fish detected in the seagrasses that play key roles as secondary engineers (bioturbators) and algal control (herbivores).

Table 5

Detected Fish Families and Genera that can impact Seagrass Functionality. Station in Bel Ombre; BO Don, and Mon Choisy; MC Don. Biological traits: benthic; (b), benthopelagic; (bp), carnivore; (c), omnivore; (o), herbivore; (h), sediment sifting; (ss)

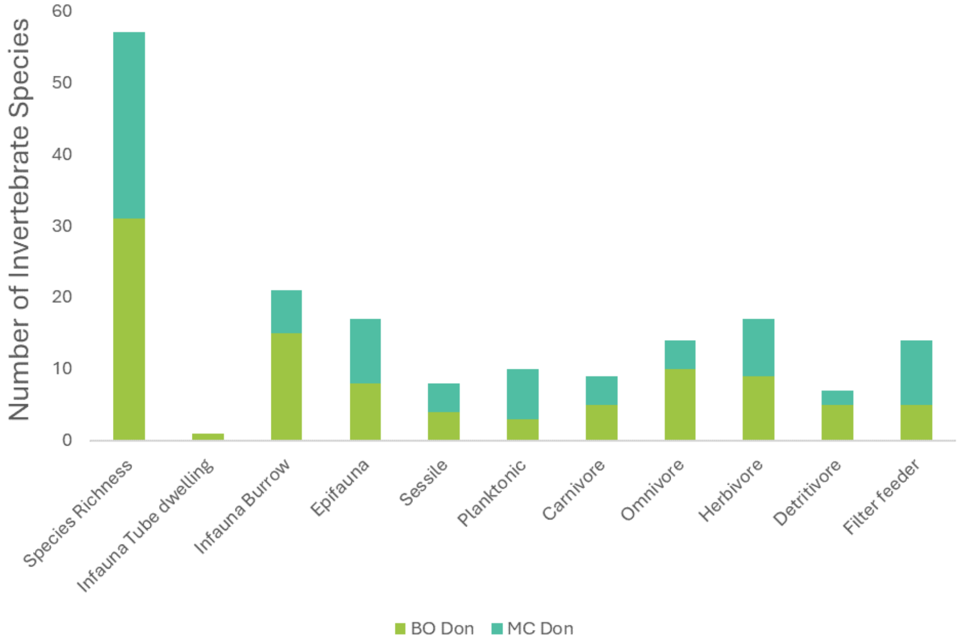
Family	Genus	b	bp	ss	c	o	h	BO_ Don	MC_ Don	Role in Ecosystem
Muraenidae	Gymnothorax	✓			✓	✓		✓	✓	**invertebrate community balance**
Ophichthidae	Myrichthys	✓			✓			✓		**invertebrate community balance**
	Leiuranus	✓			✓				✓	**invertebrate community balance**
Synodontidae	Saurida	✓			✓			✓		**invertebrate community balance**
	Synodus	✓			✓			✓	✓	**invertebrate community balance**
Mugilidae	Crenimugil		✓	✓		✓		✓	✓	**bioturbation, nutrient cycling**
	Mugil		✓	✓		✓		✓	✓	**bioturbation, nutrient cycling**
Acanthuridae	Acanthurus		✓				✓	✓	✓	**controls algae growth**
	Ctenochaetus		✓				✓	✓	✓	**controls algae growth**
	Naso		✓				✓	✓		**controls algae growth**
	Zebrasoma		✓				✓	✓		**controls algae growth**
Blenniidae	Cirripectes	✓					✓	✓		**controls algae growth**
	Exallias	✓			✓			✓		**invertebrate community balance**
	Glyptoparus	✓					✓	✓		**controls algae growth**
	Istiblennius	✓					✓	✓		**controls algae growth**
	Petroscirtes	✓					✓	✓		**controls algae growth**
Callionymidae		✓			✓			✓		**invertebrate community balance**
Cirrhitidae	Cirrhitus	✓			✓			✓		**invertebrate community balance**
Gobiidae	Amblygobius	✓		✓	✓			✓	✓	**bioturbation, nutrient cycling**
	Asterropteryx	✓		✓	✓			✓		**bioturbation, nutrient cycling**
	Callogobius	✓			✓			✓	✓	**invertebrate community balance**
	Cryptocentrus	✓			✓				✓	**invertebrate community balance**
	Gnatholepis	✓			✓			✓	✓	**invertebrate community balance**
	Vanderhorstia	✓			✓			✓	✓	**invertebrate community balance**
Kyphosidae	Kyphosus		✓				✓	✓		**controls floating algae growth**
Pomacentridae	Plectroglyphidodon		✓			✓	✓	✓	✓	**controls algae growth, omnivore**
Scaridae	Leptoscarus		✓			✓	✓	✓	✓	**controls algae growth**
	Scarus		✓				✓	✓	✓	**controls algae growth**
Siganidae	Siganus		✓				✓	✓	✓	**controls algae growth**
Labridae	Novaculichthys	✓		✓	✓			✓		**bioturbation, nutrient cycling**
Tripterygiidae	Enneapterygius	✓				✓		✓		**benthic community balance**
Bothidae	Bothus	✓			✓			✓		**benthic community balance**
Scorpaenidae	Scorpaenodes	✓			✓			✓		**benthic community balance**
Plotosidae	Plotosus	✓		✓				✓	✓	**bioturbation, nutrient cycling**

