



## REPORT

# Chapter 5.0 Substrate, Macroflora, and Benthic Epifauna

*2024 Milne Port Marine Environmental Effects Monitoring Program (MEEMP) and Non-Indigenous Species/Aquatic Invasive Species (NIS/AIS) Monitoring Program*

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CA0026317.6821-051-R-Rev0-86000

April 9, 2025



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## ACRONYMS AND ABBREVIATIONS

| Acronym or Abbreviation | Definitions                                       |
|-------------------------|---|
| AIC                     | Akaike's Information Criterion                    |
| CD                      | Chart Datum                                       |
| cm                      | centimetre  |
| DF                      | Degrees of freedom                                |
| DFO                     | Fisheries and Oceans Canada                       |
| EEM                     | Environmental Effects Monitoring                  |
| FEIS                    | Final Environmental Impact Statement              |
| GLMM                    | Generalized Linear Mixed Models                   |
| L                       | Litres  |
| LPL                     | Lowest Practical Level                            |
| m                       | metre   |
| mm                      | millimetre  |
| m <sup>2</sup>          | square-metre                                      |
| MEEMP                   | Marine Environmental Effects Monitoring Program   |
| MEWG                    | Marine Environment Working Group                  |
| MFEAP                   | Marine Foreshore Environment Assessment Procedure |
| mL                      | millilitre  |
| NIS/AIS                 | Non-Indigenous Species/Aquatic Invasive Species   |
| No.                     | Number  |
| Ns                      | Natural spline                                    |
| org/m <sup>2</sup>      | Organism per square-meter                         |
| <i>P</i>                | P-value   |
| PC                      | Project Certificate                               |
| Q                       | Quadrat   |
| QA/QC                   | Quality Assurance and Quality Control             |
| ROV                     | Remotely Operated Vehicle                         |
| SDI                     | Simpson's Diversity Index                         |
| SE                      | Standard Error                                    |

| Acronym or Abbreviation | Definitions  |
|-------------------------|--|
| sp.                     | Single or unconfirmed multiple species within a genus, used when the specimen has not been identified to the species level |
| UNB                     | University of New Brunswick  |
| UTM                     | Universal Trans Mercator   |
| VIF                     | Variance Inflation Factor  |
| ZOI                     | Zone of Influence  |
| %                       | Percent  |
| >                       | Greater than   |
| <                       | Less than  |

## 5.0 SUBSTRATE, MACROALGAE, AND BENTHIC EPIFAUNA

### 5.1 Introduction

This chapter presents the results of the substrate, macroalgae, and benthic epifauna monitoring program, a component of the larger Marine Environmental Effects Monitoring Program (MEEMP) conducted in Milne Inlet during the 2024 open-water season. The MEEMP is designed to evaluate potential Project-related effects on the marine environment as predicted in the Final Environmental Impact Statement (FEIS) and FEIS addenda (Baffinland 2012, 2013). This component was also developed in consideration of the monitoring requirements outlined in the Project Certificate (PC) Conditions described in Chapter 1.0, Table 1-2. PC Conditions related to the monitoring of substrate, macroalgae, and epifauna included PC Conditions No. 76, 83 (a), 87, 99 (a), and 99 (c).

#### 5.1.1 Objectives

The MEEMP objectives are outlined in Section 1.3 of Chapter 1.0 (Program Overview). Objectives specific to the substrate, macroalgae, and benthic epifaunal component are as follows:

- Monitor for changes in substrate conditions or in the macroalgae and benthic epifaunal community at Milne Port and at a nearby reference area for the purpose of identifying potential Project-related effects.
- Verify predictions made in the FEIS, and subsequent addenda, regarding effects on Arctic char (*Salvelinus alpinus*) habitat.
- Recommend necessary and appropriate changes to survey methodology for future years, if warranted.

### 5.2 Study Design

#### 5.2.1 Background

The 2014 to 2017 MEEMP study design monitored for changes to the benthic community with epifauna<sup>1</sup> and macroalgae<sup>2</sup> as indicators, using towed underwater video transect surveys. The use of epifauna and macroalgae as effect indicators deviated from the standard Environmental Effects Monitoring (EEM) methodology (Environment Canada 2012) and presented a number of challenges, including 1) high temporal and spatial variability due to the mobile and transient nature of many epifaunal species, 2) typically low resolution of video survey data compared to laboratory analysis for species identification, enumeration and substrate classification, and 3) difficulty in distinguishing between live macroalgae (e.g., kelp) and detrital vegetation debris using video survey methods, which can result in inaccurate results.

In 2018, a new survey design was implemented, based on a Before-After-Control-Impact (BACI) approach. A reference area had been established in 2013 and selected for its proximity to Milne Port while residing outside of the main zone of influence (ZOI) of Project activities (SEM 2014). Towed underwater video transects were replaced with five belt transects (1 m x 5 m plots) permanently installed on the seabed in each exposure (impact) and reference (control) areas. The belt transects deployed were composed of two 1 m-long, 5 cm-diameter

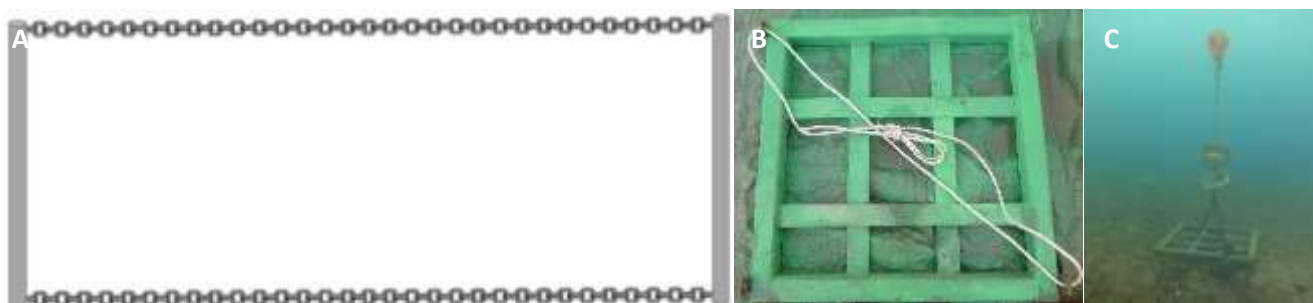
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<sup>1</sup> Benthic invertebrates or other organisms living on the substrate.

<sup>2</sup> Marine vegetation attached to the substrate (e.g. kelp).



aluminum pipes filled with concrete connected by two steel chains (5 m in length) attached to both ends of the pipes (Figure 5-1A). Monitoring was conducted using a remotely operated vehicle (ROV) underwater video system. In addition to informing this component of the MEEMP, taxonomic data were also used to inform the Non-Indigenous Species and Aquatic Invasive Species (NIS/AIS) program (Chapter 8.0). In 2019, underwater video monitoring of epifauna and macroalgae communities within permanent belt transects continued for a second year. In 2019, it was determined that the flexible design was not suitable for the environment, as five of the ten deployed transects had been dragged from their original position due to presumed interactions with the sea-ice during spring break-out or had become embedded in the sediment and thus obscured from video detection (Golder 2020, 2021).



**Figure 5-1: A) Diagram of Belt Transects Used for Macroalgae and Epifauna Surveys in 2018 – 2019; B) Example of 1 x 1 m Steel Quadrat Deployed in 2021; and C) Underwater Photo of Quadrat (Q4) With Attached Settlement Substrates for NIS/AIS Monitoring.**

The program was modified in 2020 to replace the belt transects, which had been determined to be ineffective due to being dragged out of position. Modifications to the program in 2020 included the use of divers to undertake biophysical surveys of permanent, heavy-duty steel quadrats to improve the resolution of taxonomic identification. In previous years, taxonomic resolution was relatively coarse because of poor visibility due to suspended particles in the water column and the use of a ROV-based underwater video survey for monitoring. Survey of the quadrats was performed by a combination of divers and ROV in 2020<sup>3</sup>. The rationale for discontinuing the ROV was that divers were more accurately able to distinguish unique taxa, differentiate between detrital algae or non-living organisms versus living organisms, move vegetation aside to observe the underlying substrate and marine organisms, and collect specimens from the quadrats for identification purposes.

A total of ten 1 m x 1 m square quadrats (Q) were fabricated onsite in 2020 and installed on the sea bottom in Milne Port, five in the exposure area (Q1 through Q5) and five in the reference area (Q6 through Q10) (Figure 5-1B,C; Figure 5-2; Appendix 5B – Photo 1). Each quadrat was deployed with chains (Appendix 5B – Photo 1) and marked with fluorescent spray paint to aid in relocating them in subsequent surveys. Additionally, starting in 2020 as part of the Chapter 8.0 NIS/AIS program, settlement substrates were co-located and deployed with the quadrats to monitor for short- and medium-term recruitment of encrusting taxa. Settlement substrates consisted of settlement plates attached to the center quadrat deployment chain/line with a subtidal buoy, and cobble-filled settlement baskets attached to a corner of the quadrat, as described in Chapter 8.0 (Figure 5-1C; Appendix 5B – Photo 2).

<sup>3</sup> Divers surveyed quadrats in the reference area (Q6, Q8, Q9, Q10), but were unable to survey the quadrats in the exposure area due to time constraints in the field program (these were subsequently completed using ROV-video surveys).



In 2021, survey methods were modified to be conducted solely via divers to maintain taxonomic resolution. An additional ten square steel quadrats were fabricated and deployed with polyurethane line (Appendix 5B – Photo 2) in 2021 (Q11 through Q20; five in each exposure and reference area). These quadrats were generally deployed to be paired with existing quadrats (e.g., Q1 with Q11; Figure 5-2), doubling the total number of quadrats relative to 2020, to ten in each area in 2021. Surveys conducted in 2020 indicated that Q9 in the reference area had been originally deployed over hard substrate (boulder) and supported different ecological communities relative to the soft substrate quadrats. Therefore, in 2021, Q9 was relocated to a different area to maintain comparability between quadrats.

During the 2021 field program, divers were unable to locate Q2 after undertaking a thorough search between the -3 and -12 m CD (chart datum) depth contours (Q2 was deployed at approximately -10 m CD), extending approximately 25 m to the west and east of the original location. This quadrat was assumed to have been dragged from its original position by sea-ice. A replacement quadrat (Q2) was deployed at -12 m CD in 2022 with co-located settlement substrates. Surveys conducted in 2021 also noted that Q12 had been deployed too shallow (-6 m CD) for desired target depth (-7 to -9 m CD) and, consequently, the Q12 quadrat data were not included in analyses in 2021. Quadrat Q12 was relocated to deeper water in 2022 (with co-located settlement substrates) to maintain comparability between quadrats, as per recommendations in Golder (2022).

In 2022, six new permanent quadrats, Q21 through Q26, were deployed (three in each of the exposure and reference areas), to increase statistical power and address Marine Environment Working Group (MEWG) Comment No. 05 from the 2021 MEEMP and NIS/AIS Monitoring Program Report (Golder 2022). This modification expanded the total number of quadrats to 13 in each area (Figure 5-2). Additional quadrats deployed in 2022, Q21 through Q26, were not co-located with Chapter 8.0 NIS/AIS settlement substrates.

During the 2022 field program, the vessel anchor had been deployed and caught on Q16, causing it to drag across the bottom to a new location in -9.1 m CD from the original -5.7 m CD depth. Divers observed that the sediment surface was disturbed inside and outside the quadrat and the data collected during the 2022 quadrat survey indicated that less silt was observed compared to 2021 and that infauna, such as clams, had been brought up to the sediment surface. Therefore, Q16 results from 2022 were considered an outlier and removed from analyses.

During the 2023 field program, one objective was to relocate Q16 to a new location at a similar depth as the original location in 2021 (-5.7 m CD) with a new quadrat name to restart the quadrat data series. Relocation was not possible in 2023 due to field program constraints (e.g., program delay due to late ice break up, vessel repairs).

### 5.2.2 Modifications to the 2024 Program

In total, 24 of the 26 quadrats were surveyed in 2024. Two quadrats, Q21 and Q22 (deployed at -6 and -10 m CD, respectively), could not be located despite multiple prolonged search efforts between the -3 and -12 m CD depth contours near the deployment coordinates. Divers recovered Q16 which had been dragged into deeper water in 2022, and, using a diver-assisted 56 kg lift bag, relocated it to its approximate original depth but offset from its original position by approximately 10 m. This quadrat was renamed as Q27 and surveyed two days after being relocated. The Q16 timeseries was discontinued and a separate timeseries for Q27 created in 2024 for data analysis.

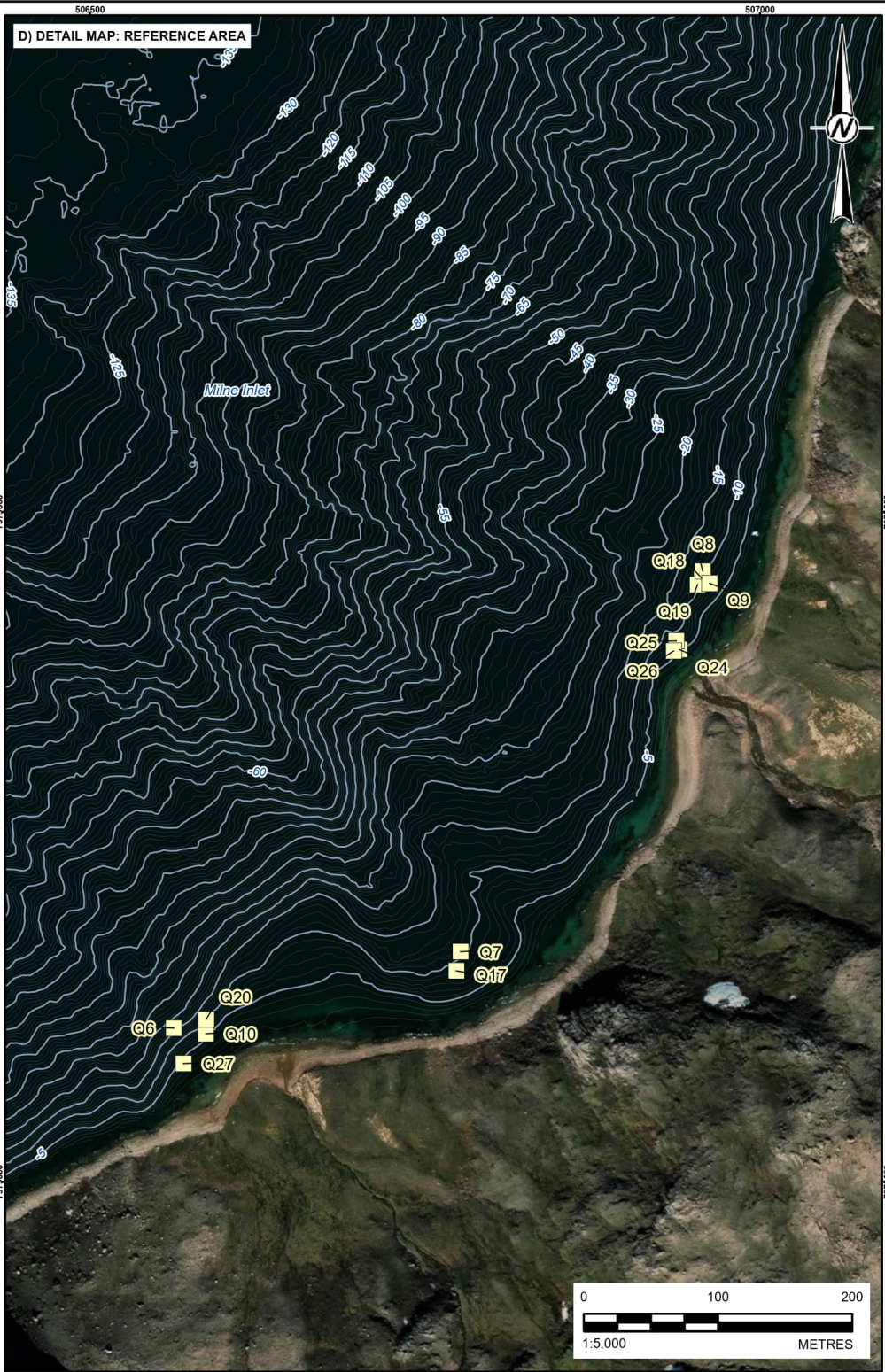
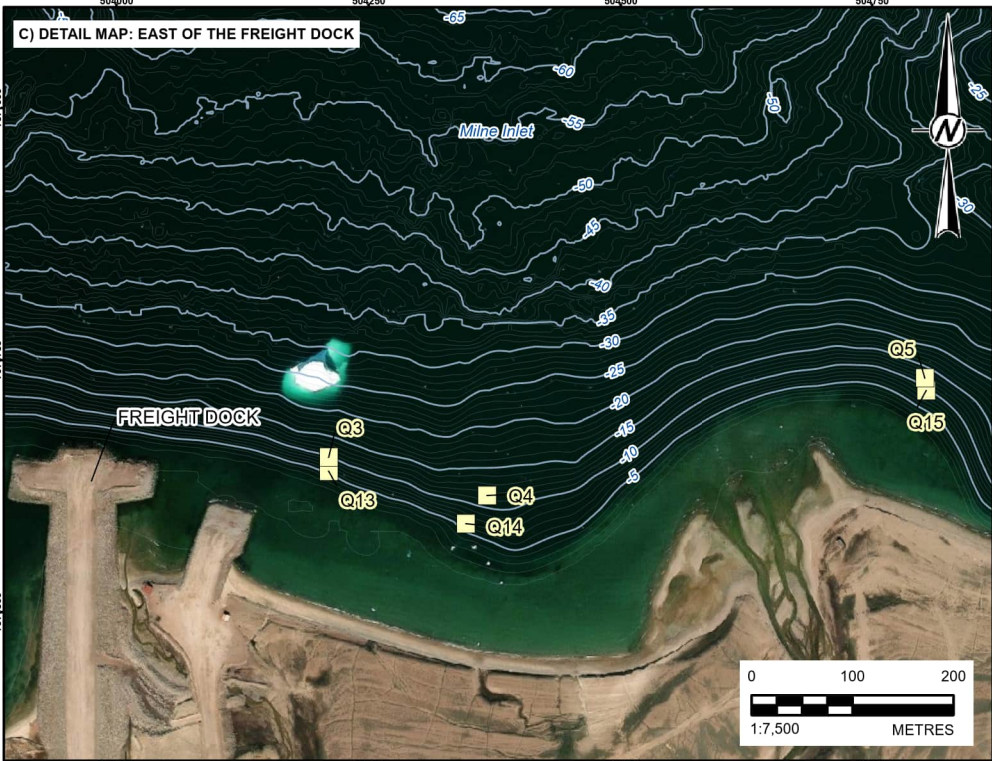
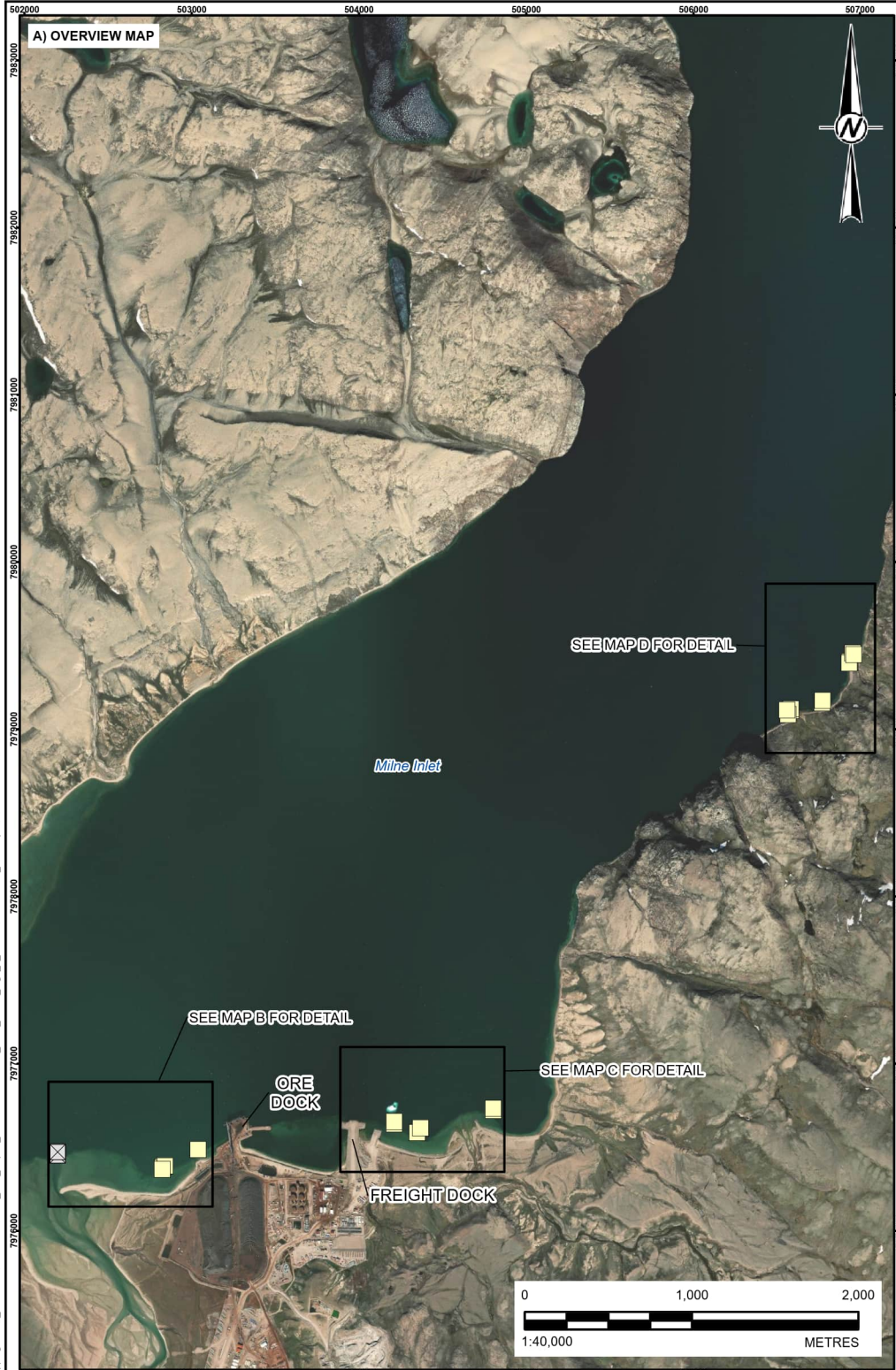
To assist in site identification in future years (e.g., in case quadrats are moved from the original coordinates), white hard plastic cards, approximately 5 cm x 5 cm, were attached to each quadrat in 2024 with their respective site identification (e.g., 'Q1') written on the card in permanent marker (Appendix 5B – Photo 3). The cards were attached on the settlement plate buoy line above the quadrats so as not to interfere with colonization within the quadrat.

### **5.2.3 Indicators**

Effect indicators selected to evaluate potential Project-induced changes in substrate, macroalgae and epifauna include taxa richness (number of unique taxa present), relative abundance, Simpson's Diversity Index (SDI), density (motile taxa) and percent cover (macroalgae and sessile invertebrates). These indicators are described in detail in Section 5.3.2.1. The indicators were calculated from data collected in both reference and exposure areas and analyzed statistically to evaluate Project-related effects within the study area.

Changes in field methodologies over time (as explained in Section 5.2.1), preclude the ability to make quantitative temporal comparisons to years prior to 2021. The 2021 quadrat survey results served as a benchmark for quantitative comparisons for future monitoring years so long as field methodologies remained consistent.