3.3.10 Invertebrate Community Biological Traits in Pure Seagrass Stations

When comparing invertebrate species, and key biological traits recorded for the two pure seagrass stations, species richness was very similar between the two stations, where 31 species were detected in Bel Ombre and 26 species were detected Mon Choisy (Figure 27).

Figure 27

Key Biological Traits represented by Invertebrate Seagrass Associated Species at Bel Ombre and Mon Choisy



Of these, 16 infauna species were detected for Bel Ombre, but where only one species represented infauna tube builders, by a polychaete worm from the family Terebellidae. This should be compared with the six infauna species recorded in the in Mon Choisy that was dominated by gastropods. These taxa included; Ptychoderidae (Acorn worm), Tellinidae (Bivalve mollusc), Bullidae (Bubble snail), Nassariidae (Nassa mud snails or dog whelks), and Cerithiidae (Cerith snails). At both locations Bel Ombre and Mon Choisy, Cerithiidae were the most abunadant infauna with an average relative abundace of 16.35% at Bel Ombre and 13.56% calculated for Mon Choisy.

Regarding epifauna and sessile species, similar numbers were detected at the two pure seagrass meadows in Bel Ombre and Mon Choisy with eight and nine epifaunal invertebrates recorded repsepectively. Of these, one species of epifauna belonging to the family Aglajidae (Headsheild slug) has the function of being a bioturbator. In addition, four sessile taxa were recorded at both seagrass stations where the hard coral of the genus *Porites* was identified. Further taxa detected included two classes of sponges, Haplosclerida and Poecilosclerida, which included the boring sponge Clionaidae, and two families of mollusks, Siphonariidae commonly known as false limpets as well as Ostreidae from the genus *Dendostrea.* All taxa listed here play significant functional roles in filtering water.

Furtheremore, planktonic species were dominated by copepods. These copepods belonged to five different families: Centropagidae, Clausocalanidae, Paracalanidae, Tortanidae and Oithonidae. These planktonic species predominantly consume phytoplankton, except for species in the family Tortanidae, which actively hunts smaller zooplankton. Besides, the number of carnivores together with herbivores detected in the two seagrass meadowes include only one family of the swimming crab Portunidae, while all other carnivores belonged to the class Gastropoa except for a marine planocerid flatworm Planoceridae. Concerning the grazers, eight of the nine herbivores detected belonged to the class Gastropoda with one family of sea urchin Echinometridae, that was identified in the meadow in Bel Ombre. The gastropod from the family Strombidae (True conchs) was identified at both locations. In summary, herbivores, omnivores together with detritivores dominated, and were the most species rich as well as having most abundant familes represented at both locations (Table 6).