CLIENT  
BAFFINLAND IRON MINES CORPORATION

CONSULTANT



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| YYYY-MM-DD | 2025-04-09 |
| DESIGNED   | NO         |
| PREPARED   | AA         |
| REVIEWED   | NO         |
| APPROVED   | AL         |

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PROJECTED COORDINATE SYSTEM: NAD 1983 UTM ZONE 17N

PROJECT  
MARY RIVER PROJECT

**TITLE**  
**DEPLOYMENT LOCATIONS FOR THE PERMANENT QUADRATS FOR MONITORING SUBSTRATE, MACROFLORA AND BENTHIC EPIFAUNA IN MILNE PORT, 2024**

|                |         |      |        |
|----------------|---------|------|--------|
| PROJECT NO.    | CONTROL | REV. | FIGURE |
| CA0026317.6821 | 86000   | 0    | 5-2    |



5.3 Materials and Methods

5.3.1 Field Methodology

Twenty-six 1 m x 1 m square steel quadrats<sup>4</sup> were fabricated on site and lowered to the sea floor from a vessel: thirteen quadrats in each of the exposure area and reference area (Table 5-1; Figure 5-2). Ten quadrats (Q1 through Q10) were deployed in 2020, ten additional quadrats were deployed in 2021 (Q11 through Q20) generally co-located with the 2020 quadrats, and six additional quadrats were deployed in 2022 (Q21 through Q26). In each case, annual surveying commenced in the year of deployment and/or in the year of their redeployment or movement, as necessary (see Section 5.2.2). The 2020 quadrats were deployed from the field vessel at the locations of the previous belt transects, in water depths of approximately -4 to -18 m CD. Quadrats were marked over time using various methods with quadrat-specific identifying features to identify quadrat number to the divers<sup>5</sup> for aid in relocating them in subsequent surveys. With the exception of Q12, quadrats were deployed in pairs or clusters so that more than one quadrat could be surveyed on a single dive.

Field surveys of the quadrats were conducted between July 29 and August 11, 2024 by WSP’s occupational (SCUBA-based) dive team composed of marine biologists. The dive team is certified in accordance with Canadian Standard Association Z275:4-97, and WorkSafe BC Regulations Part 24. Dive surveys were conducted from Baffinland’s 9.75 m research vessel.

Field surveys included the following components:

- Subtidal dive quadrat surveys to quantitatively evaluate macroalgae, sessile and motile invertebrates and fish occurrence (commonly termed epifauna) within both the exposure area and reference area.
- Opportunistic observations<sup>6</sup> of macroalgae, fish and motile/sessile invertebrates during quadrat surveying.
- Opportunistic specimen collection of macroalgae, fish and motile/sessile invertebrates to enhance taxonomic resolution, particularly for taxonomic groups capable of hull fouling or ballast water transport.

Table 5-1: Quadrat Locations in Milne Port (2024)

| Area                  | Quadrat | UTM Coordinates (17W) |              | Depth (-m below CD) <sup>1</sup> | Deployment Date | Survey Date (2024) |
|-----------------------|---------|-----------------------|--------------|----------------------------------|-----------------|--------------------|
|                       |         | Easting (m)           | Northing (m) |                                  |                 |                    |
| Milne Port (Exposure) | Q1      | 502828                | 7976382      | 8.8                              | August 12, 2020 | July 31            |
|                       | Q2      | 502839                | 7976390      | 10.3                             | August 9, 2022  | July 31            |
|                       | Q3      | 504210                | 7976655      | 10.3                             | August 12, 2020 | August 11          |
|                       | Q4      | 504367                | 7976617      | 11.7                             | August 12, 2020 | August 11          |
|                       | Q5      | 504802                | 7976734      | 11.5                             | August 12, 2020 | August 6           |

<sup>4</sup> The quadrat frames are approximately 1 m x 1 m but the survey area excludes the metal sub-grid bars (see Section 5.3.1.1). 2020/2021-fabricated quadrats have a survey area 0.44 m<sup>2</sup>; the 2022-fabricated quadrats have a survey area 0.64 m<sup>2</sup>.

<sup>5</sup> Quadrats Q1 through Q10 have anchor chain suspending the settlement plates, while quadrats Q11 through Q20 have polyurethane line. Quadrats Q21 through Q26 do not have co-located settlement plates and have unique buoys associated with each quadrat. All quadrats were given attached permanent plastic cards in 2024, with the exception of Q21 and Q22.

<sup>6</sup> Opportunistic observations refer to observations that were recorded during diver-collected photo or video to document presence/absence in a qualitative manner rather than quantitatively assessed during the quadrat survey.

| Area           | Quadrat          | UTM Coordinates (17W) |              | Depth (-m below CD) <sup>1</sup> | Deployment Date | Survey Date (2024) |
|----------------|------------------|-----------------------|--------------|----------------------------------|-----------------|--------------------|
|                |                  | Easting (m)           | Northing (m) |                                  |                 |                    |
|                | Q11              | 502823                | 7976378      | 7.5                              | August 10, 2021 | July 31            |
|                | Q12              | 503036                | 7976488      | 11.4                             | August 6, 2022  | July 31            |
|                | Q13              | 504210                | 7976641      | 6.7                              | August 10, 2021 | August 11          |
|                | Q14              | 504346                | 7976589      | 7.7                              | August 6, 2021  | August 11          |
|                | Q15              | 504803                | 7976721      | 9.0                              | August 6, 2021  | August 6           |
|                | Q21*             | 502197*               | 7976453*     | 6*                               | August 6, 2022  | Not surveyed       |
|                | Q22*             | 502198*               | 7976473*     | 10*                              | August 6, 2022  | Not surveyed       |
|                | Q23              | 502822                | 7976368      | 4.3                              | August 6, 2022  | July 31            |
| Reference Area | Q6               | 506562                | 7979116      | 15.7                             | August 13, 2020 | August 8           |
|                | Q7               | 506776                | 7979173      | 9.1                              | August 13, 2020 | August 6           |
|                | Q8               | 506957                | 7979457      | 9.9                              | August 13, 2020 | July 29            |
|                | Q9               | 506962                | 7979448      | 8.3                              | August 11, 2021 | July 29            |
|                | Q10              | 506586                | 7979112      | 6.4                              | August 13, 2020 | August 6           |
|                | Q17              | 506773                | 7979159      | 8.6                              | August 11, 2021 | August 6           |
|                | Q18              | 506956                | 7979452      | 9.8                              | August 11, 2021 | July 29            |
|                | Q19              | 506953                | 7979447      | 9.6                              | August 11, 2021 | July 29            |
|                | Q20              | 506586                | 7979123      | 10.0                             | August 8, 2021  | August 6           |
|                | Q24              | 506940                | 7979399      | 5.2                              | August 7, 2022  | August 7           |
|                | Q25              | 506937                | 7979405      | 7.4                              | August 7, 2022  | August 7           |
|                | Q26              | 506935                | 7979397      | 6.4                              | August 7, 2022  | August 7           |
|                | Q27 <sup>2</sup> | 506569                | 7979090      | 5.0                              | August 6, 2024  | August 8           |

Note: "\*" indicates the quadrats were determined to be lost after multiple recognisance efforts and subsequently not surveyed in 2024. Depths and coordinates recorded above indicate the last observed locations and depth of the quadrats.

<sup>1</sup> Diver depth gauge was converted to meters chart datum (CD), estimated using tide table for Milne Inlet, Nunavut (<https://tides.gc.ca/en/stations/05791> [accessed September 2024]). The negative (-) numbers indicate 'below' CD.

<sup>2</sup> Q16 was repositioned to its approximate original depth (-5.0 m CD) after having been moved into deeper water (-9.1 m CD) in 2022 due to vessel anchor dragging the quadrat. This quadrat was removed from analysis/discontinued and renamed Q27 with a new time series.

### 5.3.1.1 *Quadrat Survey*

Biophysical data within each quadrat were recorded by one diver while another diver collected representative photographs<sup>7</sup> of the quadrat (Appendix 5B – Photo 1). Observations were made of the quadrat as a whole as opposed to surveying each sub-quadrat individually (as was done in 2021) to increase survey efficiency and remain standardized for multi-year comparisons. Due to the three-dimensional space created by the quadrat steel frame, the deployment chain/line, and the vertical settlement plate line, and to remain consistent with previous years, the survey quantification excluded the following areas, unless organisms moved (wholly or partially) within the quadrat during the survey:

- Outside of the quadrat steel frame.
- Within the frame structure (i.e., outside hollow frame) of quadrat.
- On top of the metal crossbars inside the main frame or on top of the outer frame.
- Attached to the quadrat deployment chain/line above the height of the quadrat frame (attachment to the chain or line strung through the quadrat was included).

Quantitative data were collected in general accordance with Fisheries and Oceans Canada's (DFO) Marine Foreshore Environmental Assessment Procedure (MFEAP; Appendix 5A). Quadrat data were recorded on project-specific datasheets, and included the following information (Appendix 5B – Photo 1):

- Substrate type, visually estimated according to the following size ranges derived from Wentworth (1922) and recorded as relative composition (i.e., as a percent areal coverage): bedrock; boulder (>256 mm diameter); cobble (64 to 256 mm); gravel (2 to 64 mm); and soft substrate<sup>8</sup> (<2 mm).
- Total percent cover of detritus and debris, calculated separately from substrate composition as it overlaid the existing substrate. Detritus and debris were categorized into three groups: detrital veneer, detrital macroalgae, and other debris (i.e., metal). Detrital veneer is organic material and appears to consist of phytoplankton/diatoms usually with silt.
- Macroalgae, visually estimated to total areal coverage and identified to the lowest practical level (LPL).
- Sessile invertebrates (e.g., clams and mussels), visually estimated to total areal coverage and identified to LPL (as above).
- Motile invertebrates (e.g., urchins, limpets) and fish, identified to LPL and enumerated<sup>9</sup>.

Photographs showing representative biological features and aiding in species identification were also taken.

<sup>7</sup> Underwater imagery collected using a SONY RX100 V camera in Fantasea underwater housing and video light for all underwater surveys. The camera has high-definition video capability and still photography features.

<sup>8</sup> Sand (0.0625 to 2 mm) and silt/mud/clay (<0.0625 mm) were estimated as a single category of soft sediment starting in 2023 due to constraints in the field determining exact grain size through dive gloves and misalignment of Chapter 5.0 and Chapter 3.0 (Sediment Quality) results in previous years. Data from 2022 and 2021 were adjusted to follow this change.

<sup>9</sup> Abundance was estimated if relatively large numbers (typically, numbers exceeding 100, depending on size and behaviour of organisms) of motile species were present.

### 5.3.1.2 *Opportunistic Specimen Collection*

Opportunistic samples of epifauna and macroalgae were collected to improve species identifications, which is particularly important if this sampling method is to effectively detect species that might be non-indigenous to the area. Specimens were collected using the following protocol:

- Divers collected specimens in sealed plastic bags or small vials and brought these to the surface in a mesh bag.
- Discretion was used to sample one to two representative individual(s) to avoid over-harvesting from the quadrats which could have future implications on the community assemblage (experimental design interaction) and/or to collect samples from just outside the quadrat.
- The majority of macroalgae samples were processed by a master's student from Dr. Gary Saunder's laboratory at the University of New Brunswick (UNB), who was on site for the majority of surveys. Samples were separated and laid out within a metal dish with a thin layer of water for visual identification. Pieces of samples were then dried with Kimwipes and placed into 1.25 mL centrifuge tubes filled with silica gel to preserve specimens for DNA analysis. Samples were sent to UNB for further analysis.
- Macroalgae samples collected when the master's student was not on site were preserved in either in the same manner as above or placed in placed into appropriately sized containers (e.g., 1 L jar) and preserved with 80% ethanol.
- Epifauna samples were placed into appropriately sized containers (e.g., 50 mL vials, 120 mL clear glass jars) and preserved either in a 10% buffered formalin solution or 80% ethanol, both solutions confirmed to be appropriate for initial preservation of specimens for taxonomic analysis and/or DNA analysis by the Canadian Centre for DNA Barcoding. The jars were then sealed and inverted several times to promote homogenization and saturation with the preservative. Jars were labelled internally and externally with water-resistant labels.
- Samples were sent to Biologica Environmental Services Ltd. (Biologica) for taxonomic identifications.

### 5.3.2 *Data Analysis*

Diver-collected quadrat data were entered into an electronic database by one biologist and verified by a second biologist to reduce transcription errors. Some taxa observed were neither epifauna nor benthic, such as pelagic taxa (mysid shrimp, sea angels), and hence considered "incidental" and not included in data analysis. Field-based identifications were updated where lab identifications of opportunistically sampled specimens resulted in improved taxonomic resolution. Further, identifications were verified and updated, as necessary, with photographs taken from the field.

Data analysis was based on separating biota by three assemblages, defined as taxa that share an attribute of habitat or taxonomic similarity, and representing subsets of a biological community: macroalgae, sessile epifauna, and motile epifauna. Results are therefore presented by these three assemblages in Section 5.4.

Statistical analysis was based on four indicators: taxa richness (to the lowest practicable level), Simpson's Diversity Index (SDI), organism density (motile epifauna) and percent cover (macroalgae and sessile epifauna). In addition, assemblages were explored using relative abundance, by grouping related taxa into broader taxonomic classifications (e.g., grouping all fish taxa into the broader classification 'fish') to evaluate how proportions of these



major classifications might differ between exposure and reference area. Quantitative statistical analyses were performed comparing results from 2021 through 2024. Due to inconsistent sampling methodologies in previous survey years (see Section 5.2.1), a quantitative statistical analysis was not possible for survey years before 2021.

5.3.2.1 Community Indicators

Taxa Richness

Richness is defined as the total number of unique<sup>10</sup> taxa per quadrat. This metric provides an indication of the diversity (number of different species) in the local ecological community. A higher richness value typically indicates a healthier and more balanced community. Mean taxa richness and standard error (SE) of the mean was calculated based on number of taxa by area (exposure, reference).

Simpson's Diversity Index

Simpson's Diversity Index (SDI) measures the proportional distribution of organisms in the community. The SDI considers the abundance patterns and taxonomic richness of the community. Certain conditions may favour one taxon over another, resulting in the community being dominated by a few taxa, which is reflected in decreased diversity (Simpson 1949). The SDI values range between zero and one, where lower values indicate a less diverse community, and higher values indicate a more diverse community. The SDI was calculated using the following formula provided by Krebs (1999):

$$SDI = 1 - \sum_{i=1}^S (p_i)^2$$

Where:

- S = the total number of taxa
- p<sub>i</sub> = the proportion of the i<sup>th</sup> taxon (of each unique taxon out of the total abundance of the sample)

For categorization, SDI values were considered to range from very low diversity to high diversity, as described in Table 5-2. Mean SDI and SE of the mean were calculated for each exposure and reference areas.

Table 5-2: Diversity Categories for Simpson's Diversity Index (SDI) Values

| SDI Value           | Diversity Category |
|---------------------|--------------------|
| <0.250              | Very Low           |
| 0.250 through 0.499 | Low                |
| 0.500 through 0.750 | Moderate           |
| >0.750              | High               |

<sup>10</sup> Did not include higher order taxa for which there exists a lower order identification. For example, does not include *Mya* sp. if *Mya truncata* is found in the same quadrat.

## Organism Density

For motile invertebrates and fish (collectively motile epifauna), density was standardized to organisms (org)/m<sup>2</sup> using a correction factor for quadrat size to maintain comparability. Newly fabricated quadrats in 2022 were larger than those fabricated in 2020 and 2021. The survey area (excluding the metal sub-grid bars; Figure 5-1B) of the 2020/2021 quadrats was 0.44 m<sup>2</sup> while the 2022-fabricated quadrats had a survey area of 0.64 m<sup>2</sup>. Therefore, correction factors have been applied to quadrats during data analysis to standardize results to 1 m<sup>2</sup>. Mean density (org/m<sup>2</sup>) and SE of the mean were calculated separately for exposure and reference areas.

## Percent Cover

For macroalgae and sessile epifauna, mean percent areal cover (total cover) and SE of the mean was calculated separately by area (exposure, reference). Relative abundance was calculated as percent cover standardized out of 100% for substrate, macroalgae, sessile and motile epifauna.

## Relative Abundance

Relative abundance is the proportion that a taxa or grouping contributes to the total abundance (standardized as a percentage). Dominant taxa (e.g., wrinkled rock borers [*Hiatella arctica*], brittle stars [Family Ophiuroidea]) were kept at the lowest practical level while less commonly observed and/or easily grouped taxa were rolled into broader taxonomic classifications (e.g., grouping all fish taxa into the broader classification 'fish').

### 5.3.2.2 Statistical Analysis

#### Generalized Linear Mixed Models (GLMMs)

Differences in substrate, detritus and debris, macroalgae, and benthic epifauna indicators between the exposure and reference areas and between the survey years 2021-2024 were analyzed using Generalized Linear Mixed Models (GLMMs). These models were chosen due to their ability to account for both fixed and random effects, making them suitable for repeated measure studies where quadrats are resampled across time. The fixed effects in our models included the categorical explanatory variables of years, area (exposure and reference area), and substrate type, and the continuous explanatory variable of depth; the random effect was quadrat. The models incorporated an interaction term (Year\*Area) to assess whether area effects varied across different years. Model selection for finding the best fitting model was performed using Akaike's Information Criterion (AIC) weights. The three models compared included the following explanatory variables<sup>11</sup>:

- 1)  $y \sim \text{Year} * \text{Area} + (1 \mid \text{Quadrat})$
- 2)  $y \sim \text{Year} * \text{Area} + \text{ns}(\text{Substrate}) + (1 \mid \text{Quadrat})$
- 3)  $y \sim \text{Year} * \text{Area} + \text{ns}(\text{Depth}) + (1 \mid \text{Quadrat})$

For count and density data, Poisson or negative binomial distributions were used, while beta regression was employed for percent and substrate data. To ensure the absence of collinearity among the explanatory variables, Variance Inflation Factor (VIF) values were calculated, with values below three indicating no collinearity, as per the guidelines of Zuur et al. (2007).

<sup>11</sup> ns = natural splines

The models were further checked for overdispersion and outliers. Pairwise comparisons were conducted (1) between years within quadrats and (2) between areas within a year, to describe effect sizes. After fitting the GLMM, estimated marginal means were calculated to perform pairwise comparisons to compare areas within each year and across all years.

A p-value <0.05 is considered to indicate significance between groups. The statistical analyses were conducted using R Studio (R version 4.3.2; R Core Team 2025) with the packages glmmTMB (Version: 1.1.8; Brooks et al. 2017), DHARMA (Version: 0.4.6; Hartig 2022), and emmeans (Version 1.10.0; Lenth 2020).

### **Taxa Accumulation**

A taxa accumulation curve was calculated for quadrats surveyed in each year from 2021 through 2024 to provide an estimate of the effort required to characterize the epibenthic community assemblage. A taxa accumulation curve illustrates how the number of unique taxa (or species) increases as the number of samples are accumulated; in other words, the harder one looks (i.e., the higher the sampling effort), the more unique taxa are found. The curve reaches an asymptote when all taxa within the given community assemblage have been sampled and the community assemblage is assumed to have been fully described. The observed species (or taxa) curve is plotted and the sample (i.e., quadrat) order is randomized and permuted 999 times, resulting in an averaged curve describing a smooth relationship of the average number of species (or taxa) for each number of replicates and the standard deviation of the mean (i.e., permutations). This is equivalent to station-based rarefaction curves. Analysis was conducted using R Studio (R version 4.3.2; R Core Team 2025).

### **Power Analysis**

A power analysis was performed using the 2021 through 2024 data using simulation methods. A detailed methodology is provided in Appendix 5E.

## **5.3.3 Quality Management**

Quality assurance and quality control (QA/QC) procedures were applied to the field collection, data analysis, and reporting tasks within the chapter component to verify that the data presented were valid and of acceptable quality to address objectives stated in Section 5.1.1.

### **Field QA/QC**

QA/QC measures for quantitative and qualitative data collected during quadrat surveys included the following:

- Field survey data sheets were checked and cross-validated in the field.
- Taxonomic identifications, including common and species name, were verified using references<sup>12</sup>.
- Dive survey video, photographs and datasheets were saved to a laptop computer and external hard drive at the end of each field day. Once in the office, the survey data were uploaded to an internal SharePoint site.

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<sup>12</sup> References used during the surveys included but were not limited to: Coad and Reist 2018; Golder 2021, 2022; Küpper et al. 2016; Guiry and Guiry 2025; Saunders 2023; WoRMS 2025; WSP 2023, 2024.

## Laboratory and Data Analysis QA/QC

The following QA/QC measures were implemented:

- Taxa common name/species name and recorded observations were verified using references.
- Transcribed diver-collected data was reviewed for transcription errors by a second biologist.
- Calculations were verified by a second biologist for errors as part of the data review process.

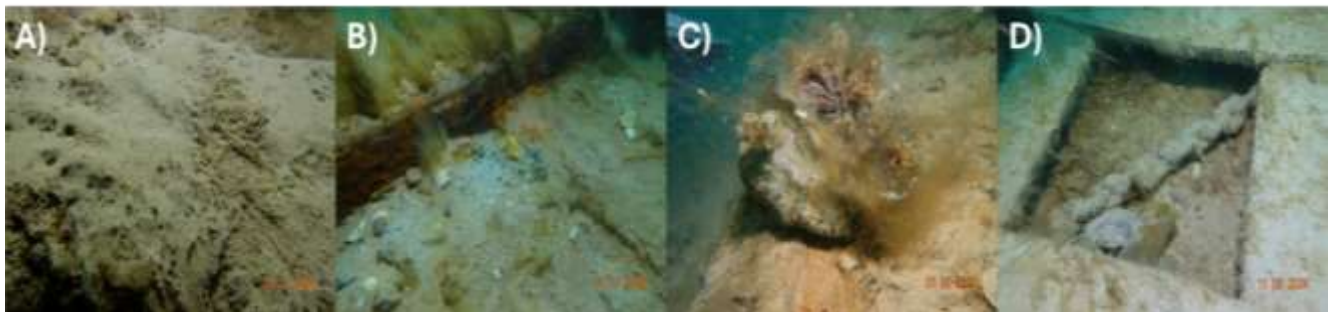
## 5.4 Results

This section presents results from the 2024 quadrat sampling program at Milne Port, with a quantitative comparison to 2021, 2022 and 2023 data. Representative photographs are provided in Appendix 5B. Quadrat/transect data in tabulated form are presented in Appendix 5C for 2021 through 2024. Results of statistical analysis of substrate macroalgae, and benthic epifauna endpoints are provided in Table 5-3, with post hoc pairwise comparison results provided in Table 5-4. A taxa list with common and scientific names is provided in Appendix 5D. Taxonomic results in relation to the objective of monitoring for non-indigenous species are presented in Chapter 8.0 (NIS/AIS). Power analysis methods and results are provided in Appendix 5E.

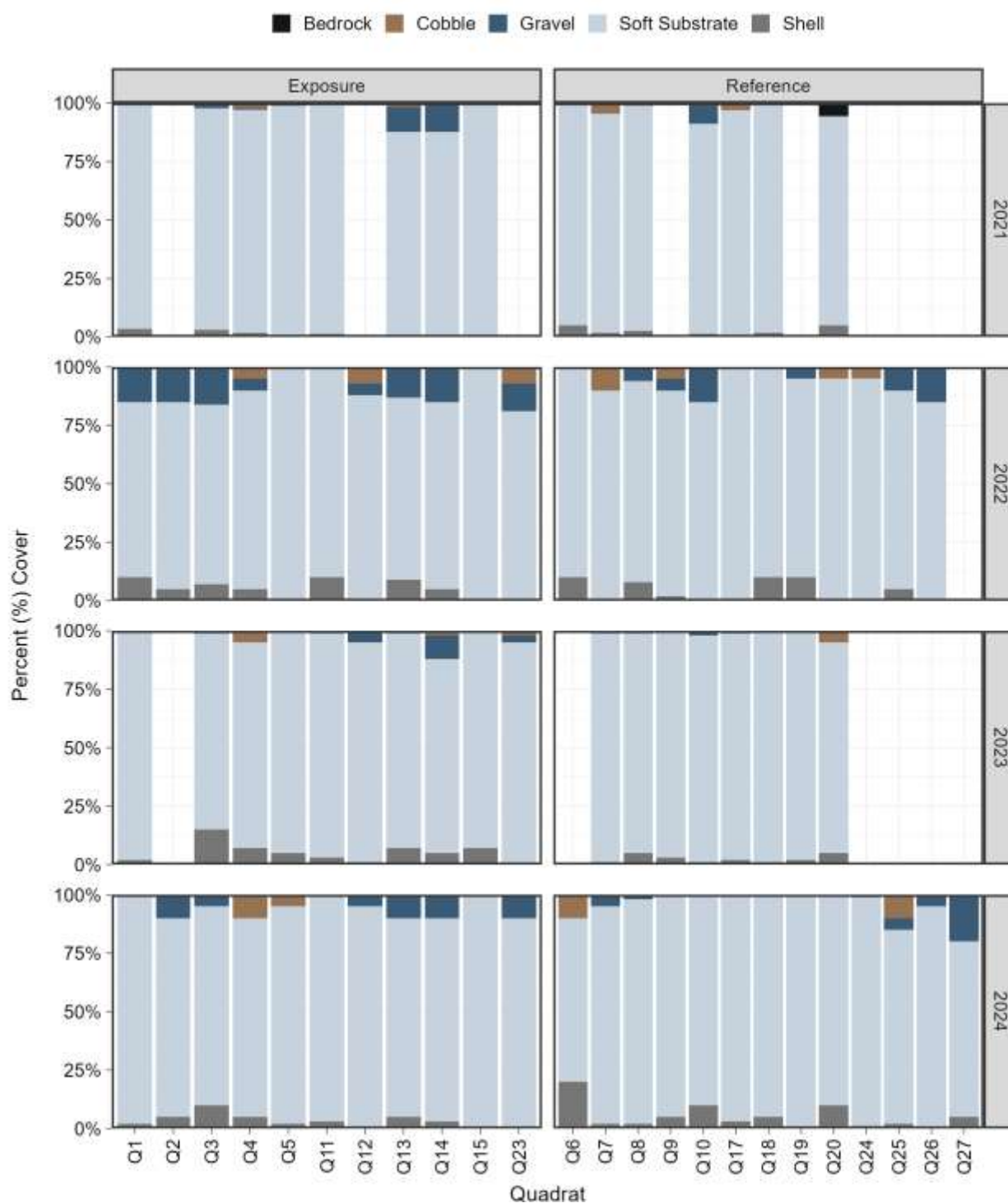
### 5.4.1 Substrate

#### Substrate Composition

Substrate, as visually estimated by the divers using a modified Wentworth scale (Wentworth, 1922), was composed predominantly of soft substrate (silt and sand; Figure 5-3) for quadrats in both the exposure and reference areas, as observed in previous years (Figure 5-4). Quadrats Q3 and Q13 within the exposure area contained mainly sand (65 – 70%) while quadrats Q4, Q14, and Q23 in the exposure area and quadrats Q24, Q25, and Q26 in the reference area contained nearly equal amounts of sand and silt. Silt was more dominant in the other quadrats within both areas (Appendix 5C).



**Figure 5-3: Substrate Types Observed in Survey Quadrats at Milne Port, Showing Areas Dominated By: A) Soft Substrate (Sand/Silt); B) Gravel; C) Cobble; and D) Anthropogenic Debris (e.g., chain and rust).**



**Figure 5-4: Relative Abundance of Substrate Types from Quadrats Surveyed during Quadrat Surveys in Milne Port, 2021 – 2024.**

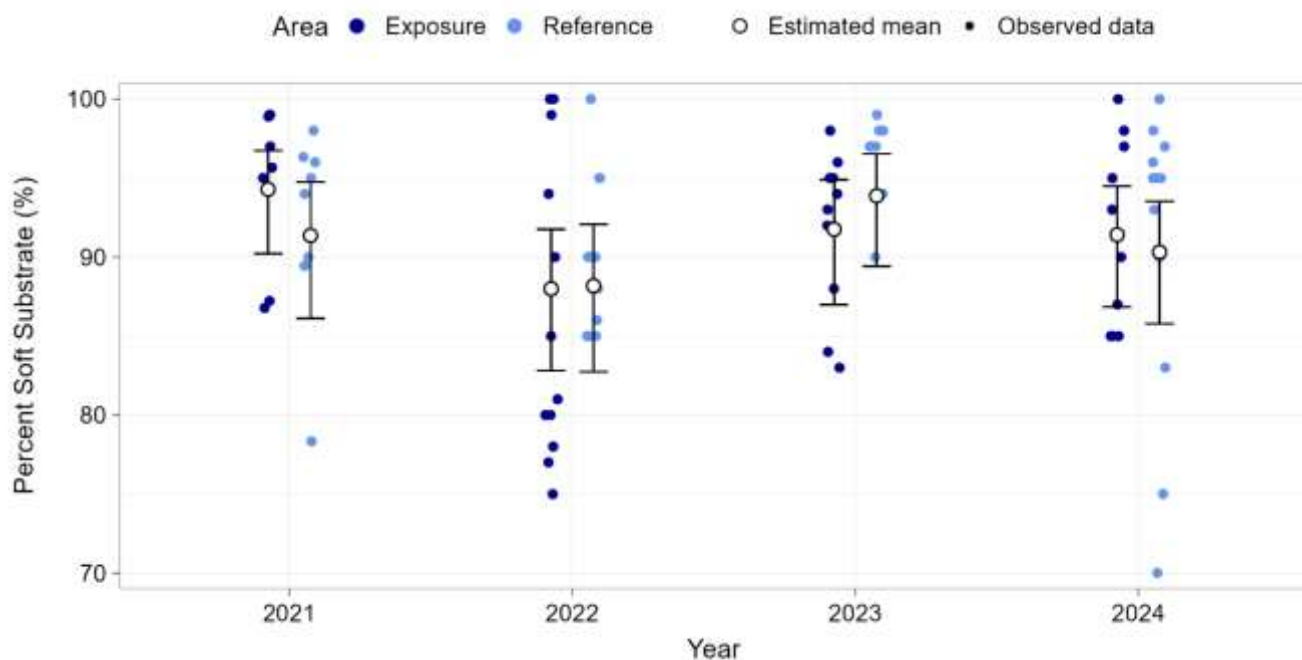
Note: Only quadrats sampled in 2024 are represented throughout the years.

Other substrate types present in small proportions within each area in 2024 included gravel (0 to 20%), cobble (0 to 10%), and shell (0 to 20%) (Figure 5-3B and C; Figure 5-4). Consistent with 2022 and 2023 results, bedrock was not observed within the quadrats in 2024.

Soft substrate cover varied significantly among sampling years ( $P=0.011$ ), while neither the interaction nor the main effect of area was significant ( $P>0.3$  for both; Table 5-3). That is, soft substrate was not found to differ between areas, and interannual differences in soft substrate cover did not differ between the reference and exposure areas (Figure 5-5; Table 5-3).

In pairwise comparisons between years within each area, effect sizes ranged from -51% to +125%; the only significant difference was between 2021 and 2022 in the exposure area, where soft substrate cover in 2021 was 125% higher than in 2022 ( $P=0.022$ ; Table 5-4). In 2024, soft substrate cover was 14% higher in the exposure area compared to the reference area but the difference was not significant ( $P=0.7$ ; Table 5-4).

In the analysis of soft substrate, statistical power was sufficient to detect a significant year and area interaction at effect sizes of -50% and +120% (Appendix 5E). That is, when the 2024 exposure area had mean soft substrate content that was 50% lower or 120% higher than the 2024 reference area, the model could detect a significant interaction between year and area. There was not sufficient power to detect the interaction given the 2024 effect size. The model had higher power to detect decreases than increases in soft substrate, due to the high values of soft substrate observed and the bounding of the variable by 0% and 100%.



**Figure 5-5: Interannual Differences in Soft Substrate Percent Cover in Milne Port, 2021 – 2024.**

Note: Soft substrate includes sand and silt. Observed (solid points) and estimated marginal mean percent cover (open points) and 95% confidence interval (error bars) are presented for each sampling area and year.

**Table 5-3: Results of Generalized Linear Mixed Models for Substrate, Macroalgae, and Benthic Epifauna in Milne Port, 2021 – 2024**

| Indicator                                | Variable           | Chi square | DF | P-value          |
|--|--------------------|------------|----|------------------|
| <b>Substrate Composition</b>             |                    |            |    |                  |
| Percent soft substrate                   | Year               | 11.13      | 3  | <b>0.011</b>     |
|  | Area               | 0.05       | 1  | 0.819            |
|  | Year x Area        | 3.16       | 3  | 0.368            |
| <b>Macroalgae</b>                        |                    |            |    |                  |
| Percent Cover                            | Year               | 33.29      | 3  | <b>&lt;0.001</b> |
|  | Area               | 2.20       | 1  | 0.138            |
|  | Year x Area        | 7.53       | 3  | 0.057            |
| Taxa Richness                            | Year               | 32.24      | 3  | <b>&lt;0.001</b> |
|  | Area               | 1.82       | 1  | 0.177            |
|  | Year x Area        | 1.38       | 3  | 0.710            |
| Simpson's Diversity Index <sup>(b)</sup> | Year               | 28.66      | 3  | <b>&lt;0.001</b> |
|  | Area               | 0.28       | 1  | 0.599            |
|  | Year x Area        | 7.43       | 3  | 0.059            |
| <b>Sessile Epifauna</b>                  |                    |            |    |                  |
| Percent Cover <sup>(b)</sup>             | Year               | 9.31       | 3  | <b>0.025</b>     |
|  | Area               | 1.01       | 1  | 0.315            |
|  | Depth <sup>a</sup> | 5.71       | 2  | 0.057            |
|  | Year x Area        | 5.99       | 3  | 0.112            |
| Taxa Richness                            | Year               | 15.83      | 3  | <b>0.001</b>     |
|  | Area               | 3.15       | 1  | 0.076            |
|  | Depth <sup>a</sup> | 12.53      | 2  | <b>0.002</b>     |
|  | Year x Area        | 0.10       | 3  | 0.991            |
| Simpson's Diversity Index <sup>(b)</sup> | Year               | 15.26      | 3  | <b>0.002</b>     |
|  | Area               | 1.19       | 1  | 0.274            |
|  | Soft Substrate     | 5.76       | 1  | <b>0.016</b>     |
|  | Year x Area        | 1.67       | 3  | 0.644            |
| <b>Motile Epifauna</b>                   |                    |            |    |                  |
| Density <sup>(b)</sup>                   | Year               | 22.09      | 3  | <b>&lt;0.001</b> |
|  | Area               | 0.06       | 1  | 0.804            |
|  | Depth <sup>a</sup> | 46.68      | 2  | <b>&lt;0.001</b> |
|  | Year x Area        | 18.33      | 3  | <b>&lt;0.001</b> |



| Indicator                 | Variable    | Chi square | DF | P-value          |
|---------------------------|-------------|------------|----|------------------|
| Taxa Richness             | Year        | 5.82       | 3  | 0.121            |
|                           | Area        | 0.00       | 1  | 0.993            |
|                           | Depth       | 28.49      | 1  | <b>&lt;0.001</b> |
|                           | Year × Area | 5.10       | 3  | 0.164            |
| Simpson's Diversity Index | Year        | 2.10       | 3  | 0.552            |
|                           | Area        | 0.04       | 1  | 0.849            |
|                           | Depth       | 3.13       | 1  | 0.077            |
|                           | Year × Area | 2.71       | 3  | 0.439            |

Note: Cells highlighted in **blue** indicate statistically significant results ( $P$ -value  $<0.05$ ); where the interaction between year and area is significant, the significance of the main effects is not interpretable and main effect cells are not highlighted. DF = degrees of freedom.

(a) Indicates a variable that was modelled using natural splines in order to account for non-linearity.

(b) Datapoints were excluded from the model due to outliers or influence.

**Table 5-4: Post hoc Comparisons of Estimated Marginal Means and Effect Sizes for Generalized Linear Mixed Models for Substrate, Macroalgae, and Benthic Epifauna in Exposure and Reference Areas of Milne Port, 2021 – 2024**

| Indicator <sup>1</sup>  |                      | Post hoc Comparison <sup>2</sup> |                         |                         |                     |                         |              |
|-------------------------|----------------------|----------------------------------|-------------------------|-------------------------|---------------------|-------------------------|--------------|
|                         |                      | 2021-2022                        | 2021-2023               | 2021-2024               | 2022-2023           | 2022-2024               | 2023-2024    |
| <b>Exposure Area</b>    |                      |                                  |                         |                         |                     |                         |              |
| <b>Soft Substrate</b>   | <b>% Cover</b>       | <b>0.022 (125%)</b>              | 0.548 (48%)             | 0.427 (55%)             | 0.331 (-34%)        | 0.367 (-31%)            | 0.998 (5%)   |
| <b>Macroalgae</b>       | <b>% Cover</b>       | 0.479 (-41%)                     | 0.508 (-40%)            | <b>0.009 (-69%)</b>     | 1.000 (3%)          | 0.204 (-47%)            | 0.180 (-48%) |
|                         | <b>Taxa Richness</b> | 0.707 (36%)                      | 0.703 (-25%)            | 0.348 (-34%)            | 0.085 (-44%)        | <b>0.012 (-51%)</b>     | 0.920 (-13%) |
|                         | <b>SDI</b>           | 0.952 (-15%)                     | 0.328 (-42%)            | 0.195 (-47%)            | 0.571 (-31%)        | 0.374 (-38%)            | 0.989 (-9%)  |
| <b>Sessile Epifauna</b> | <b>% Cover</b>       | 0.987 (10%)                      | 0.859 (-21%)            | 0.477 (57%)             | 0.619 (-28%)        | 0.607 (43%)             | 0.102 (98%)  |
|                         | <b>Taxa Richness</b> | 0.050 (77%)                      | 0.990 (7%)              | 0.976 (9%)              | 0.097 (-40%)        | 0.101 (-39%)            | 1.000 (2%)   |
|                         | <b>SDI</b>           | 0.834 (38%)                      | 0.895 (-24%)            | 0.155 (-57%)            | 0.302 (-45%)        | <b>0.005 (-69%)</b>     | 0.388 (-44%) |
| <b>Motile Epifauna</b>  | <b>Density</b>       | 0.140 (54%)                      | <b>0.022 (89%)</b>      | <b>0.001 (149%)</b>     | 0.805 (23%)         | 0.211 (62%)             | 0.729 (32%)  |
|                         | <b>Taxa Richness</b> | 0.978 (14%)                      | 0.750 (-28%)            | 0.773 (43%)             | 0.380 (-37%)        | 0.910 (25%)             | 0.174 (98%)  |
|                         | <b>SDI</b>           | 0.825 (69%)                      | 0.941 (-31%)            | 0.890 (60%)             | 0.308 (-59%)        | 1.000 (-5%)             | 0.451 (132%) |
| <b>Reference Area</b>   |                      |                                  |                         |                         |                     |                         |              |
| <b>Soft Substrate</b>   | <b>% Cover</b>       | 0.586 (42%)                      | 0.691 (-31%)            | 0.971 (13%)             | 0.087 (-51%)        | 0.767 (-20%)            | 0.355 (64%)  |
| <b>Macroalgae</b>       | <b>% Cover</b>       | <b>0.003 (-78%)</b>              | <b>&lt;0.001 (-87%)</b> | <b>&lt;0.001 (-91%)</b> | 0.343 (-42%)        | <b>0.013 (-59%)</b>     | 0.714 (-29%) |
|                         | <b>Taxa Richness</b> | 0.110 (88%)                      | 0.771 (-21%)            | 0.126 (-38%)            | <b>0.006 (-58%)</b> | <b>&lt;0.001 (-67%)</b> | 0.628 (-22%) |
|                         | <b>SDI</b>           | 0.150 (98%)                      | 0.771 (-28%)            | 0.061 (-56%)            | <b>0.012 (-64%)</b> | <b>&lt;0.001 (-78%)</b> | 0.455 (-39%) |
| <b>Sessile Epifauna</b> | <b>% Cover</b>       | 0.278 (-44%)                     | <b>0.008 (-64%)</b>     | 0.212 (-45%)            | 0.441 (-35%)        | 1.000 (-1%)             | 0.379 (53%)  |
|                         | <b>Taxa Richness</b> | <b>0.040 (74%)</b>               | 0.933 (13%)             | 0.883 (15%)             | 0.189 (-35%)        | 0.166 (-34%)            | 1.000 (1%)   |
|                         | <b>SDI</b>           | 1.000 (-3%)                      | 0.456 (-44%)            | 0.282 (-48%)            | 0.464 (-42%)        | 0.197 (-46%)            | 0.997 (-7%)  |
| <b>Motile Epifauna</b>  | <b>Density</b>       | <b>&lt;0.001 (383%)</b>          | 0.571 (44%)             | 0.172 (78%)             | <b>0.002 (-70%)</b> | <b>0.014 (-63%)</b>     | 0.886 (24%)  |
|                         | <b>Taxa Richness</b> | 0.105 (107%)                     | 0.961 (18%)             | 0.997 (6%)              | 0.402 (-43%)        | 0.134 (-49%)            | 0.986 (-10%) |
|                         | <b>SDI</b>           | 0.831 (67%)                      | 0.804 (69%)             | 0.995 (15%)             | 1.000 (2%)          | 0.913 (-31%)            | 0.885 (-32%) |

| Indicator <sup>1</sup>          |               | Post hoc Comparison <sup>2</sup> |           |   |           |           |
|---------------------------------|---------------|----------------------------------|-----------|---|-----------|-----------|
|                                 |               | 2021-2022                        | 2021-2023 | 2021-2024   | 2022-2023 | 2022-2024 |
| Within 2024: Exposure-Reference |               |                                  |           |   |           |           |
| Soft Substrate                  | % Cover       | 0.689 (14%)                      |           | Note: Cells highlighted in <b>blue</b> indicate statistically significant results ( <i>P</i> -value <0.05). Pairwise comparisons were conducted for (1) between years within areas and (2) between areas within a year (2024), to describe effect sizes.<br><br><sup>1</sup> Soft substrate refers to soft substrate composition.<br><br><sup>2</sup> Values are shown as: <i>P</i> -value (Effect Size, %). SDI = Simpson's Diversity Index; % = percent. Effect sizes, e.g., for Year1-Year2, were calculated as follows: ([Year1 / Year2] – 1)×100% using marginal means on the response scale for richness and motile epifauna counts, and on the odds scale for percent cover and SDI. |           |           |
| Macroalgae                      | % Cover       | 0.090 (-57%)                     |           |   |           |           |
|                                 | Taxa Richness | 0.145 (-24%)                     |           |   |           |           |
|                                 | SDI           | 0.313 (-29%)                     |           |   |           |           |
| Sessile Epifauna                | % Cover       | 0.085 (-48%)                     |           |   |           |           |
|                                 | Taxa Richness | 0.410 (-14%)                     |           |   |           |           |
|                                 | SDI           | 0.995 (0%)                       |           |   |           |           |
| Motile Epifauna                 | Density       | 0.281 (-32%)                     |           |   |           |           |
|                                 | Taxa Richness | 0.118 (-40%)                     |           |   |           |           |
|                                 | SDI           | 0.446 (-34%)                     |           |   |           |           |

Note: Cells highlighted in **blue** indicate statistically significant results ( $P$ -value  $< 0.05$ ). Pairwise comparisons were conducted for (1) between years within areas and (2) between areas within a year (2024), to describe effect sizes.

<sup>1</sup> Soft substrate refers to soft substrate composition.

<sup>2</sup> Values are shown as:  $P$ -value (Effect Size, %). SDI = Simpson's Diversity Index; % = percent. Effect sizes, e.g., for Year1-Year2, were calculated as follows:  $([Year1 / Year2] - 1) \times 100\%$  using marginal means on the response scale for richness and motile epifauna counts, and on the odds scale for percent cover and SDI.

### **Detritus and Debris**

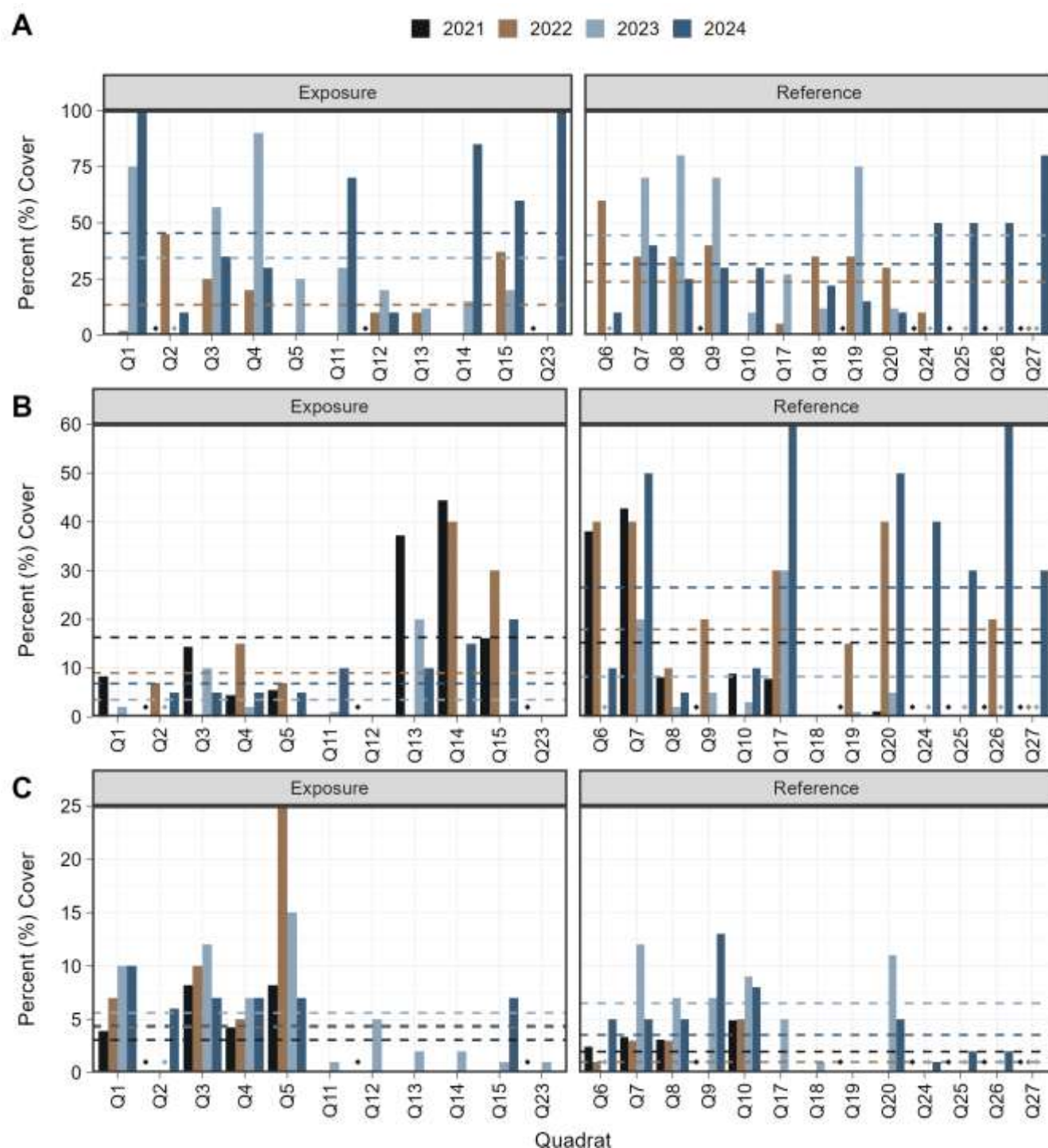
A detrital veneer, consisting of plankton with silt and/or diatoms, was present at most quadrats in 2024, consistent with previous years' results (Figure 5-6A). The percent cover range was similar between areas but slightly wider within the exposure area, similar to 2023 (exposure area: 0 to 100% vs. reference area: 0 to 80%). Notably, Q23 appeared to have high cover of detrital veneer with a large silt component over most of the quadrat, which may have impeded colonization of other epiflora and fauna (Appendix 5D – Photo 4). Overall, the percent detrital veneer in 2024 was similar to 2023, with both years being increased from 2022 and 2021 surveys.

Detrital (or drift) macroalgae was present in three-quarters of the quadrats (Figure 5-6B), with the reference area having a higher average percent cover of detrital algae compared to the exposure area (consistent with previous years' results). Moreover, quadrats generally had higher detrital macroalgae cover in 2024 compared to previous years within the reference area. Highest percent cover was recorded in Q26, Q7, and Q24 (with 60%, 50%, and 40%, respectively). Overall, the percent detrital algae increased in 2024 compared to the previous years.

Other debris consisted of metal pieces (Figure 5-3D) from the deployment chain strung through the quadrat to maintain the NIS/AIS monitoring settlement plate (Chapter 8.0) above the 2020-deployed quadrats (Q1 through Q10)<sup>13</sup>, rust pieces from the quadrat's steel frame, aluminum piping from the belt transects observed in Q5 that has been observed every survey year since 2020, and a fish skeleton noted in Q9 in 2024 (Figure 5-6C). Overall, the percent of debris in 2024 was comparable to previous years.

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<sup>13</sup> Quadrats deployed in later years did not use chain as part of the experimental design.



**Figure 5-6: A) Total Percent Cover of Detrital Veneer, B) Detrital Macroalgae, and C) Other Anthropogenic Debris from Quadrats Surveyed during Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed lines represent the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.