Table 6

Invertebrate Families detected for seagrass meadows, Stations BO Don and MC Don and Biological traits: infauna (in), epifauna (epi), sessile (se),planktonic (plk), carnivore (c), omnivore (o), herbivore (h), detritivore (d) and filter feeder (f)

Family	Common Name	in	epi	se	plk	c	o	h	d	f	BO_ Don	MC_ Don	Role in Ecosystem
Terebellidae		✓							✓	✓	0.63		nutrient cycling, tube building, bioturbation sediment mixing and oxygenation
Centropagidae	Copepods - zooplankton				✓					✓		0.27	Indicator of water quality
Clausocalanidae	Copepods - zooplankton				✓					✓		0.44	Indicator of water quality
Paracalanidae	Copepods - zooplankton				✓					✓	0.64		Indicator of water quality
Paracalanidae	Copepods - zooplankton				✓					✓		0.62	Indicator of water quality
Tortanidae	Copepods - zooplankton				✓	✓						0.02	controls smaller zooplankton populations.
Oithonidae	Copepods - zooplankton				✓		✓				1.36		transfers nutrients to higher levels
Oithonidae	Copepods - zooplankton				✓		✓					15.87	transfers nutrients to higher levels
Portunidae	Swimming crab		✓			✓			✓		0.07		maintains populations
Poritidae	Hard Coral			✓						✓	0.66	0.06	foundation species
Campanulariidae	Hydrozoans			✓						✓	0.20		helps to form coral reef complexity
Echinometridae	Rock boring urchin			✓				✓			0.17		controls algae on coral reef
Ptychoderidae	Acorn worm	✓							✓		0.06		nutrient cycling, bioturbator, mixes and aerates the sediment
Tellinidae	Bivalve mollusc	✓							✓			0.02	bioturbator - mixes and aerates the sediment, nutrient cycling and water clarity
Ostreidae	Oysters			✓					✓			1.07	maintains good water quality
Aplysiidae	Sea hare		✓					✓				0.07	controls algal growth
Aglajidae	Headshield slug		✓			✓					0.58	0.04	controls populations of invertebrates. Bioturbator mixes and aerates sediment
Bullidae	Bubble snail	✓						✓	✓		0.28	0.03	controls microalgae, nutrient cycling, bioturbator mixes and aerates sediment, sensitive to pollution
Haminoeidae	Sea slug		✓					✓	✓		2.24		control algae growth and nutrient cycling
Cycloneritida_sp1	Gastropods Nerites		✓					✓	✓		0.31	0.17	control algae growth and nutrient cycling