## 5.4.2 Macroalgae

Macroalgae identified in quadrats belonged to three larger taxonomic groups: Ochrophyta (brown algae), Rhodophyta (red algae), and Chlorophyceae (green algae) (Appendix 5C). There were no taxa new to the survey collected within quadrats in 2024.

Brown algae were resolved to eight distinct taxa in 2024, five of which were defined to species level: sugar kelp (*Saccharina latissima*), rockweed (*Fucus distichus*), brown filamentous algae *Halosiphon tomentosus*, brown branched algae *Dictyosiphon foeniculaceus*, and brown branched algae *Stictyosiphon tortilis* (Appendix 5B – Photos 5 to 6). The other three brown algae were identified to genus level - *Pylaiella* sp., acid weed (*Desmarestia* sp.), and brown branched algae *Battersia* sp. (Appendix 5B – Photos 7 to 8). Red algae observed within the quadrats that could be identified to the species level were *Coccotylus truncatus*, *Savoiea arctica*, and *Phycodrys fimbriata* (Appendix 5B – Photos 6, 9, and 10). Other red algae observed included encrusting coralline algae (Order Corallinales) and red filamentous algae *Polysiphonia* sp. (Appendix 5C). Green algae comprised two species, *Chaetomorpha melagonium* and *Spongomorpha aeruginosa* (Appendix 5B – Photos 11 and 12), while others were unidentified filamentous green algae. Three macroalgae taxa observed in previous years (2021 to 2023) were not observed in 2024, including sieve kelp (*Agarum clathratum*), brown filamentous algae *Coelocladia arctica*, and red foliose *Dilsea socialis* (Appendix 5C; Golder 2022; WSP 2023, 2024).

### Percent Cover

Macroalgae percent cover varied greatly among quadrats in both the exposure and reference areas (Figure 5-7). The exposure area percent cover ranged from 2 to 75% in individual quadrats while the reference area ranged from 20 to over 100% (when accounting for epiphytic algae [corrected to 100% for analyses]). Q15 in the exposure area had the highest percent cover (75%), followed by Q11 (58%) while the other quadrats in the exposure area were all below 55%. Half of the quadrats surveyed within the reference area in 2024 contained coverage greater than 50%, resulting in a higher average percent cover compared to the exposure area. Percent cover in the exposure area in 2024 was nearly double that of any previous year (2024 = 40.1%, 2023 = 23.8%; 2022 = 23.9%; 2021 = 17.8%) and percent cover in the reference area showed a trend of increasing cover from 18.2% in 2021 to 55.0% in 2024 (Table 5-5).

Quadrat Q12 had the lowest macroalgae cover (2%) of quadrats surveyed. During the survey, the quadrat appeared to be partially buried in the soft substrate, with a portion of the quadrat frame and bars not visible at the surface (Appendix 5B – Photo 13). Due to its location near West Beach and the Ore Dock, this quadrat may be impacted by tug wash and ship movement or by natural movements of sediment, either of which might impede macroalgae and epifaunal attachment and colonization.

The most abundant (by coverage) macroalgae type in 2024 was brown algae (acid weed) and ephemeral *Pylaiella* sp. across both areas (Table 5-5; Figure 5-7). Rockweed was present in high abundance within the reference area. Sugar kelp, *C. melagonium*, and *S. arctica* were also present in lower proportions in several quadrats in both areas. Overall, relative abundance of major algae classifications was comparable between years; however, sugar kelp appeared to increase in abundance in 2024 compared to previous years in both areas while *Pylaiella* sp. appeared to increase in abundance in 2024 in the reference area compared to previous years (Figure 5-7).

Macroalgae percent cover increased over time in both exposure and reference areas, although the increase was steeper and more consistent for the reference area compared to the exposure area (Figure 5-8). In three of the four sampling years, percent cover was higher in the reference area compared to the exposure area although the data were highly variable. In the analysis of percent cover, mean macroalgae percent cover significantly differed

between years ( $P < 0.001$ ) but not between areas ( $P = 0.138$ ) with no significant interaction between area and year ( $P = 0.057$ ; Table 5-3). Based on multiple comparisons, macroalgae cover in the exposure area was 41–69% lower in 2021 when compared to 2022–2024 ( $P = 0.5$ ,  $P = 0.5$ , and  $P = 0.009$ , respectively), and, in the reference area was 78–91% lower in 2021 than 2022–2024 ( $P \leq 0.003$  for all; Table 5-4). Macroalgae cover was 47–69% lower in 2021–2023 when compared to 2024 in the exposure area, although the difference between 2024 and other years was only significant for 2021 (Table 5-4). In the reference area, macroalgae cover was 29–91% lower in 2021–2023 than in 2024, although the difference between 2024 and other years was only significant for 2021 and 2022 (Table 5-4).

In the analysis of macroalgal percent cover, there was sufficient power ( $\geq 0.8$ ) to detect an effect of +270% (i.e., value in exposure area higher than in reference area), but none of the negative effect sizes (i.e., value in exposure area lower than in reference area) (Appendix 5E). When translated into percent cover, a 270% increase means that the percent cover in the exposure area would need to be 84% (compared to the 59% value estimated in the 2024 reference area). The low statistical power was likely due to the high variability in percent cover of macroalgae, leading to high uncertainty and lack of statistical significance.

### Diversity

Macroalgae diversity (taxa richness and SDI) was similar between the areas in 2024 but slightly higher within the reference area for both metrics. Taxa richness ranged between one to eight taxa in the exposure area and four to ten taxa in the reference area, and SDI ranged from very low ( $< 0.250$ ) to high ( $> 0.750$ ) in the exposure area but ranged from moderate (0.500 to 0.750) to high ( $> 0.750$ ) in the reference area (Table 5-5; Figure 5-9A). Both taxa richness and SDI means and ranges were higher for their respective areas in 2024 compared to both previous years except for SDI in the exposure area, where the mean value decreased slightly from 0.632 to 0.600 (Figure 5-9B; Golder 2022; WSP 2023, 2024).

Macroalgae mean taxa richness increased over time in both areas from a series low in 2022; in all years except 2022, taxa richness was higher in the reference area compared to the exposure area (Figure 5-8). Macroalgae taxa richness significantly differed between years ( $P < 0.001$ ) but not between areas ( $P = 0.177$ ) with no significant interaction between area and year ( $P = 0.710$ ; Table 5-3). Based on multiple comparisons, macroalgae taxa richness in 2022 was 26–51% lower than in 2021, 2023, and 2024 in the exposure area ( $P = 0.012$  for 2024,  $P > 0.08$  for all others), and 47–67% lower than in 2021, 2023, and 2024 in the reference area ( $P = 0.1$  for 2022 and  $P \leq 0.006$  for 2021 and 2024 (Table 5-4). Compared to 2024, macroalgae taxa richness was 13–51% lower in 2021–2023 in the exposure area and 22–67% lower in 2021–2023 in the reference area, although the comparisons were only significant for 2022 in both areas (Table 5-4; Figure 5-8).

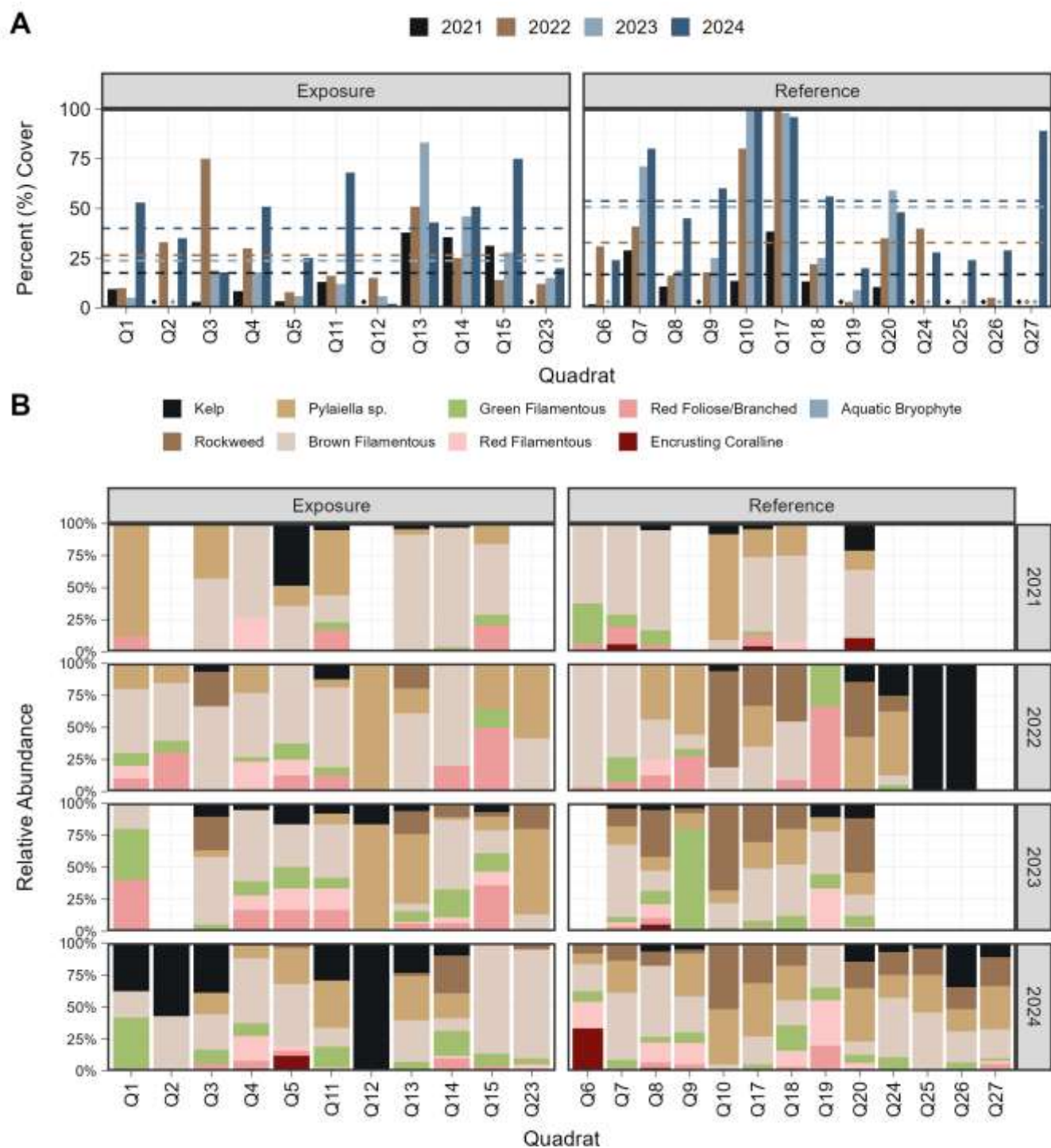
In the analysis of macroalgal species richness, statistical power was sufficient to detect a significant year and area interaction at effect sizes of -62% or +80% (Appendix 5E). That is, when the 2024 exposure area had mean macroalgae richness 62% lower or 80% higher than the 2024 reference area, the model could detect a significant interaction between year and area. When translated into richness values, this means that in the exposure area, there would need to be an average of 2.2 macroalgae species or an average of 10.5 macroalgae species (compared to the 5.9 value estimated in the 2024 reference area). The observed effect size (-24%) did not have sufficient power.

Macroalgae SDI in the exposure area increased over time but SDI was variable in the reference area. Macroalgae SDI significantly differed between years ( $P < 0.001$ ) but not between areas ( $P = 0.599$ ) with no significant interaction between area and year ( $P = 0.059$ ; Table 5-3). Macroalgae SDI was 29% lower in the exposure area than in the



reference area ( $P>0.1$ ) in 2024 (Table 5-4; Figure 5-8). In the exposure area, macroalgae SDI was 15–47% lower in 2021 when compared to 2022–2024 ( $P>0.1$  for all years) and SDI was 9–47% lower in 2021–2023 when compared to 2024 ( $P>0.1$  for all; Table 5-4; Figure 5-8). In the reference area, macroalgae taxa richness in 2022 was 50–78% lower than in 2021, 2023, and 2024, respectively ( $P>0.1$  [2021],  $P=0.012$  [2023],  $P<0.001$  [2024]; Table 5-4; Figure 5-8). Additionally, in the reference area, macroalgae SDI was 39–78% lower in 2021–2023 when compared to 2024, although the comparison was only significant for 2022 (Table 5-4; Figure 5-8).

In the analysis of macroalgal SDI, statistical power was sufficient to detect a significant year and area interaction at effect sizes of -38% or +240% (Appendix 5E). When translated into SDI values, this means that in the exposure area, macroalgae SDI would need to be an average of 0.6 or 0.893 (compared to the 0.7 value estimated in the 2024 reference area). The observed effect size in 2024 was -29%, and statistical power to detect it was low ( $<0.7$ ).



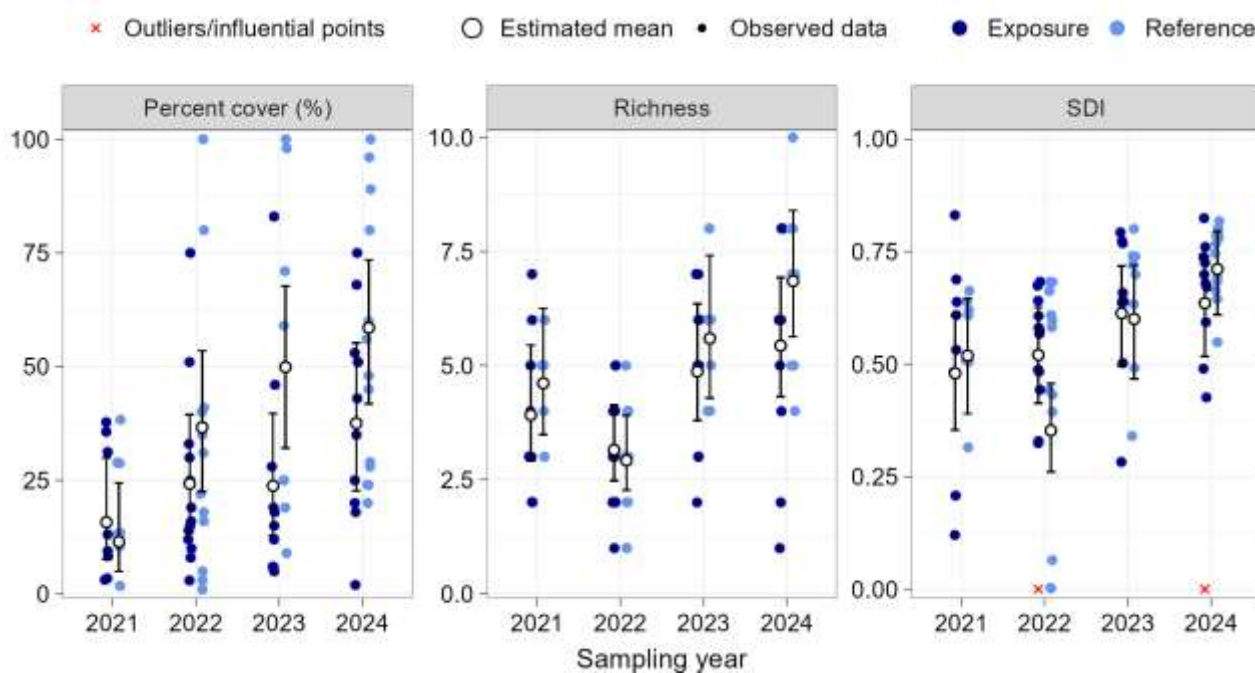
**Figure 5-7: A) Total Percent Cover and B) Relative Abundance of Macroalgae Recorded During Quadrats Surveyed in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed line in top figure represents the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.

**Table 5-5: Quadrat Survey Results for Macroalgae in Milne Port (2024)**

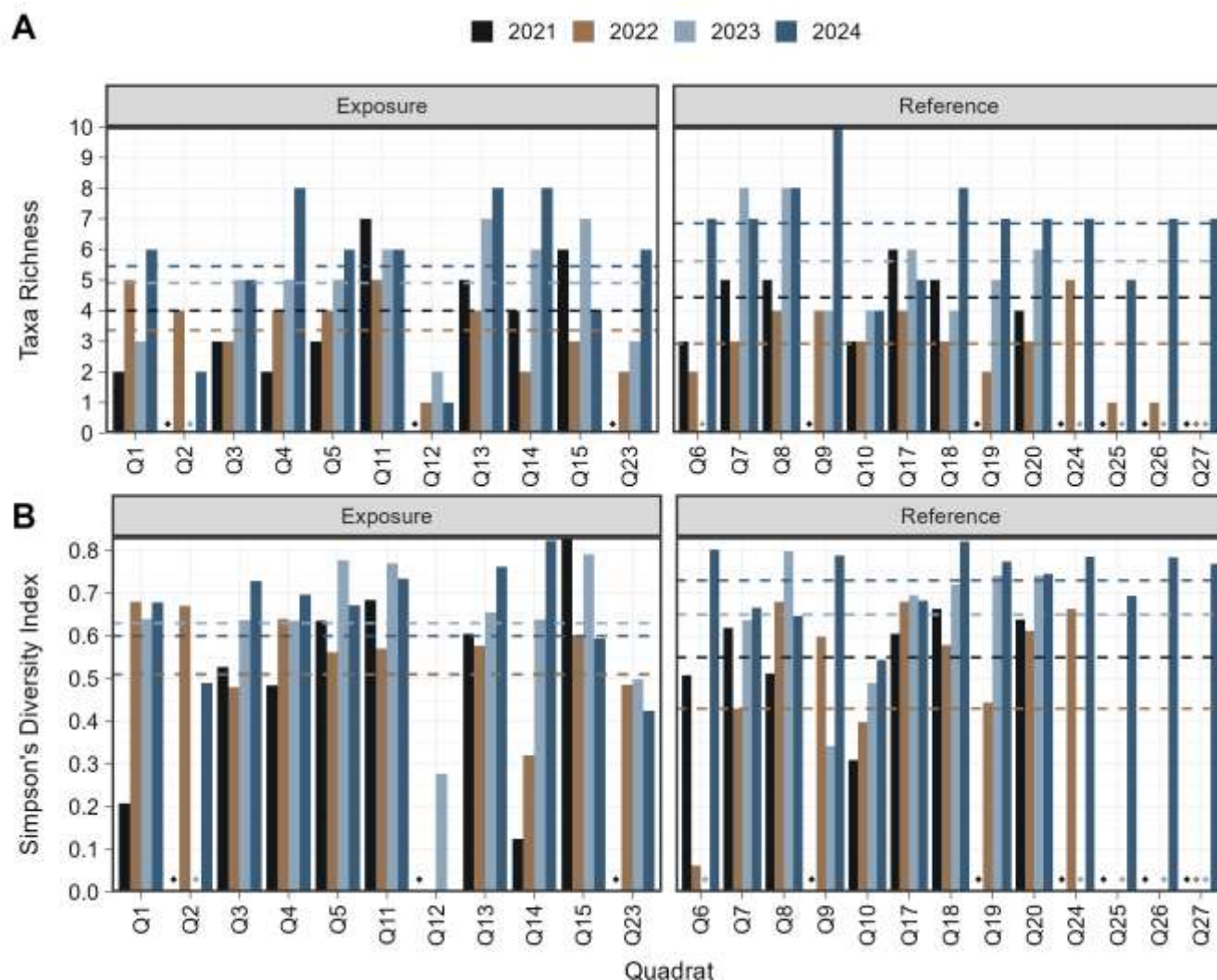
| Survey Area | Quadrat          | Macroalgae         |                   |                      |   |
|-------------|------------------|--------------------|-------------------|----------------------|---|
|             |                  | Total Cover (%)    | Taxa Richness     | SDI                  | Dominant Taxa                                       |
| Exposure    | Q1               | 53                 | 6                 | 0.679                | Sugar kelp, <i>Chaetomorpha melagonium</i>          |
|             | Q2               | 35                 | 2                 | 0.490                | Sugar kelp, acid weed                               |
|             | Q3               | 18                 | 5                 | 0.728                | Sugar kelp, acid weed                               |
|             | Q4               | 51                 | 8                 | 0.697                | Acid weed, <i>Savoiea arctica</i>                   |
|             | Q5               | 25                 | 6                 | 0.672                | Acid weed, <i>Pylaiella</i> sp.                     |
|             | Q11              | 68                 | 6                 | 0.734                | <i>Pylaiella</i> sp., sugar kelp                    |
|             | Q12              | 2                  | 1                 | 0.000                | Sugar kelp  |
|             | Q13              | 43                 | 8                 | 0.761                | <i>Pylaiella</i> sp., acid weed, sugar kelp         |
|             | Q14              | 51                 | 8                 | 0.824                | Rock weed, <i>Pylaiella</i> sp                      |
|             | Q15              | 75                 | 4                 | 0.594                | Acid weed, <i>Dictyosiphon foeniculaceus</i>        |
|             | Q21              | -                  | -                 | -                    | -   |
|             | Q22              | -                  | -                 | -                    | -   |
|             | Q23              | 20                 | 6                 | 0.425                | Acid weed   |
|             | <b>Mean ± SE</b> | <b>40.1 ± 6.75</b> | <b>5.5 ± 0.71</b> | <b>0.600 ± 0.070</b> | <b>Acid weed, sugar kelp, <i>Pylaiella</i> sp.</b>  |
| Reference   | Q6               | 24                 | 7                 | 0.802                | Acid weed, <i>Polysiphonia</i> sp.                  |
|             | Q7               | 80                 | 7                 | 0.667                | Acid weed, <i>Pylaiella</i> sp., rockweed           |
|             | Q8               | 45                 | 8                 | 0.647                | Acid weed, <i>S. arctica</i>                        |
|             | Q9               | 60                 | 10                | 0.788                | <i>Pylaiella</i> sp., acid weed, <i>S. arctica</i>  |
|             | Q10              | 116                | 4                 | 0.545                | Rock weed, <i>Pylaiella</i> sp                      |
|             | Q17              | 96                 | 5                 | 0.683                | <i>Pylaiella</i> sp., rockweed, acid weed           |
|             | Q18              | 56                 | 8                 | 0.822                | <i>Pylaiella</i> sp., rockweed, acid weed           |
|             | Q19              | 20                 | 7                 | 0.775                | Acid weed, <i>S. arctica</i>                        |
|             | Q20              | 48                 | 7                 | 0.745                | Rock weed, acid weed, <i>Pylaiella</i> sp.          |
|             | Q24              | 28                 | 7                 | 0.786                | <i>S. arctica</i>                                   |
|             | Q25              | 24                 | 5                 | 0.694                | <i>Halosiphon tomentosus</i> , <i>Pylaiella</i> sp. |
|             | Q26              | 29                 | 7                 | 0.785                | Sugar kelp  |
|             | Q27              | 89                 | 7                 | 0.769                | <i>Pylaiella</i> sp., rockweed, acid weed           |
|             | <b>Mean ± SE</b> | <b>55.0 ± 8.74</b> | <b>6.8 ± 0.42</b> | <b>0.731 ± 0.022</b> | <b><i>Pylaiella</i> sp., rockweed, acid weed</b>    |

Note: SE = Standard error; % = percent; '-' indicates the quadrat was not surveyed in 2024. Simpson Diversity Index (SDI) values are color-coded by category, **red** = very low (<0.250), **blue** = low (0.250 to 0.499), **yellow** = moderate (0.500 to 0.750), **green** = high (>0.750).



**Figure 5-8: Interannual Differences in Macroalgae Percent Cover, Taxa Richness, and Simpson's Diversity Index in Milne Port, 2021 – 2024.**

Note: Observed (solid points) and estimated marginal mean percent cover (open points) and 95% confidence interval (error bars) are presented for each sampling area and year.



**Figure 5-9: A) Taxa Richness and B) Simpson's Diversity Index of Macroalgae Recorded During Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed lines represent the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.

### 5.4.3 Benthic Epifauna

Benthic epifauna identified in the quadrats in 2024 belonged to six phyla: Annelida, Arthropoda, Cnidaria, Chordata, Echinodermata, and Mollusca. All observed taxa had been previously reported from the Milne Port area (i.e., overall area encompassing both exposure and reference areas); however, observed taxa recorded for the first time during quadrat surveys were previously observed during benthic infauna surveys, including spaghetti worm (Family Terebellidae), Greenland cockle (*Serripes groenlandicus*), nut clam (*Nuculana* sp.), and scale worm identified to species level (*Harmothoe imbricata*; Appendix 5B – Photo 14).

The majority of species identified in the quadrats belonged to the phylum Mollusca, with nine taxa identified to species level: wrinkled rock-borer clam (*Hiatella arctica*), blunt gaper clam (*Mya truncata*), Icelandic scallop (*Chlamys islandica*), discord mussel (*Musculus discors*), Greenland scallop (*Similipecten greenlandicus*),

Northern Astarte (*Astarte borealis*), Greenland cockle, nut clam, and common tortoise limpet (*Testudinalia testudinalis*) (Appendix 5B – Photos 8, 11, 15, and 16). Other molluscs included bushy-backed sea slug (*Dendronotus* sp.) and margarite snail (*Margarites* sp.).

Phylum Annelida was represented by five distinct taxa: cone worm (*Cistenides granulata*), scale worm, spaghetti worm, and two morphologically distinct sabellid polychaetes (Family Sabellidae)<sup>14</sup> (Appendix 5B – Photos 10, 14, and 17). Phylum Arthropoda (subphylum Crustacea) was only represented by the order Amphipoda while phylum Nemertea was represented by one unidentified individual (Appendix 5B – Photo 18). Phylum Echinodermata included brittle stars (Class Ophiuroidea), green urchin (*Strongylocentrotus droebachiensis*), and six-rayed sea star (*Leptasterias* sp.) (Appendix 5B – Photos 19 to 20) and the phylum Cnidaria was represented by burrowing anemone (Subclass Ceriantharia).

Phylum Chordata included one species of tunicate (Subphylum Tunicata) and six distinct fish taxa: Shorthorn Sculpin (*Myoxocephalus scorpius*), Saddled Eelpout (*Lycodes mucosus*), Slender Eelblenny (*Lumpenus fabricii*), Eelblenny (*Lumpenus* sp.), Fish Doctor (*Gymnelus viridis*) (Appendix 5B – Photo 7), and a juvenile Lumpfish (*Cyclopterus lumpus*) (Appendix 5B – Photos 6, 21 to 23).

#### 5.4.3.1 Sessile Epifauna

Wrinkled rock-borer clams (one of the species used for fish health monitoring at Milne Inlet; Chapter 7.0) and cone worms were the dominant sessile epifauna taxa in the majority of quadrats in both areas in 2024, consistent with results from previous years (Table 5-6; Figure 5-10). Blunt gaper clams, sabellid worms, and mussels were also frequently observed in quadrats in both areas. One sessile epifaunal taxon observed in previous years, an orange tunicate (*Polycarpa* sp.), was not observed in 2024 (Golder 2022; WSP 2023, 2024).

#### Percent Cover

Total percent cover of sessile epifauna varied among quadrats in both exposure and reference areas but on average was slightly higher in the reference area ( $19.8 \pm 4.8\%$ ) than in the exposure area ( $24.8 \pm 5.0\%$ ; Table 5-6; Figure 5-10). Sessile cover in the exposure area ranged from 2% (Q15) to 62% (Q4). Total sessile percent cover was lowest in Q15 and Q23 (3%) in the exposure area; Q23 sessile cover has increased annually since the quadrat was deployed in 2022 (0%) while sessile cover in Q15 has been highly variable (2021 = 33.7%, 2022 = 5%, 2023 = 23%; Table 5-6; Appendix 5C). In the reference area, sessile cover ranged from 7% (Q24) to 73% (Q20). Q24 and Q26 (8%) had the lowest sessile cover in the reference area. These quadrats are located in front of a freshwater outflow and have had variable but generally low cover since their deployment in 2022 (Figure 5-10; Appendix 5C).

Sessile epifauna percent cover varied over time in both the exposure and reference areas, but in three of the four sampling years, percent cover was higher in the reference area than the exposure area (Figure 5-8). In the analysis of percent cover, the model that included a non-linear effect of depth as a covariate was the best supported based on AIC and was selected for interpretation. Mean sessile cover significantly differed between years ( $P=0.025$ ) but not between areas ( $P=0.315$ ) with no interaction between area and year ( $P=0.112$ ); the relationship with depth was also not statistically significant ( $P=0.057$ ) Table 5-3). Within 2024, sessile cover was 48% lower in the exposure area than in the reference area, but the result was not significant (Figure 5-8).

<sup>14</sup> A large tube casing collected in 2022 was identified by the laboratory as *Pista maculata* (Family Terebellidae); however, since this organism was not observed to be alive in the field, it is not completely linked to the large sabellid worm (sp. 2) that has been observed in all years 2021 to 2023.



In the analysis of sessile epifauna percent cover, there was sufficient power ( $\geq 0.8$ ) to detect an effect of +275%, but none of the negative effect sizes (Appendix 5E), likely due to the high variability associated with the data (Figure 5-11). When translated into percent cover, a 275% increase means that the sessile percent cover in the exposure area would need to be 60% (compared to the 29% value estimated in the 2024 reference area). The observed effect size in 2024 was -48%, and statistical power to detect the effect was low.

Relative abundance of sessile epifauna also varied between the quadrats but remained within the same ranges between the exposure and reference areas in 2024, as well as being comparable to previous years (Figure 5-10; Appendix 5C). Tunicate and scallop percent cover increased in 2024 compared to previous years while generally clam (e.g., wrinkled rock-borer, blunt gaper) abundance decreased (Figure 5-10).

### **Diversity**

Mean taxa richness was similar between the exposure ( $4.6 \pm 0.6$  taxa) and reference areas ( $5.1 \pm 0.4$  taxa) in 2024 (Figure 5-12) and both were within the standard error of 2023 results, with the reference area having slightly lower mean taxa richness in 2024 (Figure 5-12). Sessile taxa richness ranged from one to seven taxa in the exposure area and from three to seven taxa in the reference area. In the exposure area, Q15 had the lowest taxa richness, represented only by cone worms, while in the reference area, Q26 and Q27 had the lowest taxa richness, represented by cone worms, sabellid worms, and various bivalve taxa (Appendix 5C). Five quadrats contained the highest number of taxa (seven): Q1 and Q5 in the exposure area and Q6, Q8, and Q20 in the reference area. These quadrats all had higher taxa richness in 2024 compared to 2023, except for Q6, which was not surveyed in 2023 (Appendix 5C).

Sessile epifauna mean taxa richness was variable over time, with the highest mean values in both areas observed in 2021 and the lowest mean values in both areas observed in 2022. Higher taxa richness was observed in the reference area compared to the exposure area in all years (Figure 5-8). In the analysis of taxa richness, the model that included a non-linear effect of depth as a covariate was the best supported based on AIC and was selected for interpretation. Sessile taxa richness significantly differed between years ( $P=0.001$ ) but not between areas ( $P=0.076$ ) with no significant interaction between area and year ( $P=0.991$ ; Table 5-3). Additionally, sessile mean taxa richness had a significant positive non-linear relationship with depth ( $P=0.002$ ; Table 5-4). Based on multiple comparisons, sessile taxa richness in 2021 was higher than in 2022-2024 in both areas, ranging from 7-77% higher in the exposure area ( $P>0.3$  for all years), and 13-74% higher in the reference area ( $P>0.8$  except in 2021 [ $P=0.040$ ]; Table 5-4; Figure 5-8). In 2024, richness in the exposure area was 14% lower than in the reference area ( $P=0.4$ ; Table 5-4).

In the analysis of sessile epifauna richness, there was sufficient statistical power to detect a significant interaction at effect sizes of -68% or +62% (Appendix 5E). That is, when the 2024 exposure area had mean sessile epifauna richness 68% lower or 62% higher than the 2024 reference area, the model could detect a significant interaction between year and area. When translated into richness values, this means that in the exposure area, there would need to be an average of 1.8 sessile species or an average of 9.3 sessile species for detection of a difference (compared to the average of 5.7 sessile species estimated in the 2024 reference area). The observed effect size in 2024 was small (-14%; Figure 5-11).

Values of SDI ranged from very low ( $<0.250$ ) to high ( $>0.750$ ) in the exposure area and from low ( $0.250-0.499$ ) to high ( $>0.750$ ) in the reference area (Figure 5-12), with only minor differences in mean values between areas (exposure =  $0.609 \pm 0.070$ ; reference =  $0.650 \pm 0.029$ ) (Table 5-6). SDI was very low (0.000) for Q15 due to very low abundance of only one taxon (cone worms) and high cover of detrital algae and diatoms within the quadrat (Appendix 5C). SDI was high in Q1 and Q5 within the exposure area and Q6 and Q17 within the reference area due to even abundance of observed taxa (Table 5-6; Appendix 5C).



Sessile epifauna SDI in the reference area slowly increased over time, while in the exposure area SDI was more variable (Figure 5-8). In the analysis of SDI, the model that included the effect of soft substrate composition as a covariate was the best supported based on AIC and was selected for interpretation. Sessile SDI significantly differed between years ( $P=0.002$ ) but not between areas ( $P=0.274$ ) with no significant interaction between area and year ( $P=0.644$ ; Table 5-3). The effect of soft substrate composition was significant ( $P=0.016$ ; Table 5-4). Compared to 2024, sessile epifauna SDI was 44-69% lower in 2021–2023 in the exposure area and 7–48% lower in 2021–2023 in the reference area, although the comparisons were only significant for 2022 in the exposure area (Table 5-4; Figure 5-8).

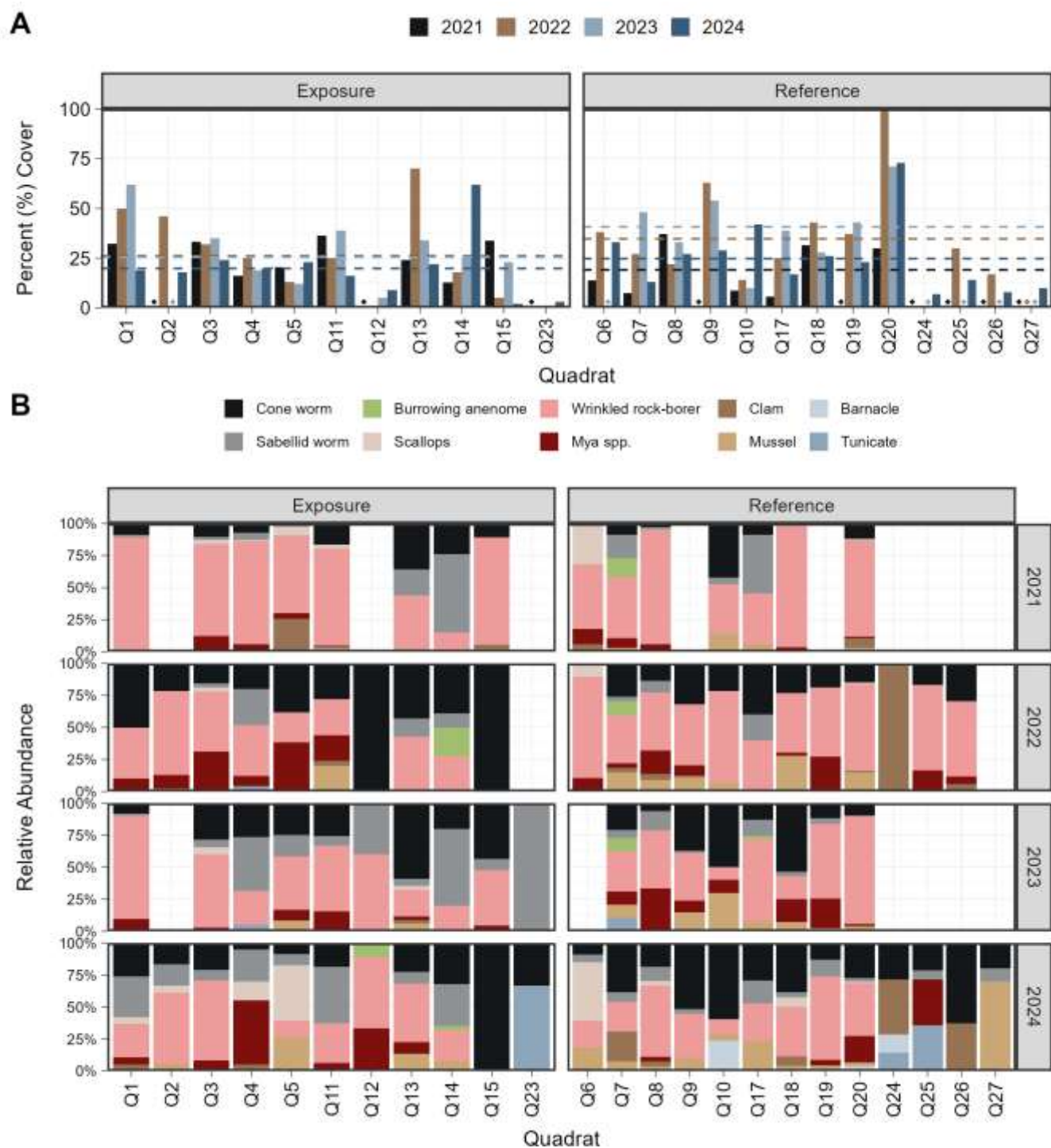
In the analysis of sessile epifauna SDI, there was sufficient statistical power to detect a significant interaction at an effect size of +155%, but not at any of the examined negative effect sizes, ranging up to -90% (Appendix 5E). That is, the 2024 sessile SDI at the exposure area would have to be 155% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into SDI values, this means that in the exposure area, sessile SDI would need to be an average of 0.810 (compared to the 0.626 value estimated in the 2024 reference area). The observed effect size in 2024 was small (0.3%), and statistical power to detect the effect was low (<0.3).

**Table 5-6: Quadrat Survey Results for Sessile Epifauna in Milne Port (2024)**

| Survey Area | Quadrat          | Sessile Epifauna  |                  |                      |   |
|-------------|------------------|-------------------|------------------|----------------------|---|
|             |                  | Total Cover (%)   | Taxa Richness    | SDI                  | Dominant Taxa   |
| Exposure    | Q1               | 19                | 7                | 0.803                | Wrinkled rock-borer, cone worm                        |
|             | Q2               | 18                | 6                | 0.642                | -   |
|             | Q3               | 24                | 4                | 0.552                | Wrinkled rock-borer                                   |
|             | Q4               | 20                | 5                | 0.660                | <i>Mya truncata</i> , sabellid worm                   |
|             | Q5               | 23                | 7                | 0.836                | <i>Similipecten greenlandicus</i> , Icelandic scallop |
|             | Q11              | 16                | 5                | 0.750                | Wrinkled rock-borer, sabellid worm                    |
|             | Q12              | 9                 | 3                | 0.568                | Wrinkled rock-borer, blunt gaper                      |
|             | Q13              | 22                | 6                | 0.715                | Wrinkled rock-borer, cone worm                        |
|             | Q14              | 62                | 5                | 0.726                | Sabellid worm, cone worm, wrinkled rock-borer         |
|             | Q15              | 2                 | 1                | 0.000                | Cone worm   |
|             | Q21              | -                 | -                | -                    | -   |
|             | Q22              | -                 | -                | -                    | -   |
|             | Q23              | 3                 | 2                | 0.444                | Tunicate  |
|             | <b>Mean ± SE</b> | <b>19.8 ± 4.8</b> | <b>4.6 ± 0.6</b> | <b>0.609 ± 0.070</b> | <b>Wrinkled rock-borer, cone worm</b>                 |

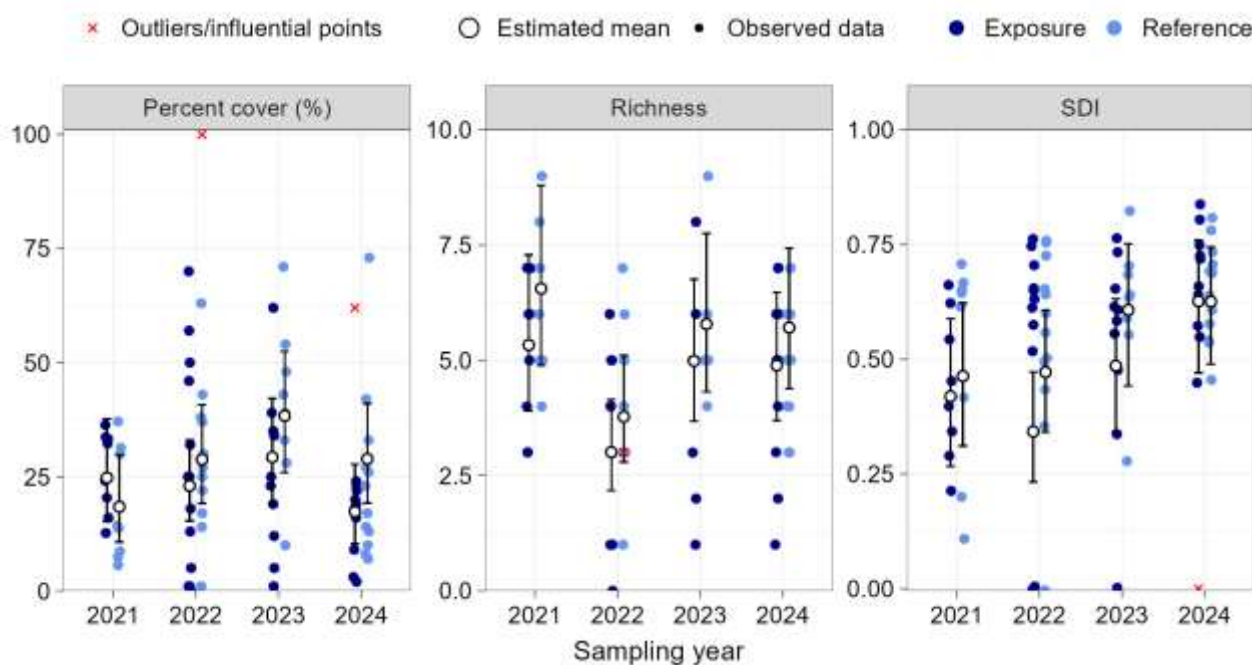
| Survey Area | Quadrat          | Sessile Epifauna  |                  |                      |   |
|-------------|------------------|-------------------|------------------|----------------------|---|
|             |                  | Total Cover (%)   | Taxa Richness    | SDI                  | Dominant Taxa                               |
| Reference   | Q6               | 33                | 7                | 0.804                | Icelandic scallop, mussel                   |
|             | Q7               | 13                | 5                | 0.734                | Cone worm                                   |
|             | Q8               | 27                | 7                | 0.639                | Wrinkled rock-borer, cone worm              |
|             | Q9               | 29                | 5                | 0.606                | Cone worm, wrinkled rock-borer              |
|             | Q10              | 42                | 4                | 0.573                | Cone worm, barnacle                         |
|             | Q17              | 17                | 6                | 0.782                | Wrinkled rock-borer, cone worm              |
|             | Q18              | 26                | 6                | 0.689                | Cone worm, wrinkled rock-borer              |
|             | Q19              | 23                | 5                | 0.537                | Wrinkled rock-borer                         |
|             | Q20              | 73                | 7                | 0.710                | Wrinkled rock-borer, cone worm, blunt gaper |
|             | Q24              | 7                 | 4                | 0.694                | Northern astarte, cone worm                 |
|             | Q25              | 14                | 4                | 0.694                | Blunt gaper, cone worm                      |
|             | Q26              | 8                 | 3                | 0.531                | Cone worm                                   |
|             | Q27              | 10                | 3                | 0.460                | Mussel                                      |
|             | <b>Mean ± SE</b> | <b>24.8 ± 5.0</b> | <b>5.1 ± 0.4</b> | <b>0.650 ± 0.029</b> | <b>Cone worm, wrinkled rock-borer</b>       |

Note: SE = Standard error; % = percent; '-' indicates the quadrat was not surveyed in 2024. Simpson Diversity Index (SDI) values are color-coded by category, **red** = very low (<0.250), **blue** = low (0.250 to 0.499), **yellow** = moderate (0.500 to 0.750), **green** = high (>0.750).



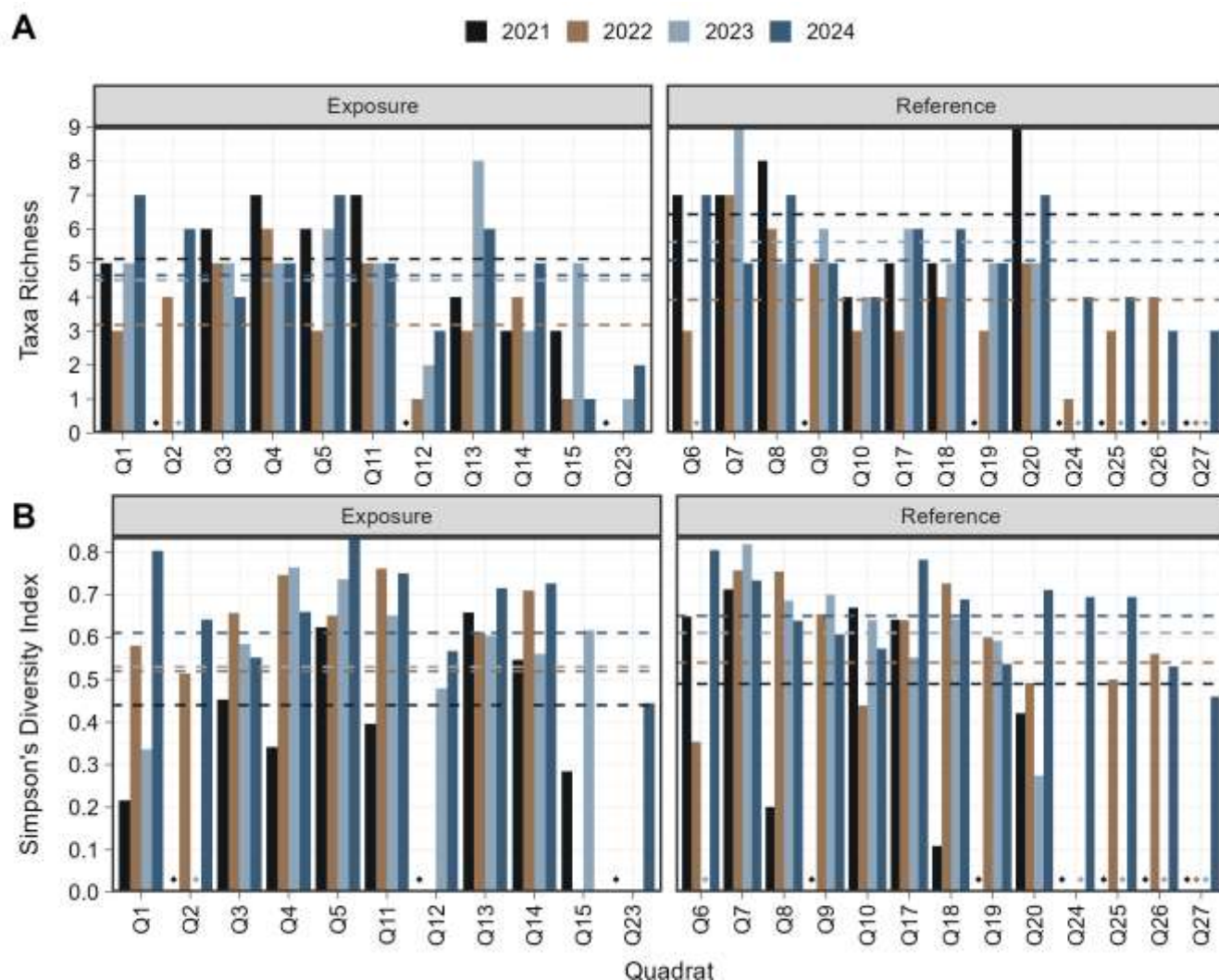
**Figure 5-10: A) Total Percent Cover and B) Relative Abundance of Sessile Epifauna Recorded in Quadrats Surveyed During Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed line in top figure represents the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.



**Figure 5-11: Interannual Differences in Sessile Epifauna Percent Cover, Taxa Richness, and Simpson's Diversity Index in Milne Port, 2021 – 2024.**

Note: Observed (solid points) and estimated marginal mean percent cover (open points) and 95% confidence interval (error bars) are presented for each sampling area and year.



**Figure 5-12: A) Taxa Richness and B) Simpson's Diversity Index of Sessile Epifauna Recorded in Quadrats Surveyed During Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed lines represent the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.