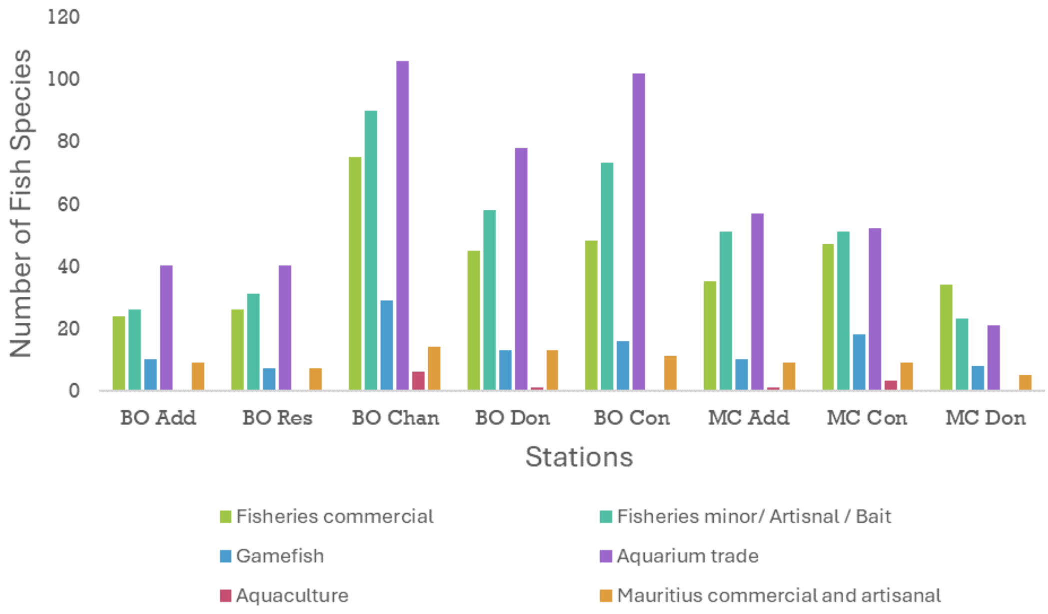
Cypraeidae	**Sea snail - gold ring cowrie**		✓			✓		✓		**1.03**		**control algae growth, nutrient cycling, empty shells used by hermit crabs**
Rissoinidae	Rissoinid snails		✓			✓		✓		0.60		control algae growth and nutrient cycling
Strombidae	True conchs		✓				✓	✓		0.34	0.03	control algae growth and aids in nutrient cycling
Columbellidae	Dove snails or dove shells		✓			✓		✓			0.13	control algae growth and aids in nutrient cycling
Nassariidae	Nassa mud snails or dog whelks	✓						✓			0.05	nutrient cycling, bioturbator, bioindicators of water quality and substrate conditions
Discodorididae	Nudibranch		✓		✓					0.20		controls sponge populations, Indicative of a healthy reef
Polyceridae	Nudibranch		✓		✓						0.19	preys on bryozoans, indicator of ecosystem health
Siphonariidae	False limpets			✓			✓				0.48	controls algae indicators of changes in water quality, pollution, and habitat conditions
Cerithiidae	Cerith snails	✓				✓	✓	✓		16.35	13.56	controls microalgae, nutrient cycling, bioturbator sensitive to pollution /habitat degradation.
Plakobranchidae	Sap-sucking sea slugs		✓				✓				0.02	controls algae
Pyramidellidae	Pyram snails		✓		✓						0.13	controls invertebrates indicator of invertebrate health
Gastropoda_sp6										3.79	0.03	
Planoceridae	Planocerid flatworms		✓		✓					0.33		controls invertebrates presence indicator of good water quality
Clionaidae	Boring sponges			✓					✓	0.10		bioeroders - reef and shell erosion negative effect if too many
Haplosclerida_sp	Sponges			✓					✓		0.03	nutrient cycling and water quality maintenance.
Poecilosclerida_sp	Sponges			✓					✓	3.27		nutrient cycling and water quality maintenance.

3.4 Commercial Fish Species found at the Locations and in the Seagrass Meadows

The economic value of the fish species detected for each station was analysed according to the value assigned to each species using FishBase (Figure 28).

Figure 28

Number of Fish Species of Commercial Value Globally and Locally Detected for each Station



Bel Ombre had many more fish species detected, with 944 species counted for all the stations, compared to 411 species counted for the stations in Mon Choisy. It is noted that fewer stations were sampled in Mon Choisy compared to Bel Ombre. However, when numbers of species are compared amongst the stations, more fish species of economic value are found at the channel station and the lagoon patch reef station, followed by the seagrass station in Bel Ombre, compared to all three stations in Mon Choisy. The number of fish species detected at each station, for all economic value categories, are shown in Table 7.

Table 7

Number of Detected Fish Species with Global Commercial Value compared to Fish Species of Local Value in Mauritius

	Commercial Value - World Wide					
Stations	**Fisheries commercial**	**Fisheries Minor/ Artisanal / Bait**	**Game fish**	**Aquarium trade**	**Aquaculture**	**Mauritius commercial and artisanal**
BO Add	24	26	10	40	0	9
BO Res	26	31	7	40	0	7
BO Cha	75	90	29	106	6	14
BO Don	45	58	13	78	1	13
BO Con	48	73	16	102	0	11
MC Add	35	51	10	57	1	9
MC Con	47	51	18	52	3	9
MC Don	34	23	8	21	0	5

The channel station in Bel Ombre has the highest number of fish species detected of global fisheries value as well as at local value. The seagrass station in Bel Ombre BO Don has the second highest number of locally important fish species of commercial value recorded and is comparable to the channel BO Cha and the lagoon patch reef BO Con stations. According to the numbers of fish species detected at the different Locations, Bel Ombre lagoon has a higher economic value compared to Mon Choisy lagoon.