### 5.4.3.2 Motile Epifauna

Green urchins were the dominant motile epifauna taxa observed in quadrats in the exposure area in 2024, followed by brittle stars. In the reference area, brittle stars, followed by green urchins and Dendronotid nudibranchs were the dominant taxa in the reference area (Table 5-7; Figure 5-13; Appendix 5C). Brittle stars and green urchins that were observed at high densities in 2022 and 2021 but only observed at low densities in 2023 were observed at slightly higher densities in 2024 (Figure 5-13). Shrimp observed at high density in 2023 were rarely observed in 2024, potentially due to interannual variability. Eight distinct motile epifauna taxa observed in previous years (2021 and/or 2022) were not observed in 2023, including polychaetes, flat worms (Phylum Platyhelminthes), whelks (Family Buccinidae), sea cucumbers (Family Holothuroidea), and Fourhorn Sculpin (*Myoxocephalus quadricornis*) (Appendix 5C; Golder 2022; WSP 2023, 2024).

## Density

Motile epifauna density in the exposure area was lower in 2024 ( $4.8 \pm 1.3$  org/m<sup>2</sup>) than in previous years but was higher in the reference area in 2024 ( $8.8 \pm 4.5$  org/m<sup>2</sup>) compared to 2023 (Table 5-7; Figure 5-13; Appendix 5C). Three quadrats (Q13, Q7, and Q8) contained motile epifauna densities greater than 10 org/m<sup>2</sup>, a decrease from six quadrats in 2023 (Figure 5-13; Appendix 5C). Five quadrats (Q2, Q15, Q23, Q10, and Q25) contained no motile epifauna compared to one quadrat (Q23) in 2023 (Table 5-7). Most quadrats contained a single organism from two to three taxa (Appendix 5C).

Density of motile epifauna was highly variable. In the exposure area, density declined over time; in the reference area, density of motile epifauna decreased considerably between 2021 and 2022, followed by a subsequent increase in values and overall similar density between the two areas in 2023–2024 (Figure 5-8). In the analysis of density, the model that included a non-linear effect of depth as a covariate was the best supported based on AIC and was selected for interpretation. The interaction between year and area was significant ( $P < 0.001$ ; Table 5-3), indicating differences in trends of density between the two areas over time. Additionally, motile density had a significant non-linear relationship with depth ( $P < 0.001$ ; Table 5-4), with the highest densities generally found at deeper depths. Pairwise comparisons between years within each area indicated that motile density in the exposure area was lowest in 2024 (24–60% lower than 2021–2023,  $P > 0.09$  for all; Table 5-4). In the reference area, 2024 density was the second lowest after 2022; the 2024 mean density was 19% and 44% lower than 2021 and 2023, respectively ( $P > 0.1$  for both) and 171% higher than 2021 ( $P = 0.014$ ; Table 5-4). In 2022, when density in the reference area was at its lowest, density at the exposure area was 201% higher than in the reference area ( $P = 0.002$ ); in the remaining years, density at the exposure area was 4–32% lower than at the reference area ( $P > 0.2$  for all; Table 5-4).

In the analysis of motile epifauna density, there was sufficient power to detect a significant interaction at all of the examined effects (Appendix 5E). This was due to the large difference between reference and exposure areas in 2022, but not in 2021 (Figure 5-8), which resulted in a high proportion of simulations with a significant interaction between year and area. That is, the interaction term of the model was significant regardless of the simulated 2024 effects.

## Diversity

Diversity (taxa richness and SDI) was low overall for motile epifauna in both areas in 2024. Unlike in previous years where taxa richness was similar between areas, in 2024 motile taxa richness ranged from zero to three taxa in the exposure area but ranged from zero to seven taxa in the reference area (Figure 5-15). Q4 and Q13 in the exposure area contained three taxa each while Q6 contained the highest taxa richness in the reference area. SDI values were generally variable in both areas in 2024 (similar to 2023), with areas having either very low (0.000) or moderate (0.500–0.750) SDI with one low site each (Table 5-7). Unlike in 2023, no sites had very high ( $> 0.750$ ) SDI values in 2024 (Figure 5-15). Thirteen of the 24 quadrats (54%) had a SDI of zero, compared to 44% in 2023; these quadrats either lacked motile epifauna (three exposure [Q2, Q15, Q23] and two reference [Q10, Q25] area quadrats) or they contained one or two organisms of a single taxon (four quadrats in each area; exposure = Q1, Q3, Q11, and Q12; reference = Q17, Q18, Q20, and Q24) (Table 5-7; Figure 5-15).

Motile taxa richness was variable within each year but was generally similar between years (Figure 5-8). In the analysis of taxa richness, the model that included the effect of depth as a covariate was the best supported based on AIC. Mean motile taxa richness did not significantly differ between years ( $P = 0.121$ ) or areas ( $P = 0.993$ ) and there was no significant interaction between area and year ( $P = 0.164$ ; Table 5-3). The effect of depth was significant ( $P < 0.001$ ; Table 5-3), wherein motile taxa richness generally increased with depth. In the exposure



area, 2024 mean richness was 20–50% lower than in 2021–2023 ( $P>0.1$  for all). In the reference area, 2024 richness was 6% lower than 2021 but 12–95% higher than 2022 and 2023 ( $P>0.1$  for all). In 2024, motile taxa richness was 40% lower in the exposure area than in the reference area, but the result was not significant (Figure 5-8; Table 5-4).

In the analysis of motile epifauna richness, there was sufficient power to detect a significant interaction at effect sizes of -69% or +190% (Appendix 5E). That is, when the 2023 exposure area had mean motile epifauna richness that was 69% lower or 190% higher than the 2024 reference area, the model could detect a significant interaction between year and area. When translated into richness values, this means that in the exposure area, there would need to be an average of 0.67 motile species or an average of 6.3 motile species (compared to the 2.2 value estimated in the 2024 reference area). The observed effect size in 2024 was -40% (Figure 5-14), and statistical power to detect the effect was low.

Motile epifauna SDI, similar to taxa richness, was highly variable within each year but values were generally similar between years (Figure 5-8). In the analysis of SDI, the model that included the effect of depth as a covariate was the best supported based on AIC. Mean motile SDI did not significantly differ between years ( $P=0.552$ ) or areas ( $P=0.849$ ) with no significant interaction between area and year ( $P=0.439$ ; Table 5-3). Motile SDI generally increased with depth, although the effect was not significant ( $P=0.077$ ; Table 5-4). In the exposure area, 2024 SDI values were 38% and 57% lower than in 2021 and 2023, respectively, but 5% higher than in 2022 ( $P>0.4$  for all). In the reference area, 2024 values were 13% lower than 2021 but 45–48% higher than 2022 and 2023 ( $P>0.8$  for all). In 2024, motile taxa richness was 34% lower in the exposure area than in the reference area ( $P=0.4$ ).

In the analysis of motile epifauna SDI, there was sufficient power to detect a significant interaction at effect sizes of -80%, but not at the examined positive effect sizes, up to 600% (Appendix 5E). That is, the 2024 motile SDI at the exposure area had to be 80% lower than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into SDI values, this means that in the exposure area, motile SDI would need to be an average of 0.09 (compared to the 0.33 value estimated in the 2024 reference area) for detection of a significant interaction. The observed effect size in 2024 was -34% (Figure 5-14), and statistical power to detect the effect was low. The low power to detect increases in motile epifauna SDI was likely due to the variability associated with the observed data (Figure 5-14).

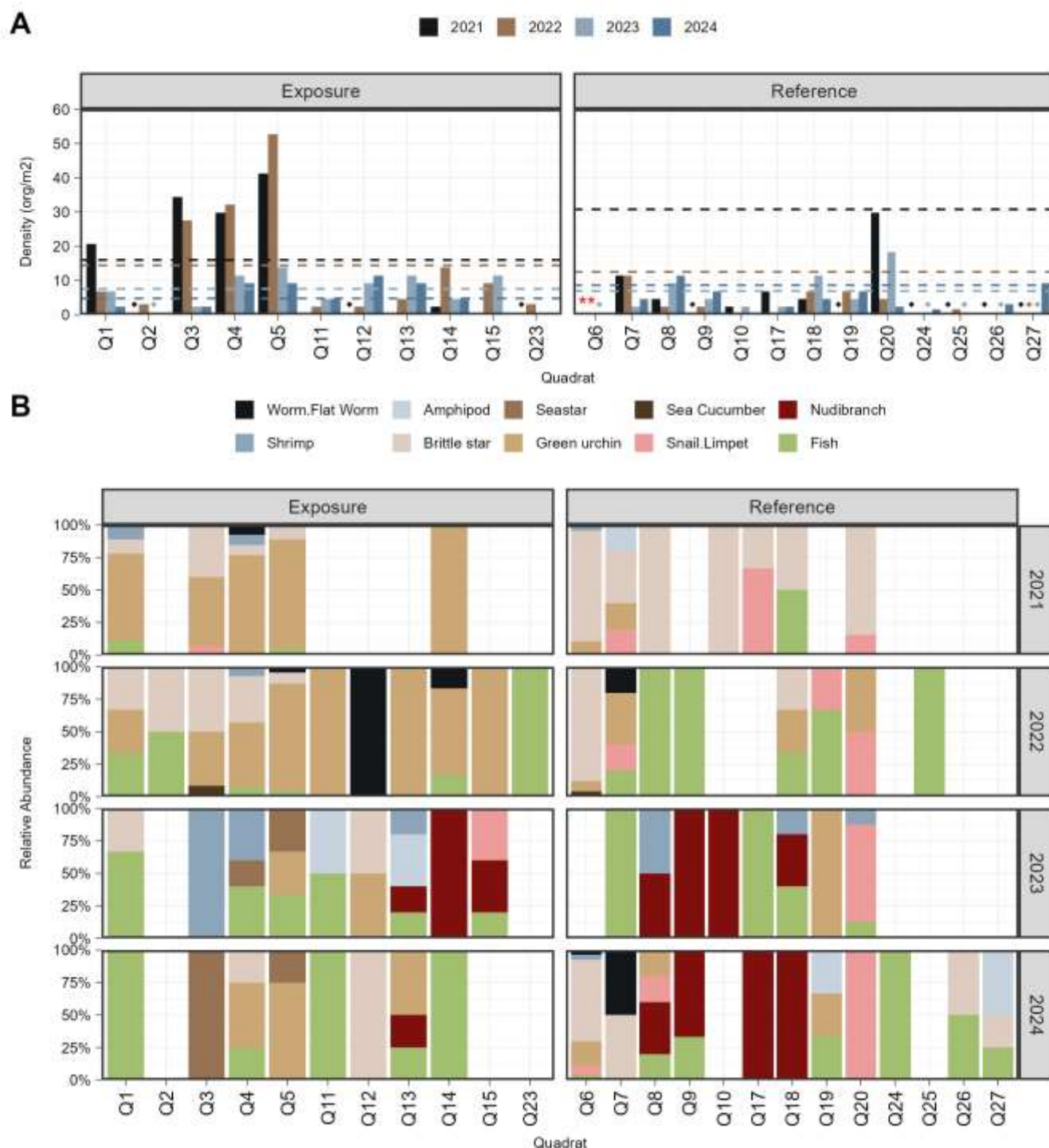
**Table 5-7: Quadrat Survey Results for Motile Epifauna in Milne Port (2024)**

| Survey Area | Quadrat | Motile Epifauna               |               |       |   |
|-------------|---------|-------------------------------|---------------|-------|---|
|             |         | Density (org/m <sup>2</sup> ) | Taxa Richness | SDI   | Dominant Taxa                           |
| Exposure    | Q1      | 2                             | 1             | 0.000 | Shorthorn Sculpin                       |
|             | Q2      | 0                             | 0             | NA    | None                                    |
|             | Q3      | 2                             | 1             | 0.000 | Six-rayed sea star                      |
|             | Q4      | 9                             | 3             | 0.625 | Green urchin, brittle star, Fish Doctor |
|             | Q5      | 9                             | 2             | 0.375 | Green urchin, six-rayed sea star        |
|             | Q11     | 5                             | 1             | 0.000 | Saddled Eelpout                         |



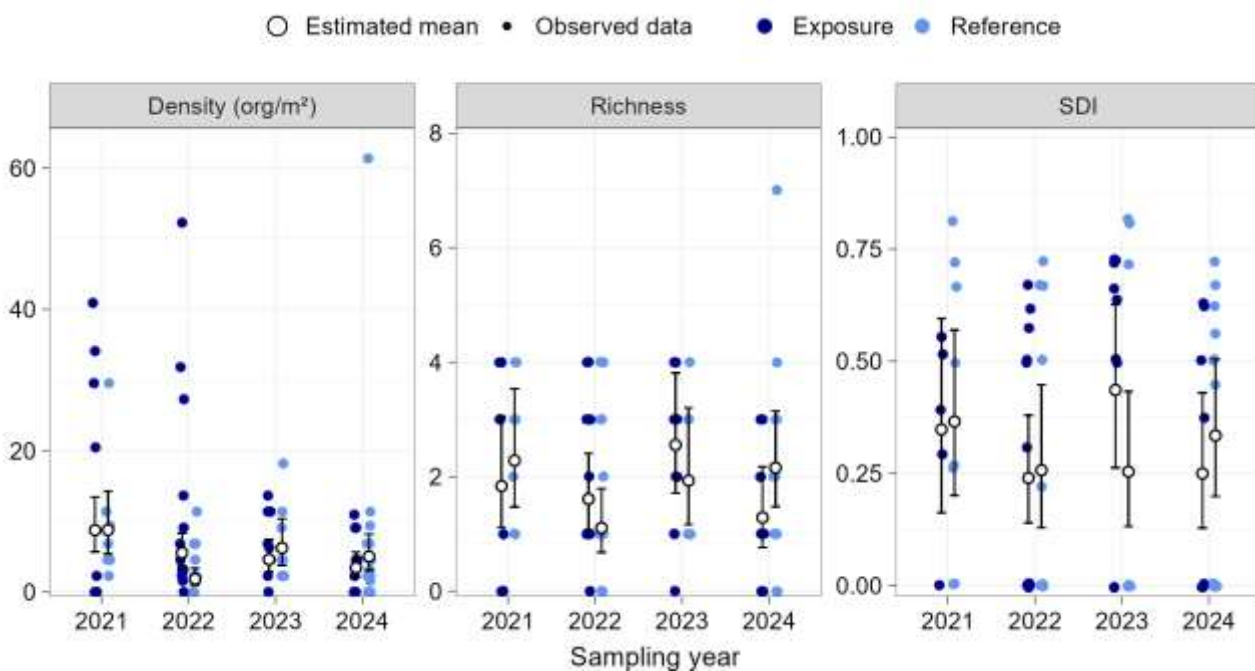
| Survey Area | Quadrat   | Motile Epifauna               |               |               |   |
|-------------|-----------|-------------------------------|---------------|---------------|---|
|             |           | Density (org/m <sup>2</sup> ) | Taxa Richness | SDI           | Dominant Taxa   |
|             | Q12       | 11                            | 1             | 0.000         | Brittle star  |
|             | Q13       | 9                             | 3             | 0.625         | Green urchin, <i>Dendronotus</i> sp., Eelblenny             |
|             | Q14       | 5                             | 2             | 0.500         | Saddled Eelpout, Fish Doctor                                |
|             | Q15       | 0                             | 0             | NA            | None  |
|             | Q21       | -                             | -             | -             | -   |
|             | Q22       | -                             | -             | -             | -   |
|             | Q23       | 0                             | 0             | NA            | None  |
|             | Mean ± SE | 4.8 ± 1.3                     | 1.3 ± 0.3     | 0.266 ± 0.104 | Green urchin, brittle star, Saddled Eelpout                 |
| Reference   | Q6        | 62                            | 7             | 0.562         | Brittle star, green urchin                                  |
|             | Q7        | 5                             | 2             | 0.500         | Brittle star, scale worm                                    |
|             | Q8        | 11                            | 4             | 0.720         | <i>Dendronotus</i> sp., <i>Margarites</i> sp., green urchin |
|             | Q9        | 7                             | 2             | 0.444         | <i>Dendronotus</i> sp., Lumpfish                            |
|             | Q10       | 0                             | 0             | NA            | None  |
|             | Q17       | 2                             | 1             | 0.000         | <i>Dendronotus</i> sp.                                      |
|             | Q18       | 5                             | 1             | 0.000         | <i>Dendronotus</i> sp.                                      |
|             | Q19       | 7                             | 3             | 0.667         | Eelblenny, green urchin, amphipod                           |
|             | Q20       | 2                             | 1             | 0.000         | <i>Margarites</i> sp.                                       |
|             | Q24       | 2                             | 1             | 0.000         | Shorthorn Sculpin   |
|             | Q25       | 0                             | 0             | NA            | None  |
|             | Q26       | 3                             | 2             | 0.500         | Brittle star, Shorthorn Sculpin                             |
|             | Q27       | 9                             | 3             | 0.625         | Amphipod, Fish Doctor, brittle star                         |
|             | Mean ± SE | 8.8 ± 4.5                     | 2.1 ± 0.5     | 0.365 ± 0.090 | Brittle star, green urchin, <i>Dendronotus</i> sp.          |

Note: '-' indicates the quadrat was not surveyed in 2024. Simpson Diversity Index (SDI) values are color-coded by category, red = very low (<0.250), blue = low (0.250 to 0.499), yellow = moderate (0.500 to 0.750), green = high (>0.750). NC = Not calculated due to no motile taxa observed.



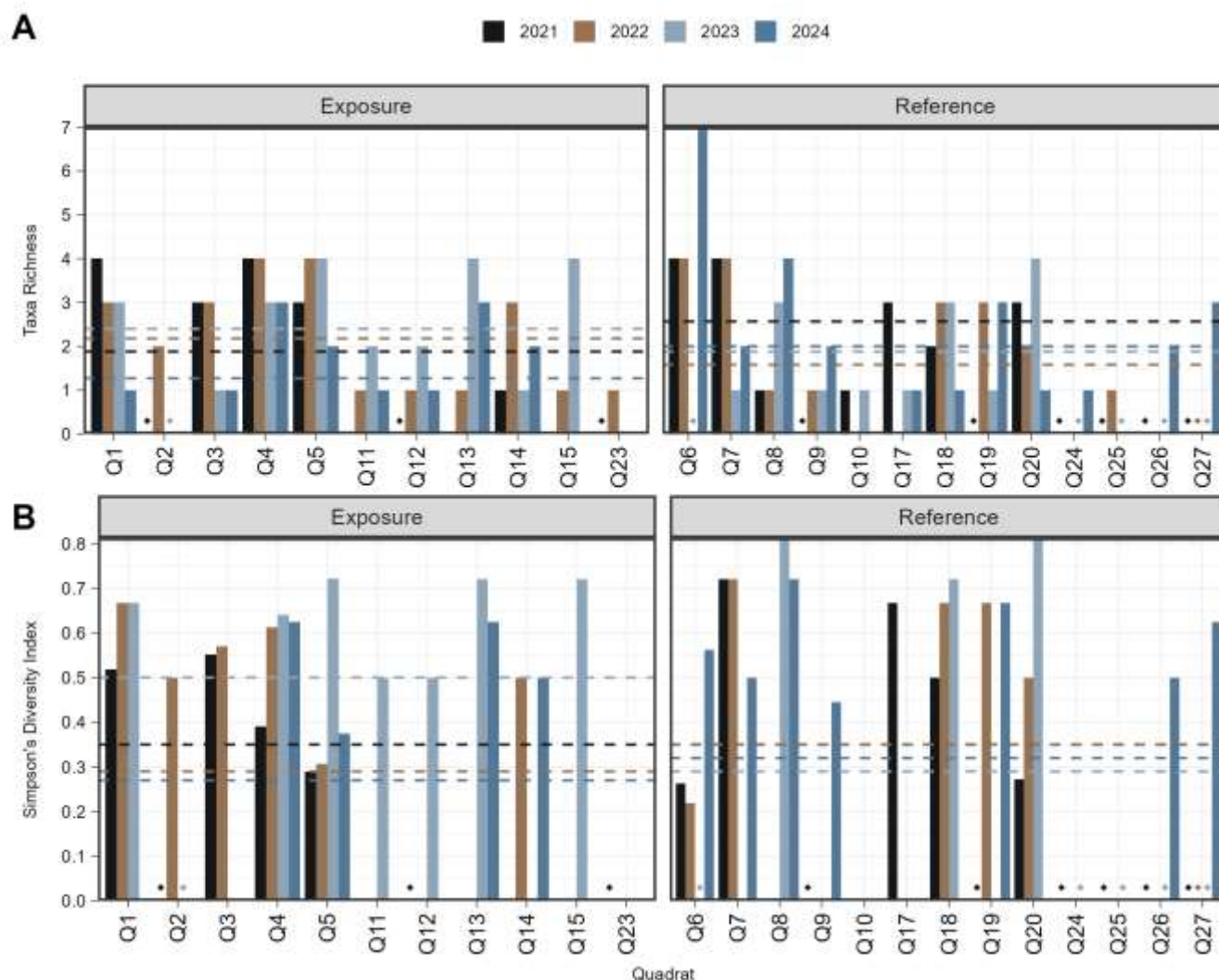
**Figure 5-13: A) Total Percent Cover and B) Relative Abundance of Motile Epifauna Recorded in Quadrats Surveyed During Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed line in top figure represents the mean percent cover for the exposure and reference quadrats for each year, diamonds represent years in which individual quadrats were not sampled, and red stars indicate data was an outlier ( $>100$  org/m<sup>2</sup>) and excluded from the plot for clarity (see Appendix 5C).



**Figure 5-14: Interannual Differences in Motile Epifauna Density, Taxa Richness, and Simpson's Diversity Index in Milne Port, 2021 – 2024.**

Note: Observed (solid points) and estimated marginal mean percent cover (open points) and 95% confidence interval (error bars) are presented for each sampling area and year. Two outliers/influential points were removed from the density plot (Q6 in 2021 and 2022) for clarity due to high values ( $>100$  org/m<sup>2</sup>).



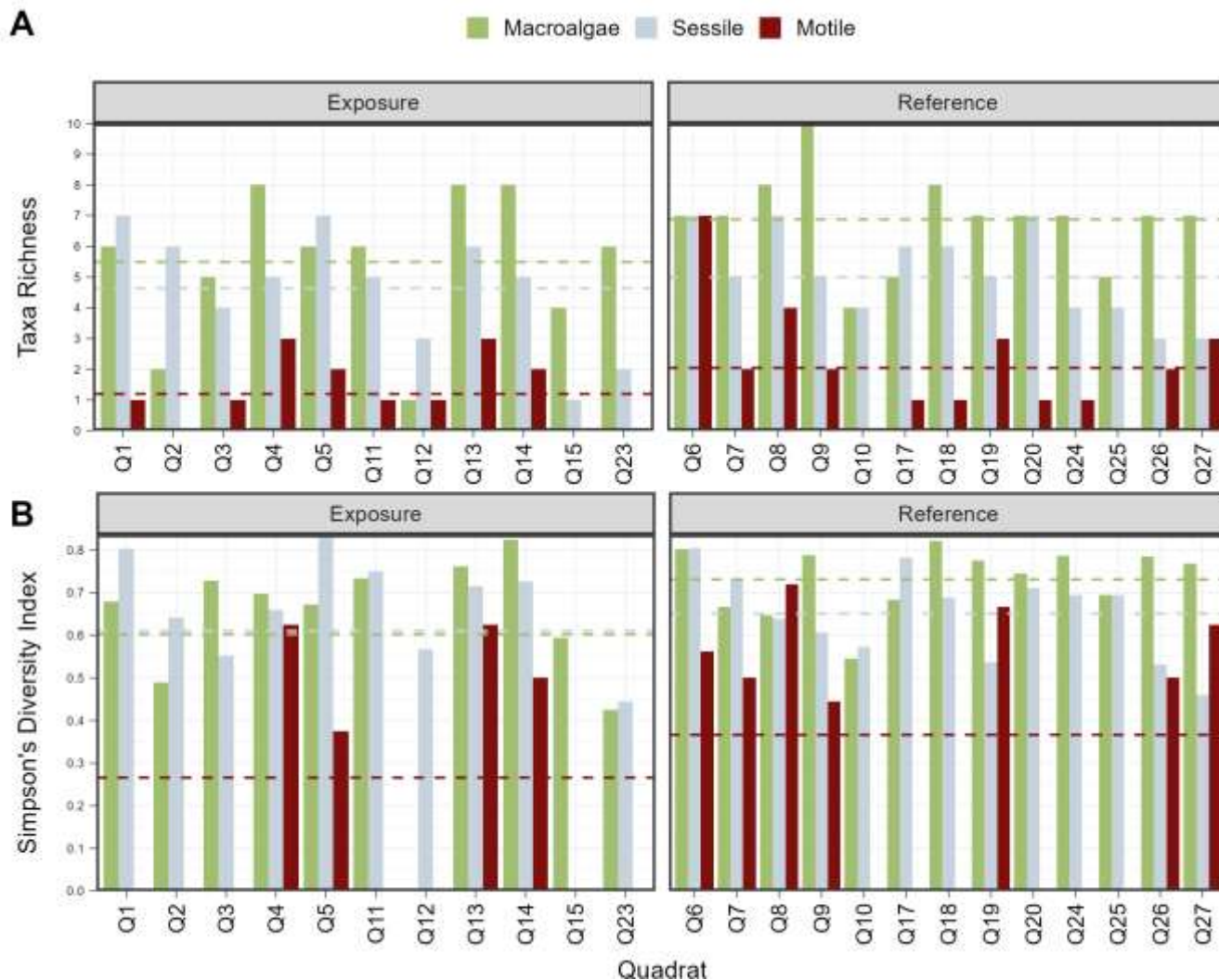
**Figure 5-15: A) Taxa Richness and B) Simpson's Diversity Index of Motile Epifauna Recorded in Quadrats Surveyed During Quadrat Surveys in Milne Port, 2021 – 2024.**

Note: Only quadrats sampled in 2024 are represented throughout the years. Dashed lines represent the mean percent cover for the exposure and reference quadrats for each year and diamonds represent years in which individual quadrats were not sampled.