In the two pure seagrass meadows, four fish species could be identified as having high artisanal value on the local market in Mauritius. These four species were common to both seagrass stations in the lagoons of Bel Ombre and Mon Choisy and included: Mugilidae (*Crenimugil seheli*), Mullidae (*Mulloidichthys flavolineatus*), Scaridae (*Leptoscarus vaigiensis*) and Sparidae (*Polysteganus baissaci*). Additional species, that are valued by fishers in Mauritius, that were identified only in the Bel Ombre seagrass meadow included; Holocentridae (*Sargocentron diadema*), Acanthuridae (*Naso unicornis*), Carangidae (*Caranx ignobilis*), two species of Labridae (*Cheilinus chlorourus*) and (*Cheilinus trilobatus*), Lethrinidae (*Lethrinus* *rubrioperculatus*), and two species of Scaridae (*Chlorurus sordidus*) and (*Scarus caudofasciatus*), and Scombridae (*Katsuwonus pelamis*). No additional fish species with respect to economic value were detected at the Northen site of Mon Choicy.

3.5 IUCN Red List of Threatened Species

With respect to red listed species, of the 202 taxa of fish detected to species level, only one species, *Albula glossodonta* (Roundjaw Bonefish), is categorised as Vulnerable (VN), while two species, *Chaetodon trifascialis* (Chevron Butterflyfish) and *Plectroglyphidodon dickii* (Blackbar devil), have been categorised as Near Threatened (NT). For the remaining detected species, 191 were scored as Least Concern (LC), seven species as Not Evaluated (NE), and one species categorised as Data Deficient (DD).

3.6 Invasive Species

Following an analysis of the fish dataset to detect invasive species in the locations Bel Ombre and Mon Choisy, and the respective seagrass stations, no known invasive species were detected in the samples after comparing for this study, the compiled species list against the Global Register of Introduced and Invasive Species (GRIS) record database for Mauritius.

Chapter 4 Discussion

4.1 Biodiversity and species richness

This study demonstrates that eDNA metabarcoding can effectively identify and provide comprehensive biodiversity records of marine taxa in dynamic and mixed habitat lagoons, and can be used to successfully describe communities and their biological traits that factor into the functioning of ecosystems and the provision of ecosystem services.

The eDNA methodology also demonstrates how powerful the technique is for detecting rare, cryptic and endangered species, which for long term impacts are instrumental for marine management and biodiversity conservation. The fact that this study, only includes two lagoons, and focuses on one habitat type (seagrass) only, it still has the capacity to discover two families, 14 genera and 26 new species never recorded before in Mauritius. Although it has previously been emphasised that the technique has been revolutionary (Seymour et al., 2019) compared to visual or invasive methods like gill net traps, it could have been expected that a set of new species for Mauritius could be recorded, that are still known for this region (Table 1). It was therefore a great surprise that one species was discovered, which has never been recorded before in the Mascarene Islands, which geographically comprises a large part of the Western Indian Ocean. The only location however, where this new taxa was discovered was at Bel Ombre where the inner lagoon has a direct connection with the ocean through the “reef channels”. Since eDNA is a non-discriminatory technique and DNA is present from various biological sources, it is possible that these rare findings could be from planktonic larvae flushed in to the lagoon through the channel, but where adult individuals never develop to the mature stage.

In addition to the new species discovery, the use of eDNA recorded 73 families, 174 genera, and 202 species of fish species in the lagoons with seagrass meadows. This should be added to the 72 families, 59 genera, and 41 species of invertebrates detected, with only four days of sampling over two sampling periods of summer and winter. Previous ecological studies of fish diversity in seagrass habitats, however using traditional survey equipment and towing gear, recorded only 16 families and 37 species of fish for Mauritius (Nakamura et al., 2006).

Still, with that said, it is important to remember that the methodology of using eDNA has never been applied to Mauritius before. For that reason, it is important to verify the occurrences of these species by following up with new eDNA surveys together with visual census surveys to provide greater confidence of the presence and possible establishment of the newly recorded species. As one species is not native to Mauritius and the region and has not previously been recorded, and although no known invasive species for Mauritius were detected amongst the fish and invertebrate species lists, this newly recorded species could be considered invasive. Its potential impact on the habitats where it occurs and native biodiversity would need to be investigated. From a management perspective, invasive species can outcompete native and endemic biodiversity and can have negative effects on the survival of native biodiversity, disrupt trophic interactions and ecosystems, and can cause negative trophic cascades in a food web (Dimitriadis et al., 2021; Kimbro et al., 2009).

4.2 Seagrass community composition fish and invertebrates

The seagrass fish community compositions for the WIO and globally were reviewed and described by Gullström et al (2002) where, dominant families in the WIO included Lethrinidae, Siganidae, Lutjanidae, Gerreidae, Labridae, Pomacentridae, Scaridae and Mullidae and from global literature the main families represented were Blenniidae, Gerreidae, Gobiidae, Labridae, Monacanthidae, Scorpaenidae, Sparidae, Syngnathidae, and Tetraodontidae. These families were also described in a study for Mauritius by Nakamura (2006) and which closely resembles the dominant fish families and community composition at both seagrass locations in this study, where all families were confirmed except Sparidae.

The dominant invertebrate phyla detected for both seagrass sites in this study included Mollusca that was represented by two families of Bivalve and 15 families of Gastropods. The present survey also recorded five families of Arthropoda, three families of Porifera, one family of Cnidaria and Echinodermata respectively. These findings confirm previous local studies in Mon Choisy, Mauritius (Daby 2003), and from other regional eDNA studies from e.g. South Africa where eDNA was also used, and traditional survey methods used in Mozambique and Madagascar (Chitará-Nhandimo et al., 2022; Herinirina et al., 2023 and Rossouw et al., 2024). However, this study presented a result with overall lower taxa detection counts for invertebrates than traditional surveys, which could stem from various sources. Simply, the WIO encompass a large ocean area, where gene flow is restricted by large geographical distances and currents preventing and allowing species larvae to spread. Differences may also occur from technical issues of using the eDNA technique, or sampling design. In this study water samples instead of sediment samples were used to collect eDNA, today we know more DNA would be expected to be present in the sediments for infauna and epifauna species as more DNA is stored in sediment compared to water. Additionally, the use of eDNA as a monitoring tool is a very new technique, particularly to the region of the Western Indian Ocean (WIO). Target species may obviously not have been previously sequenced and therefore not present in genetic databases (Rossouw et al., 2024). This would noticeably be a limiting factor in the assignment of taxa, along with the choice of primer as has been noted from many marine studies (Ruppert et al., 2019; Thomsen & Willerslev, 2015; Waters et al., 2023).

4.3 Seagrass ecosystem functioning and biodiversity present

Healthy seagrass meadows provide a number of important ecosystem functions (Lima et al., 2023). This is true for the seagrass itself, but also in combination with various associated species that contribute to ecosystem health, resilience and functionality (Duffy, 2006; Monteiro, 2016). For example, primary producers provide habitat stabilisation, carbon sequestration and habitat structure, while herbivores e.g. control seagrass growth, while predators control populations of herbivores and invertebrates in the food web. Regarding associated fauna’s habitat preference within a seagrass meadow also provides functions based on their behaviour and traits, as an e.g. bioturbators in the sediment reworking the sediment (Bouma et al., 2009; Gutiérrez et al., 2011). For seagrass meadows, the composition of associated flora and fauna may for that reason have large implications for the function of the ecosystem. As an example, a seagrass meadow can be in the state of being autotrophic, meaning the meadow sequesters more carbon than the system releases, the system will function as a sink. However, disruptions in the system, from for example climate change or anthropogenetic stress, can put the system into a heterotrophic state, meaning the meadow becomes a source of carbon (Gullström et al., 2018; Johannessen, 2022). A good example when a meadow can go from being autotrophic to a heterotrophic state is when there are imbalances in the food web. If certain key species are missing (e.g. top predators) in the system, cascade effects in the food web can increase for example bioturbators, which can change the chemical sediment composition from an anoxic condition, where the meadows sequester (Bouma et al., 2009; Diaz and Cabido, 2001).