#### 5.4.4 Relative Diversity

Taxa richness varied among quadrats for all major assemblages (macroalgae, sessile epifauna, and motile epifauna; Figure 5-16A). Generally, lower mean values and greater variability was seen within the exposure area compared to the reference area for major assemblages (Figure 5-12A). Statistical analysis indicated differences in taxa richness for macroalgae and sessile epifauna between years but not between areas (Table 5-3), consistent with 2023 results. Overall, Q9 in the reference area supported the greatest taxa richness for macroalgae; Q1, Q5, Q6, and Q8 supported the greatest taxa richness for sessile epifauna, and Q6 supported the highest taxa richness for motile epifauna (Figure 5-16A). Q12 in the exposure area supported the lowest macroalgae taxa richness consistent with 2022 and 2023, while Q15 supported the lowest sessile epifauna taxa richness and Q2, Q10, Q15, Q23, and Q24 supported the lowest motile epifauna taxa richness (Figure 5-16A).

SDI for macroalgae ranged from very low to high, sessile epifauna SDI ranged from low to high, and motile epifauna ranged from very low to moderate (Figure 5-16B); statistically significant differences in SDI were found for macroalgae and sessile epifauna between years but not among sampling areas (Table 5-3). Overall, Q4 and Q13 in the exposure area and Q8 in the reference area displayed the greatest universal diversity, where macrofauna, sessile and motile epifauna SDI values were all >0.600 (moderate to high; Figure 5-16B). Q12 was the only quadrats to have very low diversity value for macroalgae while Q23 was the only quadrat to have very low to low diversity values for the three types of assemblages (Figure 5-16B).



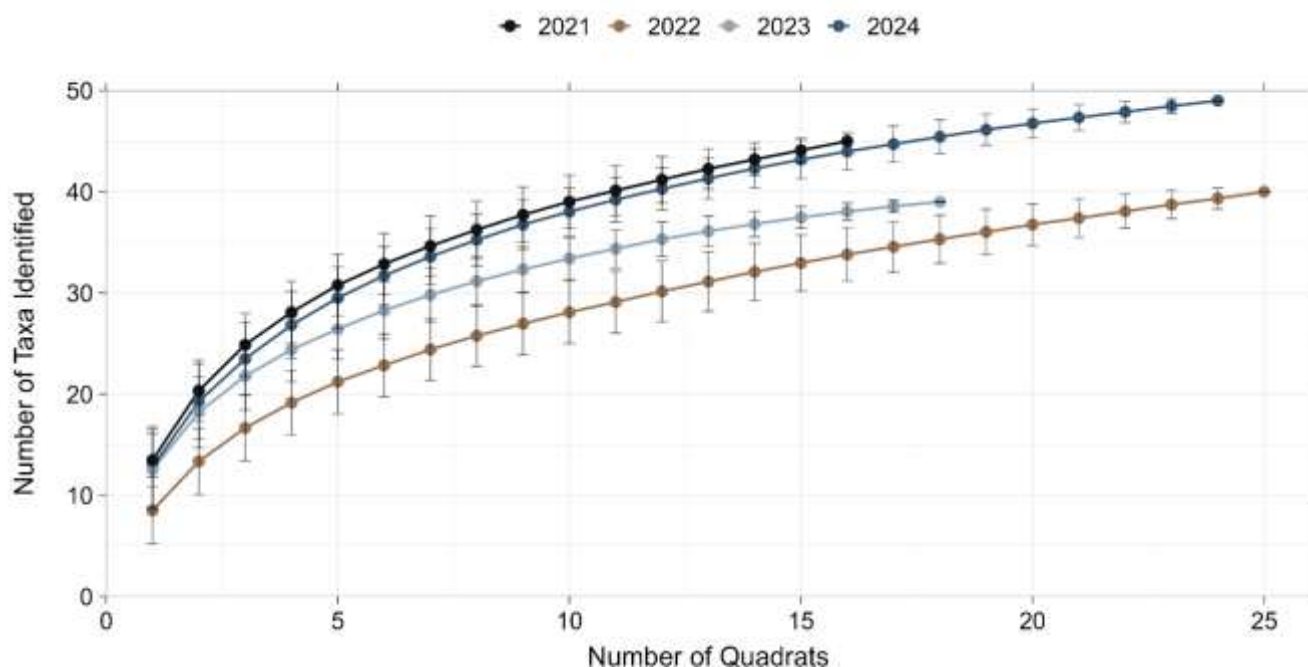
**Figure 5-16: A) Taxa Richness and B) Simpson's Diversity Index (SDI) for Macroalgae, Sessile Epifauna, and Motile Epifauna in Milne Port (2024).**

Note: Dashed lines represent the mean percent cover for the exposure and reference quadrats for each indicator.



### 5.4.5 Species Accumulation Curve and Sampling Effort

A taxa accumulation curve was calculated for quadrats surveyed in 2021 to 2024 to provide an estimate of the effort required to fully characterize the quadrat benthic community assemblage (macroalgae, sessile and motile epifauna; Figure 5-17). While reaching an asymptote within a subsample of a locality is rare, this would indicate that nearly all species have been detected within the location sampled given the level of sampling effort. For years 2021 to 2024, the four curves approached, but did not reach, an asymptote for the number of quadrats sampled in each year. This indicates that sampling in all four years did not reach levels to fully describe the overall benthic community assemblage.



**Figure 5-17: Mean Taxa Accumulation Curve for Quadrat Benthic Community Assemblage in Milne Port, 2021 – 2024.**

Note: Mean number of taxa identified (solid circles) and one standard deviation (error bars) are shown for each sampling year. Values were determined using 999 permutations.

### 5.4.6 Opportunistic Observations

While swimming to Q12, divers opportunistically found the original Q2, which was lost in 2021, west of the existing Q12 (Appendix 5B –Photo 24). The quadrat was mostly buried in soft sediment with observation of the quadrat only visible via the deployment chain and attached settlement plates and buoy. Wrinkled rock-borers, brittle stars, and very low cover of brown filamentous algae were observed on top of/within the quadrat and bobtail squid eggs (*Sepiolina* sp.<sup>15</sup>) were observed attached to the settlement plates (Appendix 5B –Photo 25). A Fourhorn Sculpin was also observed within the adjacent area to original Q2.

<sup>15</sup> Eggs were opportunistically collected near the Freight Dock and submitted to Biologica for taxonomic identification.

Saddled Eelpouts (Appendix 5B – Photo 26) were observed residing within the hollow frame of one quadrat surveyed in 2024 (Q3 in exposure area) as well as in the opportunistically observed old Q2. This is the fourth year that eelpouts have been observed within the quadrat frames. For the second year, there were no observations of deceased bivalves, some with blackened siphon ends, such as those observed within and outside of survey quadrats in both areas in 2022 (WSP 2023).

Opportunistic specimens collected for laboratory taxonomic identification around quadrats included the following:

- Mottled red chiton (*Tonicella marmorea*), colonial bryozoans of the Order Cheilostomatida (one identified to Family Calloporidae), tunicates (*Boltenia echinata* and Order Stolidobranchia), and an unidentified sponge (Class Demospongiae) observed outside/on the frame of Q6
- Scale worm (*Harmothoe imbricata*) on the frame of Q7
- Bryozoan (*Alcyonidium* sp.) attached to a settlement plate from Q7
- Mussel (*Mytilus* sp.) from Q14
- Nudibranch (Superfamily Dendronotoidea) from Q17

Epifauna opportunistically observed outside the quadrats included blunt gapers, wrinkled rock-borers, brittle stars, encrusting coralline algae, cone worms and green urchins outside of several quadrats within the exposure (Q3, Q4, Q5, Q10, Q12) and reference areas (Q6, Q28). Epifauna were also observed utilizing the settlement substrates and associated attachment lines, including a green urchin on the deployment chain of Q3, a pink shrimp on the deployment chain and bobtail squid eggs on the settlement plate above Q6, barnacles on the settlement ropes of Q7 and Q24, unidentified hydroids on the settlement plate of Q17, and *Alcyonidium* sp. on the rope of Q20. A sculpin skeleton was also observed within Q9. Mysid shrimp (Order Mysida) were observed in abundance in the water column at quadrats Q7, Q9, Q15, and Q17; several Arctic comb jellies (*Mertensia ovum*) were observed above Q9, Q10, and Q24, and a sea angel (*Clione limacina*) was observed above Q23 in the exposure area.

Nearly all quadrats were observed to have macroalgae growing on the steel quadrat frames, including sugar kelp, rockweed, and filamentous algae. Additionally, the macroalgae growing on the crossbars and within the quadrats were observed to join to create three-dimensional habitats within the perimeter of the quadrats, making exclusion of some macroalgae from analyses challenging. Numerous epifauna across 13 quadrats were also observed utilizing the quadrat frame and crossbars as habitat but were eliminated from analyses due to their location within unsurveyed areas. These organisms included green urchins, wrinkled rock-borers, dendronotid nudibranchs, brittle stars, scallops, mussels, barnacles, sabellid worms, scale worms, and cone worms.

A bryophyte specimen observed outside of the surveyed area in 2024 was identified as the short-leaved spear moss (*Pseudocalliogon brevifolium*).



## 5.5 Discussion

Substrate type was similar among quadrats and between the exposure and reference areas. Substrate within the quadrats was dominated by soft substrate (silt and sand), consistent with what has been previously documented (Golder 2022; WSP 2023, 2024). Statistically significant interannual differences in soft substrate percent cover were attributed to interannual variations in gravel as well as the quadrats (Q21-Q26) deployed in 2022, which generally contained higher percentages of coarse substrate in 2022.

Similar macroalgae and benthic epifaunal taxa were observed in 2024 as in previous years (2021 to 2023). Indicators (i.e., percent cover, density, and diversity) were variable among quadrats, expected for this component of the MEEMP, given the dynamic coastal environment of Milne Inlet: factors such as freshwater input, sediment transport processes, ice scour, and competition for attachment from annually dynamic taxa such as tube-dwelling diatoms influence the percent cover/density and distribution of species in any given year. Indicators were not significantly different between exposure and reference area in 2024; however, interannual differences were observed in most macroalgae and sessile indices, as well as interaction effects of year and area with motile density. Macroalgae indices increased in 2024 compared to years 2021-2023 for both areas whereas sessile and motile indices were more variable and generally more comparable to previous years, with the majority of significant differences primarily distinguishing the results of 2021 from those of 2022-2024. The only significant decrease in a sessile or motile index identified in 2024 was for motile density in 2024 compared to 2021 in the exposure area, driven largely by higher densities of green urchins at quadrats Q1, Q3, Q4, and Q5. Cooler average water temperatures in 2024 (Chapter 2.0 Marine Water Quality) may have resulted in suboptimal conditions for urchins (Blicher et al. 2007; Filbee-Dexter et al. 2022). No significant differences were identified between 2023 and 2024 in either area for any of the examined indicators.

Overall, there was no evidence of project-related effects on substrate, macroalgae, or benthic epifauna, demonstrated by similar temporal variation observed among both sampling areas, suggesting regional-level drivers of interannual variation.

## 5.6 Conclusions and Recommendations

Overall, macroalgae and benthic epifaunal community assemblages were comparable between exposure and reference areas though some indicators varied interannually, likely driven by natural environmental factors (e.g., temperature, salinity, ice scour, ice cover). Monitoring efforts to date revealed no evidence of overarching spatial or temporal trends that might be associated with Project-induced effects from construction or operation activities at Milne Port. Monitoring of macroalgae and benthic epifauna assemblages should continue using generally the same sampling and statistical design. However, it is recommended that in 2025, sampling design be altered to include the area on top of the quadrat outer frame and crossbars in analyses due to increased utilization of the frame components as three-dimensional habitat by macroalgae and benthic epifauna, as observed in 2024. Surveys should continue to exclude the components of the settlement substrates above the quadrat as well as organisms observed inside the hollow quadrat frame. It is also recommended that the lost quadrats Q21 and Q22, located adjacent to Phillips Creek, not be replaced in 2025. If required to sample in this area, future monitoring surveys could employ a flexible approach by first conducting a search for the lost quadrats with set time and depth constraints and if the permanent quadrats are not located, a non-permanent quadrat could be surveyed at the 2022 deployment coordinates. Additionally, it is recommended to continue to monitor for opportunistic observations of deceased bivalves (as seen in 2022) and unknown taxa and that samples should be collected, when possible, for analyses and identification.

The power analyses found sufficient power to detect larger changes in substrate, epifauna and macroalgae. Given the low statistical power to detect effect sizes that may be of biological relevance, going forward, it is recommended that conclusions are not made based on strict adherence to statistical significance. Instead, effect size, uncertainty, and statistical significance and power should be considered together before ruling out spatial and temporal changes in benthic infauna.

## 5.7 Closure

We trust this information is sufficient for your needs at this time. Should you have any questions or concerns, please do not hesitate to contact Phil Rouget, on behalf of the undersigned, at +1 250 419 4945.

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**APPENDIX 5A**

**DFO's Marine Foreshore  
Environmental Assessment  
Procedures**



## MARINE FORESHORE ENVIRONMENTAL ASSESSMENT PROCEDURE

Marine development projects have the potential to effect fish<sup>1</sup> and fish habitat<sup>2</sup>. Fisheries and Oceans Canada (DFO) is responsible for the protection and management of fish habitats under the authority of the *Fisheries Act* and may request plans, specifications and environmental assessments specific to marine projects where more detailed information is required. Assessments may be necessary for all types of projects, including, but not limited to aquaculture, log handling, industrial port development, marinas, private moorage facilities, marine repair facilities, pipeline or outfall installations, vessel launches or barge ramps, dredging projects and shoreline protection projects (breakwaters and seawalls). Presented below are standardized, transect-based assessment procedures intended to provide DFO with the basic information required to determine the potential effects of a development project on fish habitat.

### Assessment Area

For comparative purposes, the assessment area should include both the foreshore site proposed for development as well as the adjacent foreshore. This will provide a context for the project and may provide data about cumulative effects if similar developments already occur on-site. A large scale site plan, preferably an enlargement of the hydrographic chart, with a small scale insert of the general geographic location will serve as a base map of the study area.

### Tidal Height and Water Depth Measurements

The lowest normal tide (0.0 m), or chart datum, will be used as the reference point for the measurement of tidal height and water depth. Tidal height is recorded as positive relative to chart datum, while water depth below chart datum will be recorded as a negative value. For example, if the assessment is made when the tide is at 2 m, and observations are taken at a water depth of 6 m, then the depth will be recorded as -4 m. Tidal height will be corrected using the closest secondary port to the reference port found in the Canadian Tide and Current Tables, with further correction made for daylight savings time as required.

### Transect Layout

Transects should be established perpendicular to the shoreline at regular intervals both within and adjacent to the proposed or active development area so as to sample representative fish habitat conditions. A preliminary low water reconnaissance or dive survey may be advisable to establish

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<sup>1</sup> shellfish, crustaceans, marine animals and any parts of shellfish, crustaceans or marine animals, and the eggs, sperm, spawn, larvae, spat and juvenile stages of fish, shellfish, crustaceans and marine animals;

<sup>2</sup> shellfish, crustaceans, marine animals and any parts of shellfish, crustaceans or marine animals, and the eggs, sperm, spawn, larvae, spat and juvenile stages of fish, shellfish, crustaceans and marine animals;

appropriate boundaries for the assessment. Transects should begin at the highest high water mark (HHWM: distance referenced as Station 0.0 m) and, at a minimum, extend to a depth of -20 m (-30 m if the development has the potential to effect deeper benthic habitats). Though small-scale intertidal projects may only require intertidal transects, care must be taken to ensure that a representative sample is collected across the proposed development area. Procedural manuals are available from DFO if sampling of intertidal clam or benthic invertebrates is required. To ensure complete assessment of marine plants and animals in the photic zone, deeper transects may be necessary, especially to determine the effects of sunken debris or woodwaste accumulations resulting from existing developments. Transects should be spaced approximately 25 m apart, although this interval may vary depending on the width of the site. The number of transects required will depend on the nature of the foreshore development proposed, anticipated effects of the development, and local site conditions (tides and currents, geography, fetch, geology, etc.). Transects should be individually numbered and indicated on the site plan, and their commencement point referenced to benchmarks, where possible.

### **Recording Observations**

Habitat inventories should be conducted during the more productive spring and summer months. At that time, algae and saltmarsh species are more readily identifiable, enabling a better assessment of the productive capacity of the site.

Observations should be recorded every 5 m along the transect or at significant changes in habitat type. Observations should include substrate type and composition, presence and relative abundance of marine animals and plants, and any other notable features (e.g., debris accumulations) using the following format:

#### **Substrate**

Substrate types are to be subdivided into the following size class categories:

- Bedrock
- Boulder (>256 mm diameter)
- Cobble (64-256 mm diameter)
- Gravel (2-64 mm diameter)
- Sand (0.0625-2 mm diameter)
- Silt/Mud/Clay (<0.0625 mm diameter)

Substrate types are recorded cumulatively as percentages out of a total of 100% (e.g., Boulder 5%; Cobble 15%; Gravel 60%, Sand 20%)

### **Marine Plants**

Marine plants include rooted vascular vegetation (e.g., eelgrass, saltmarsh vegetation, etc.) and marine algae (e.g., rockweed, kelp, etc.). Marine plant observations are recorded as percent areal coverage estimated per 5 m × 1 m transect segment. Observations can be recorded as percentages (5%, 10%, 15%, etc.) or by utilizing the following areal coverage classes:

|   |          |
|---|----------|
| + | <5%      |
| 1 | 5-25%    |
| 2 | >25-50%  |
| 3 | >50-75%  |
| 4 | >75-100% |

### **Sessile Animals**

Many marine animals permanently attached to substrates function as important fish habitat (e.g., barnacles, bay mussels, etc.). Sessile animals are recorded as percent areal coverage along the transect line using either estimated percentages or by areal coverage classes, as presented above.

### **Motile Animals**

Motile animals include fish and marine invertebrates such as crabs and snails. These can be individually counted along the transect or, where too numerous, their estimated numbers can be recorded. Population estimates will most likely be applied to species such as herring or mysid shrimp that naturally occur in large numbers.

### **Other Features**

Accumulations of wood bark and debris, sunken logs or other waste materials arising from onsite or nearby development activities should also be recorded. For wood bark and related small size debris, observations are recorded as percent areal coverage estimates per 5 m × 1 m transect segment and estimated deposition depth (e.g., 15% / 10 cm). For larger materials (sunken logs, wood chunks, etc.), observations can be recorded by individual piece count or by estimate of percent areal coverage.

Observations should be correlated to the transect distance from the HHWM and (corrected) tidal height or water depth (e.g., Sta. 0+80 m / +4.5 m), with information compiled in tabular form, by transect. Common names of observed animals and plants are acceptable for the data table; a species list with scientific names should, however, be appended to the report.

General marine plant categories (e.g., rockweed, eelgrass, bull kelp, saltmarsh, etc.) and any other notable features should be sketched to scale directly on a copy of the site plan, drawings or photographs of the site. A site profile should be prepared for each transect showing the slope of the foreshore and the location of indicator marine plants or invertebrates. A sketch of the proposed marine development should be superimposed over the site plan so that any potential effect of the project on fish habitat is clear. Compensatory habitat proposed for offsetting altered habitat should also be sketched on site maps and profiles to enable review of the positioning of replacement habitat relative to the project.

### **Photographic Documentation**

It is essential to produce a photographic record along the intertidal and subtidal transects. A videographic record of subtidal transects is also recommended. Photos and videos provide a real-time record of characteristic fish habitat at the proposed site and can be invaluable to future post-development site monitoring. Photographic records also facilitate comparison of the productivity of natural habitats with any compensatory habitat constructed to offset habitat losses. As visibility may be a problem, careful attention should be given to appropriate tidal levels, and midday lighting conditions are recommended. Aerial photos, taken at low tide, are often useful to put the site into context with the surrounding area and to verify information provided from other sources.

Assessment reports should include photographs of representative fish habitat types. Depending upon the scope of the proposed foreshore development, an unedited, labelled copy of the assessment video may also be required for the report submission. The video footage should be referenced with pertinent information (e.g., time, date, depth, heading, etc.), and a written or recorded interpretation should accompany the video.

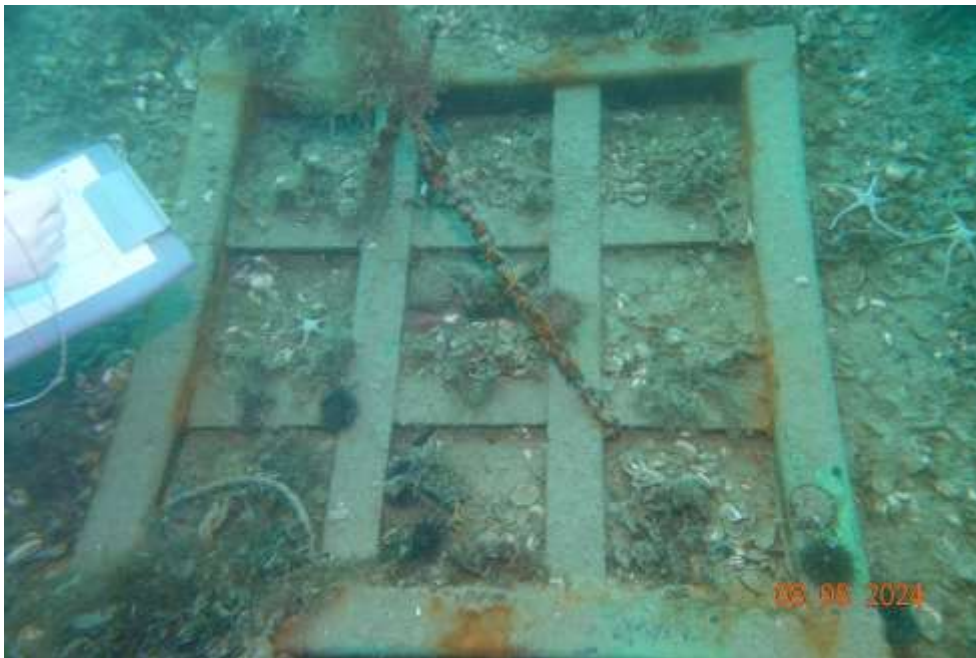
### **Summary of information to be submitted**

1. Basemap showing tenure area boundaries, surrounding area, transect locations and sampling stations
2. Shoreline video/photographs of intertidal zone
3. Underwater video/photographs of transects
4. Tabular data for each transect describing substrate type and composition, marine plants, sessile and motile marine animals, and other notable features
5. Habitat map showing location of different substrate types, plants, animals and operational infrastructure
6. Profile diagrams of each transect showing slope, sediment types and the major marine plants or animals observed
7. Photographs of site and aerial photographs if available.