An ecosystem can accordingly be robust or sensitive to external stress depending on the state of its biodiversity and species richness. If the ecosystem comprises many species with the same trait, the system is said to have a strong buffer against stress, since if a certain species disappears, another species with the same trait takes its place, known as taxonomic redundancy (Kindeberg, 2024; Micheli & Halpern, 2005). For this study, it was shown that burrowing and bioturbating species using feeding strategies that disturb or turn over the sediment, differed between the two lagoons, for both fish and invertebrates. In Bel Ombre, eight species of bioturbating fish, and 16 species of burrowing invertebrates, while in Mon Choisy, only six fish species were recorded and six species of bioturbating invertebrates were detected. This result can mean two things. The higher number of bioturbating taxa in Bel Ombre could on one hand mean Bel Ombre has a higher redundant taxonomic level in comparison with Mon Choisy, with less risk of losing that specific ecological function, as fewer bioturbators could be detected in Mon Choisy. On the other hand, if the high number of bioturbators in Bel Ombre is an effect of a disturbed food web, where the number of low trophic level species has increased due to a low number of predators, there could be a risk that the seagrass meadow can start functioning as a heterotrophic system releasing more CO_2 than it captures. However, the information to resolve these questions is not available in this study as it is out of the scope of this report.

4.4 Seagrass Ecosystem services– biodiversity present

The provisioning service of seagrasses is well documented and important to all coastal communities globally for commercial, recreational and subsistence fisheries (Nordlund et al., 2018). Seagrasses are especially important to coastal fishing communities in the Western Indian Ocean where for some countries it is a high value fishery on the international and local markets and is also an important subsistence fishery for fish and invertebrate species for food or bait (Chitará-Nhandimo et al., 2022; Gullström et al., 2002; Herinirina et al., 2023 & Nordlund et al., 2018). Within the Bel Ombre seagrass meadow, 45 species of fish were classified using the FishBase global classification as having commercial value, and 58 species were classified as artisanal. Only 13 of these species are recognised as having either commercial or artisanal value in Mauritius (FishBase https://fishbase.se/country/CountryChecklist.php?c_code=480&vhabitat=commercial&csub_code=). Comparatively for the seagrass meadow in Mon Choisy, only four fish species were classified as having local value for Mauritius, while 34 species and 23 species respectively were categorised as having commercial and artisanal value globally. If this result is accurate, and a significant number of fish species found here are commercially important elsewhere but not in Mauritius, it could represent a missed opportunity for a potential valuable resource. For the fiscal year 2022 to 2023, the coastal fishery around Mauritius from artisanal and amateur fishing combined was estimated to catch 891 tonnes (Albion Fisheries Research Center [AFRC], 2023), where the main supply of local fresh fish comes from the artisanal fishery (Sweenarain, 2012). On the other hand, the eDNA data does not provide information about which stage in the life cycle these fish species are in. It is possible that these recordings detect species in their juvenile stage, using the seagrass meadow as a nursery (Gullström et al., 2002) but migrate elsewhere as adults. No invertebrate taxa were detected that have commercial value, but obviously play a critical role of being food for fish with high commercial value.

4.5 Variation between and within the North and South Lagoons

Location proved to have a significant effect on overall fish species richness at family and genus level, but did not have a significant influence on invertebrate taxa richness indicating that Bel Ombre lagoon only supported more different fish taxa compared to the Mon Choisy lagoon. Differences in the fish community compositions were also identified through the eDNA results for both lagoons where although common fish families were detected for both seagrass habitats, unique taxa existed for both locations. Furthermore, the differences in species richness and fish assemblages between the two locations could be due to a number of factors linked to the structural complexity of the meadows and resources available to support fish diversity (Duffy, 2006; McCloskey & Unsworth 2015). Other factors could be related to the proximity of different habitats to the seagrass meadows within the same seascape (Henderson et al., 2017). Increased seascape complexity would attract diverse biodiversity to the lagoon, allowing various species to utilise the seagrass meadows and consequently influence the composition of the fish community. For example, the Bel Ombre lagoon contains four distinct habitats in close proximity; a mangrove estuary, seagrass meadows, coral patch reefs, and a deeper reef channel. The sampling stations were selected to reflect these habitat differences. In contrast, the Mon Choisy lagoon lacks mangroves, and its seagrass meadow is sheltered from the open ocean by an outer reef barrier.

Just as differences in community composition and species richness between the two lagoons, variation among stations within the lagoons can be explained by the uniqueness of the habitats which are significantly different from each other. The reef channel station within the Bel Ombre lagoon for example was significantly different in fish species richness compared to the seagrass station and the bare sand station. However, the reef channel is directly influenced by the open ocean and therefore provides unique environmental conditions, nutrients and a heterogenous habitat that offers numerous and distinct ecological niches that can support a more diverse assemblage of fish and other biota (Breckwoldt et al., 2022). It is not only a corridor for the movement of water but also for marine biota which actively move in and out of the lagoon and for larvae of many species with the rise and fall of the tides (Breckwoldt et al., 2022). Thus, it is not surprising that nine unique species were detected exclusively at the channel station and were not found at any other station.

Chapter 5 Conclusions

eDNA has seen a significant recent growth since it was introduced in the 2000's on land, rivers, lakes, and today the technique is being applied frequently in the marine realm. The methodology includes the benefits of being non-intrusive, sensitive, and relatively cost-effective, making it attractive for resource monitoring, management, and decision making (He et al. 2021). However, for Mauritius, this is the very first time eDNA has been applied to describe marine biodiversity, to my knowledge. Although the study recorded fewer numbers of invertebrates compared to other regions in the WIO (Chitará-Nhandimo et al., 2022; Herinirina et al., 2023 and Rossouw et al., 2024), the number of fish species recorded, was significantly greater compared to previous studies in Mauritius and elsewhere in the WIO region using traditional monitoring techniques (Nakamura, 2006; Rossouw et al., 2024). The technique also proved to be particularly powerful for the purpose of biodiversity conservation, since although it was only used at two shallow lagoons, the survey still had the ability to discover a number of different taxa at different taxonomic levels, and a fish species never recorded in the Indian Ocean before. Furthermore, with the knowledge of depleting fish stocks globally, which certainly is of great concern also around the Mascarene Island complex, it is therefore surprising so few species of economic importance are utilized around the island, when this study identified such a large set of fish species commercialised in other parts of the world.

Concerning local conservation strategies, this study also emphasises the differences between the North and the South of the Island. Such differences on a national level are obviously of importance in terms of localised management and conservation practises. Particularly today with a growing focus on marine spatial planning, marine protected areas, and competition for marine spaces for sustainable tourism. The study highlights the need for basic and reliable baseline data from monitoring studies, since without the knowledge of species present in an ecosystem, effective management practises become impossible, and species and critical habitats cannot be given appropriate legal protection for the future.

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Appendices

Table S1 Tukey multiple comparisons of means test with 95% family-wise confidence level for interactions of stations within Bel Ombre location. An asterisk denotes significant values (alpha level of 0.05), while a dot indicates a p-value close to the significance threshold

Station	diff	lwr	upr	p adj
BO_Cha-BO_Add	58.66667	-14.91180	132.245131	0.1623919
BO_Con-BO_Add	33.83333	-39.74513	107.411797	0.6556213
BO_Don-BO_Add	1.50000	-72.07846	75.078464	0.9999967
BO_Res-BO_Add	-11.16667	-84.74513	62.411797	0.9909005
BO_Con-BO_Cha	-24.83333	-84.90990	35.243231	0.7365017
BO_Don-BO_Cha	-57.16667	-117.24323	2.909898	**0.0673267 .**
BO_Res-BO_Cha	-69.83333	-129.90990	-9.756769	**0.0174849 ***
BO_Don-BO_Con	-32.33333	-92.40990	27.743231	0.5146992
BO_Res-BO_Con	-45.00000	-105.07656	15.076564	0.2085506
BO_Res-BO_Don	-12.66667	-72.74323	47.409898	0.9693270