**APPENDIX 5B**

**Photographs**

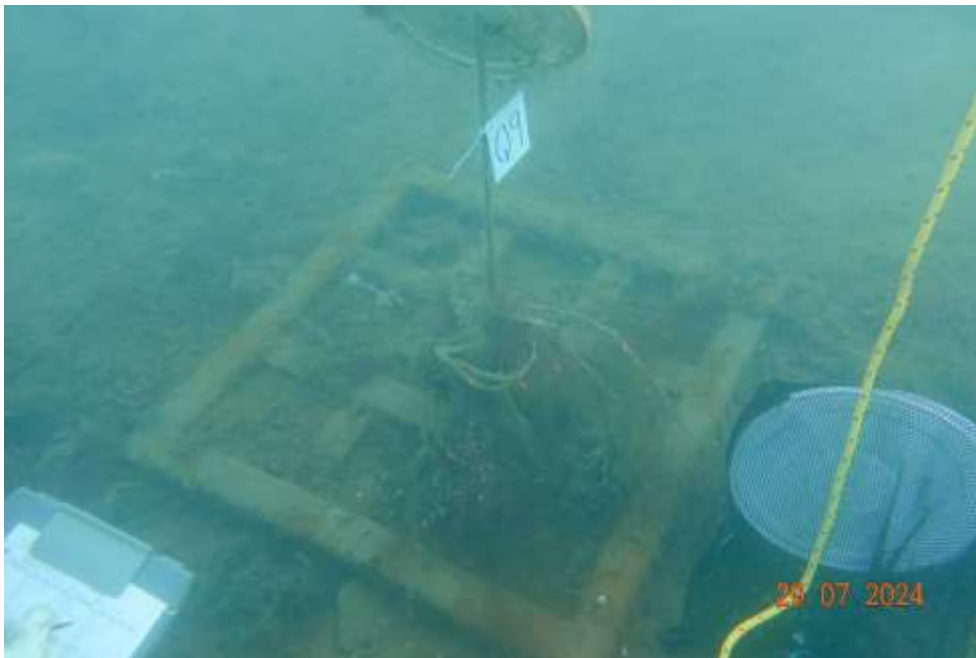




**Photo 1:** Representative quadrat (Q6) being surveyed by scientific diver in the reference area.



**Photo 2:** Representative photo of settlement plates and baskets attached to metal quadrat Q27 in the reference area.



**Photo 3:** Plastic quadrat label attached to buoy line holding settlement plates and associated float on quadrat Q9 during surveys in the reference area.



**Photo 4:** Detrital veneer (diatoms and silt) covering the substrate and quadrat cross bars of Q23 in the exposure area.



**Photo 5:** Sugar Kelp (*Saccharina latissima*) within quadrat Q1 in the exposure area.



**Photo 6:** Fish doctor (*Gymnelus viridis*) on rockweed (*Fucus distichus*) and diatoms within quadrat Q27 in the reference area.





**Photo 7:** Brown filamentous *Pylaiella* sp. and red filamentous *Savoiea arctica* growing within Q11 within the exposure area.



**Photo 8:** Acid weed (*Desmarestia* sp.) and Icelandic scallops (*Chlamys islandica*) within Q5 in the exposure area.



**Photo 9:** *Coccotylus truncates* growing within quadrat Q19 in the reference area.



**Photo 10:** Red branched *Phycodrys fimbriata* surrounded by small sabellid worms (Family Sabellidae) within quadrat Q19 in the reference area.





**Photo 11:** Mussel (*Mytilus* sp.; one included in analyses and one opportunistically observed on the quadrat frame) with green filamentous *Chaetomorpha melagonium* within Q14 in the exposure area.



**Photo 12:** Green filamentous *Spongomorpha aeruginosa* growing on the frame edge of quadrat Q9, opportunistically observed in the reference area.





**Photo 13:** Quadrat Q12 partially buried in the soft substrate offshore of West Beach within the exposure area.



**Photo 14:** Scale worm (*Harmothoe imbricata*) opportunistically observed on the quadrat frame of Q26.



**Photo 15:** Wrinkled rock-borer (*Hiatella arctica*) siphon protruding from soft sediment with sugar kelp in Q3 in the exposure area.



**Photo 16:** Blunt gaper (*Mya truncata*) within Q10 in the reference area.





**Photo 17:** Cone worms (*Cistenides granulata*) amongst deceased wrinkled rock-borer shells in quadrat Q14 in the exposure area.



**Photo 18:** Ribbon worm (Phylum Nemertea) moving into quadrat Q6 during the survey in the reference area.



**Photo 19:** Brittle star (Class Ophiuroidea) observed in quadrat Q12 within the exposure area.



**Photo 20:** Green urchin (*Strongylocentrotus droebachiensis*; left) and six-rayed sea star (*Leptasterias* sp.; right) within quadrat Q5 in the exposure area.





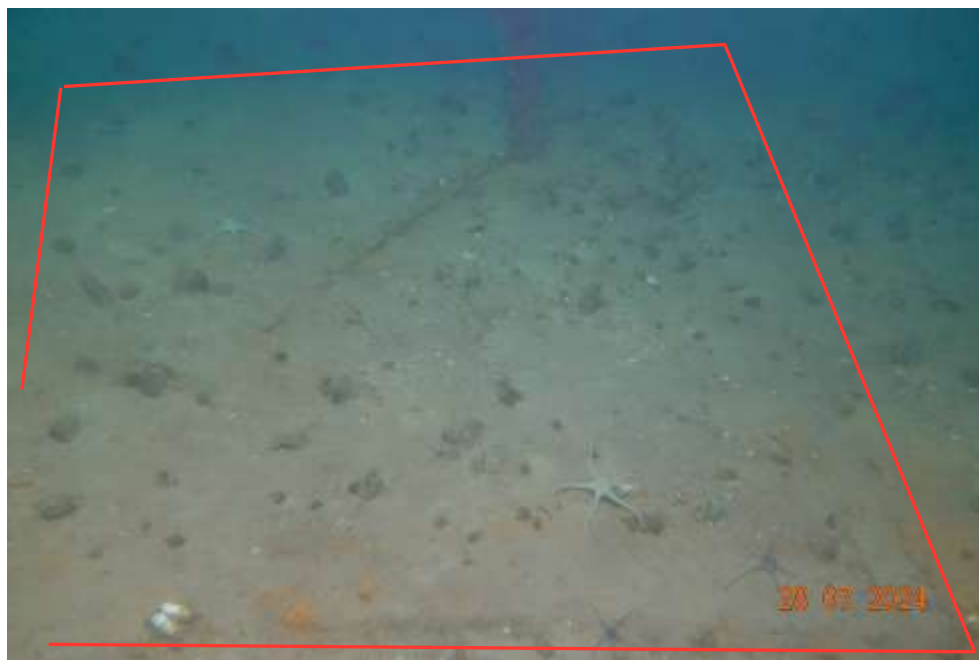
**Photo 21:** Shorthorn Sculpin (*Myoxocephalus scorpius*) within quadrat Q6 in the reference area.



**Photo 22:** Juvenile Lumpfish (*Cyclopterus lumpus*) within quadrat Q9 in the reference area.



**Photo 23:** Slender Eelblenny (*Lumpenus fabricii*) on the soft substrate within quadrat Q13 in the reference area.



**Photo 24:** Original Q2 (outlined in red) deployed in 2020 and unable to be located in 2021, opportunistically found during surveys mostly buried in the soft sediment in the exposure area.





**Photo 25:** Bobtail squid (*Sepiolina* sp.) eggs attached to settlement substrates above original Q2 in the exposure area.



**Photo 26:** Saddled eelpout (*Lycodes mucosus*) opportunistically observed within the inner frame of quadrat Q3 in the exposure area.

**APPENDIX 5C**

**Quadrat Survey Data (2021 - 2024)**

**Table 1: 2021 Quadrat Survey Data, Milne Port**

\* Tubes are likely sabellid worms, though animal inside tube was not observed during survey and not included in data analysis.

**APPENDIX 5D**

**Taxa List**

# APPENDIX 5D

## Taxa List

| Common Name                            | Scientific Name                          |
|--|--|
| <b>Macroalgae</b>                      |  |
| <b>Brown Algae / Kelp (Ochrophyta)</b> |  |
| -                                      | <i>Battersia</i> sp.                     |
| Acid weed                              | <i>Desmarestia</i> sp.                   |
| -                                      | <i>Dictyosiphon foeniculaceus</i>        |
| Rockweed                               | <i>Fucus distichus</i>                   |
| -                                      | <i>Halosiphon tomentosus</i>             |
| -                                      | <i>Pylaiella</i> sp.                     |
| Sugar kelp                             | <i>Saccharina latissima</i>              |
| -                                      | <i>Stictyosiphon tortilis</i>            |
| <b>Red Algae (Rhodophyta)</b>          |  |
| -                                      | <i>Coccotylus truncatus</i>              |
| -                                      | <i>Dilsea socialis</i>                   |
| -                                      | <i>Phycodrys fimbriata</i>               |
| -                                      | <i>Polysiphonia</i> sp.                  |
| -                                      | <i>Savoiea arctica</i>                   |
| Encrusting coralline algae             | Order Corallinales                       |
| <b>Green Algae (Chlorophyta)</b>       |  |
| -                                      | <i>Chaetomorpha melagonium</i>           |
| -                                      | <i>Spongomorpha aeruginosa</i>           |
| Green filamentous                      | Phylum Chlorophyta                       |
| <b>Marine Invertebrates</b>            |  |
| <b>Annelida</b>                        |  |
| Cone worm                              | <i>Cistenides granulata</i>              |
| Scale worm                             | <i>Harmothoe imbricata</i>               |
| Small sabellid worm sp. 1              | Family Sabellidae                        |
| Large sabellid worm sp. 2              | Family Sabellidae                        |
| Spaghetti worm                         | Family Terebellidae                      |
| <b>Arthropoda</b>                      |  |
| Pink shrimp                            | <i>Pandalus montagui</i>                 |
| Amphipod                               | Order Amphipoda                          |
| Barnacle                               | Class Balanomorpha                       |
| Mysid*                                 | Order Mysida                             |
| <b>Bryozoa</b>                         |  |
| Bryozoan*                              | <i>Alcyonidium</i> sp.                   |
| Bryozoan*                              | Family Calloporidae                      |
| Bryozoan*                              | Order Cheilostomatida                    |
| <b>Cnidaria</b>                        |  |
| Burrowing anemone                      | Order Ceriantharia                       |
| <b>Ctenophora</b>                      |  |
| Arctic comb jelly*                     | <i>Mertensia ovum</i>                    |
| Ctenophore                             | Phylum Ctenophora                        |
| <b>Echinodermata</b>                   |  |
| Six-rayed sea star                     | <i>Leptasterias</i> sp.                  |
| Green urchin                           | <i>Strongylocentrotus droebachiensis</i> |
| Brittle star                           | Family Ophiuridae                        |

| Common Name                 | Scientific Name                   |
|-----------------------------|-----------------------------------|
| <b>Marine Invertebrates</b> |                                   |
| <b>Mollusca</b>             |                                   |
| Northern astarte            | <i>Astarte borealis</i>           |
| Icelandic scallop           | <i>Chlamys islandica</i>          |
| Sea angel*                  | <i>Clione limacina</i>            |
| Bushy-backed sea slug       | <i>Dendronotus</i> sp.            |
| Wrinkled rock-borer         | <i>Hiatella arctica</i>           |
| Macoma clam                 | <i>Macoma</i> sp.                 |
| Margarite snail             | <i>Margarites</i> sp.             |
| Discord mussel              | <i>Musculus discors</i>           |
| Blunt gaper                 | <i>Mya truncata</i>               |
| Mussel                      | <i>Mytilus</i> sp.                |
| Nut clam                    | <i>Nuculana</i> sp.               |
| Bobtail squid (eggs)*       | <i>Sepiolina</i> sp.              |
| Greenland cockle            | <i>Serripes groenlandicus</i>     |
| Greenland scallop           | <i>Similipecten greenlandicus</i> |
| Common tortoise limpet      | <i>Testudinalia testudinalis</i>  |
| Mottled red chiton*         | <i>Tonicella marmorea</i>         |
| Nudibranch*                 | Superfamily Dendronotoidea        |
| <b>Nemertea</b>             |                                   |
| Ribbon worm                 | Phylum Nemertea                   |
| <b>Porifera</b>             |                                   |
| Sponge*                     | Class Demospongiae                |
| <b>Tunicata</b>             |                                   |
| Tunicate*                   | <i>Boltenia echinata</i>          |
| Tunicate*                   | Order Stolidobranchia             |
| Tunicate                    | Phylum Tunicata                   |
| <b>Fish</b>                 |                                   |
| Lumpfish                    | <i>Cyclopterus lumpus</i>         |
| Fish Doctor                 | <i>Gymnelus hemifasciatus</i>     |
| Slender Eelblenny           | <i>Lumpenus fabricii</i>          |
| Eelblenny                   | <i>Lumpenus</i> sp.               |
| Saddled Eelpout             | <i>Lycodes mucosus</i>            |
| Fourhorn Sculpin*           | <i>Myoxocephalus quadricornis</i> |
| Arctic Sculpin              | <i>Myoxocephalus scorpioides</i>  |
| Shorthorn Sculpin           | <i>Myoxocephalus scorpius</i>     |

Note: '-' indicates no existing or unknown common name;  
 "\*" indicates taxa were only observed opportunistically during transect/quadrat surveys and were not included in analyses.

**APPENDIX 5E**

# Power Analysis



## POWER ANALYSIS – BENTHIC EPIFAUNA AND MACROALGAE

This section presents the results of a power analysis undertaken for the 2021–2024 benthic epifauna and macroalgae monitoring data at Milne Port.

### METHODS

A Type I error is concluding there is a significant effect when none exists (i.e., a false positive). Alpha ( $\alpha$ ) is the probability of committing a Type I error. A Type II error is the probability of concluding there is no significant effect when there is a real effect of some specified magnitude (i.e., a false negative). Beta ( $\beta$ ) is the probability of committing a Type II error. The power of a statistical test ( $1 - \beta$ ) is the probability of detecting a real effect. In this analysis, the Type I error-rate ( $\alpha$ ), also referred to as the significance level, was set to 0.05. The desired minimum statistical power was 80%, which corresponds to a type II error-rate of 0.2. Power analyses were conducted to assess the power of statistical tests under multiple effect sizes. For each model, a set of effect sizes was created, based on preliminary power analyses, so that power >80% was achieved at the largest absolute values of effect sizes, but also so that power is assessed at a range of effect sizes. Since the analysis focused on assessment of changes to statistical power at different effect sizes, the power analysis used the observed samples sizes from the collected data.

### Data Simulation following Effect Size Application

The power to detect statistically significant effects was estimated using residual bootstrapping in R v. 4.4.2 (R 2024), following the approach of Fox and Weisberg (2018). The general approach was to simulate data based on the model selected for interpretation, the observed sample size (or the sample size of choice), and the residuals, and re-run the models that were used for the original analysis using the simulated data. The data simulation and analysis were repeated 5,000 times, and the proportion of repetitions where the *P*-values of interest were significant ( $P < 0.05$ ) was interpreted as the statistical power of the test.

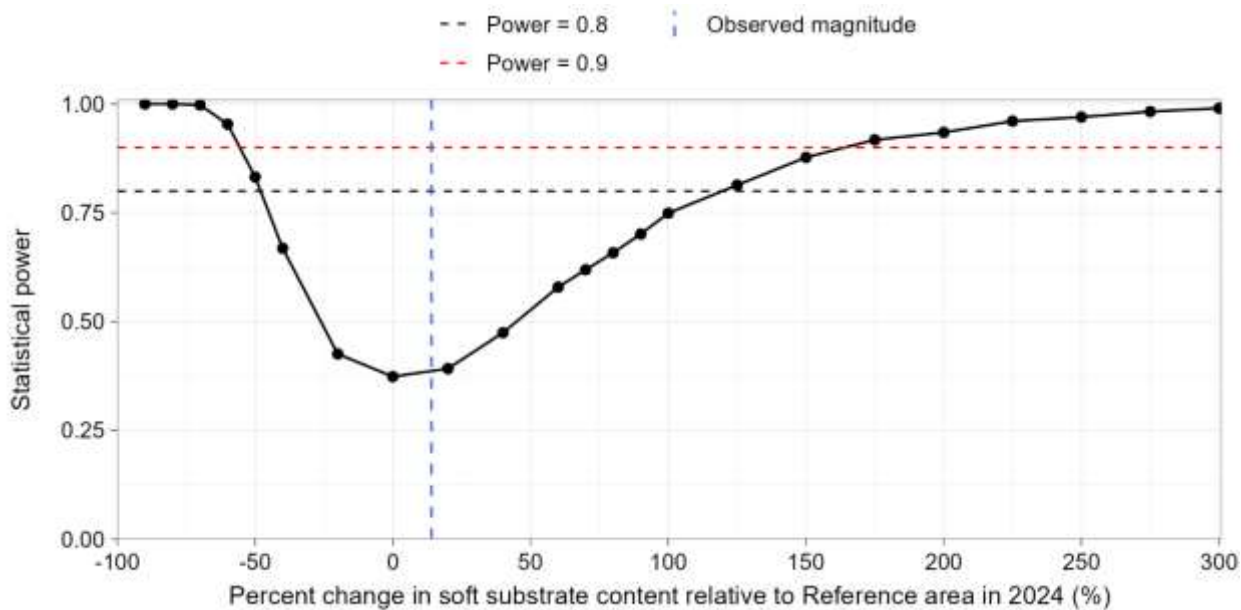
For all models, the simulated effect size was only applied to 2024 Exposure data. For models of macroalgae, sessile, and motile species richness, as well as motile density, where a Poisson mixed model was used, the effect size was applied to the incidence rate. For models of SDI and percent cover, beta mixed models were used to account for the data being restricted between 0 and 1. Thus, the effect was applied on the logit scale. For each iteration of the simulation, the predictions were estimated on the log-scale or the logit-scale, as applicable. Then, a Poisson or a beta distribution was used to generate random values using the predictions calculated above. The generation of random values was done to create random variability in the simulated data. For cases within the dataset that did not have an effect size applied to them (i.e., cases that were collected at the Reference area of prior to 2024), predictions were still used to generate a random value, resulting in simulated data that differed from the originally collected data. The simulated data were then analyzed using the same model structure as the original analysis.

In the analysis of 2021–2024 data, where the question of interest was the detection of change in response variables between exposure and the reference area, the effect was applied as percentage relative to the values predicted for the reference area. Overall, an increasing effect size resulted in higher simulated 2024 Exposure values compared to the 2024 Reference values, whereas a decreasing effect size resulted in lower 2024 Exposure values when compared to the 2024 Reference values. The simulated data were analyzed using the same model as the original analysis described in the main report, and the *P*-values for the interaction between area and year were retained. The proportion of repetitions with *P*-values less than 0.05 was interpreted as the statistical power of the overall regression for that effect size. The power analysis was performed on a range of

effect sizes. Power curves were produced, showing statistical power as a function of effect sizes. Horizontal lines were added to visualize statistical power values of 0.8 (hereafter sufficient power) and 0.9 (hereafter high power), and the observed effect size was provided in the results.

## RESULTS

There was sufficient power ( $\geq 0.8$ ) to detect an effect of -50% or +120% (Figure 1). That is, 2024 exposure soft substrate had to be 50% lower or 120% higher than 2024 reference values for the model to have sufficient power to detect a significant interaction. In comparison, the observed effect size was +14%. Statistical power to estimate the observed effects was approximately 0.3.

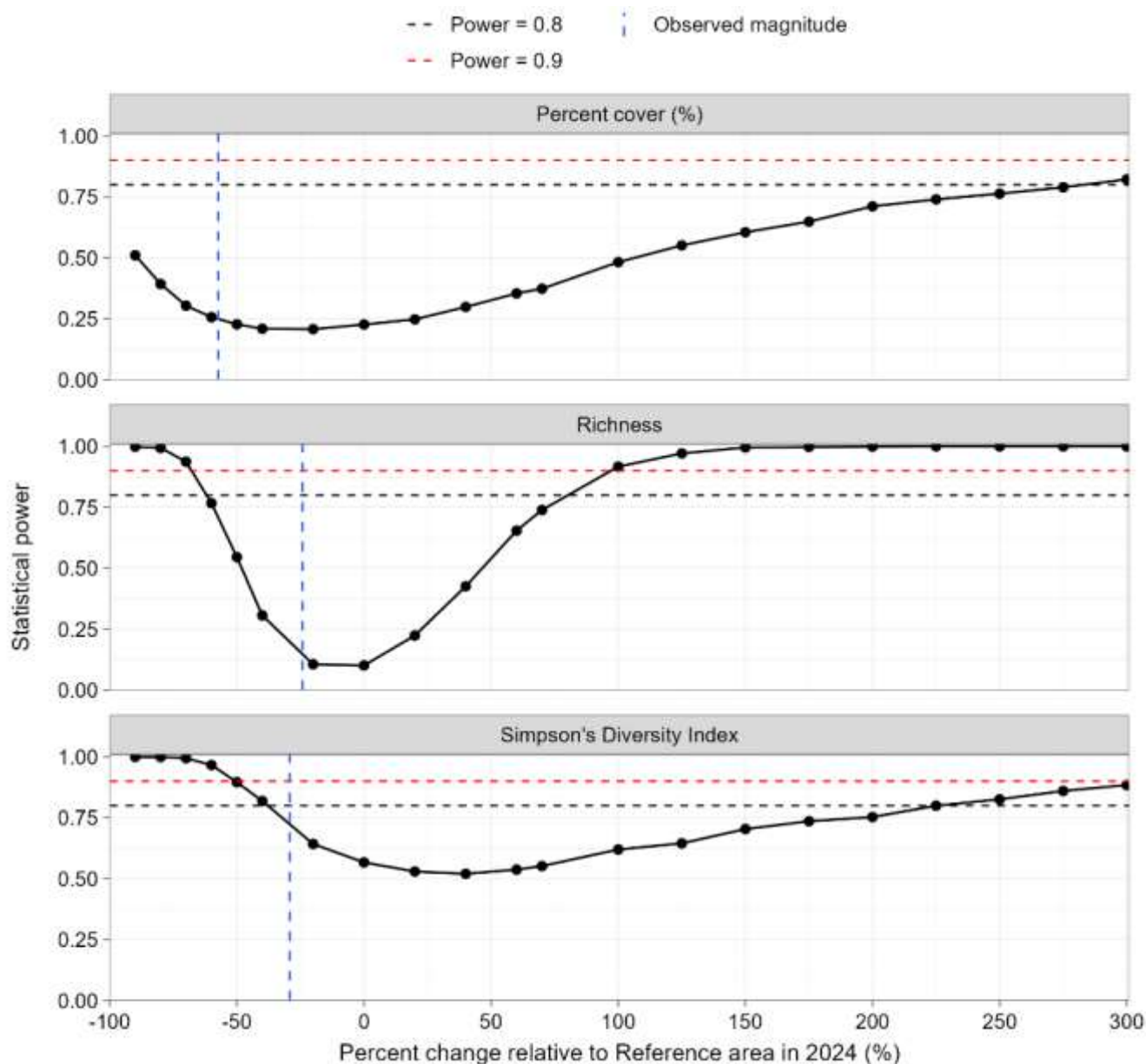


**Figure 1: Statistical Power of The Model of Percent Soft Substrate Data to Detect a Significant Interaction Between Year and Area Based on Quadrat Data Collected in 2021-2024.**

In the analysis of macroalgae percent cover, there was sufficient power ( $\geq 0.8$ ) to detect an effect of +270%, but none of the negative effect sizes (Figure 2). That is, the 2024 percent cover of macroalgae at the exposure area had to be 270% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into percent cover, a 270% increase means that the percent cover in the exposure area would need to be 84% (compared to the 59% value estimated in the 2024 reference area). In comparison, the observed effect size was -57%. Statistical power to estimate the observed effects was  $< 0.3$ .

In the analysis of macroalgae richness, there was sufficient power ( $\geq 0.8$ ) to detect an effect of -62% or +80% (Figure 2). That is, the 2024 macroalgae richness at the exposure area had to be 62% lower or 80% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into richness values, this means that in the exposure area, there would need to be an average of 2.2 macroalgae species or an average of 10.5 macroalgae species (compared to the 5.9 value estimated in the 2024 reference area). In comparison, the observed effect size was -24%. Statistical power to estimate the observed effects was  $< 0.3$ .

In the analysis of macroalgae SDI, there was sufficient power ( $\geq 0.8$ ) to detect an effect of -38% or +240% (Figure 2). That is, the 2024 macroalgae SDI at the exposure area had to be 38% lower or 240% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into SDI values, this means that in the exposure area, macroalgae SDI would need to be an average of 0.6 or 0.893 (compared to the 0.7 value estimated in the 2024 reference area). In comparison, the observed effect size was -29%. Statistical power to estimate the observed effects was 0.7.

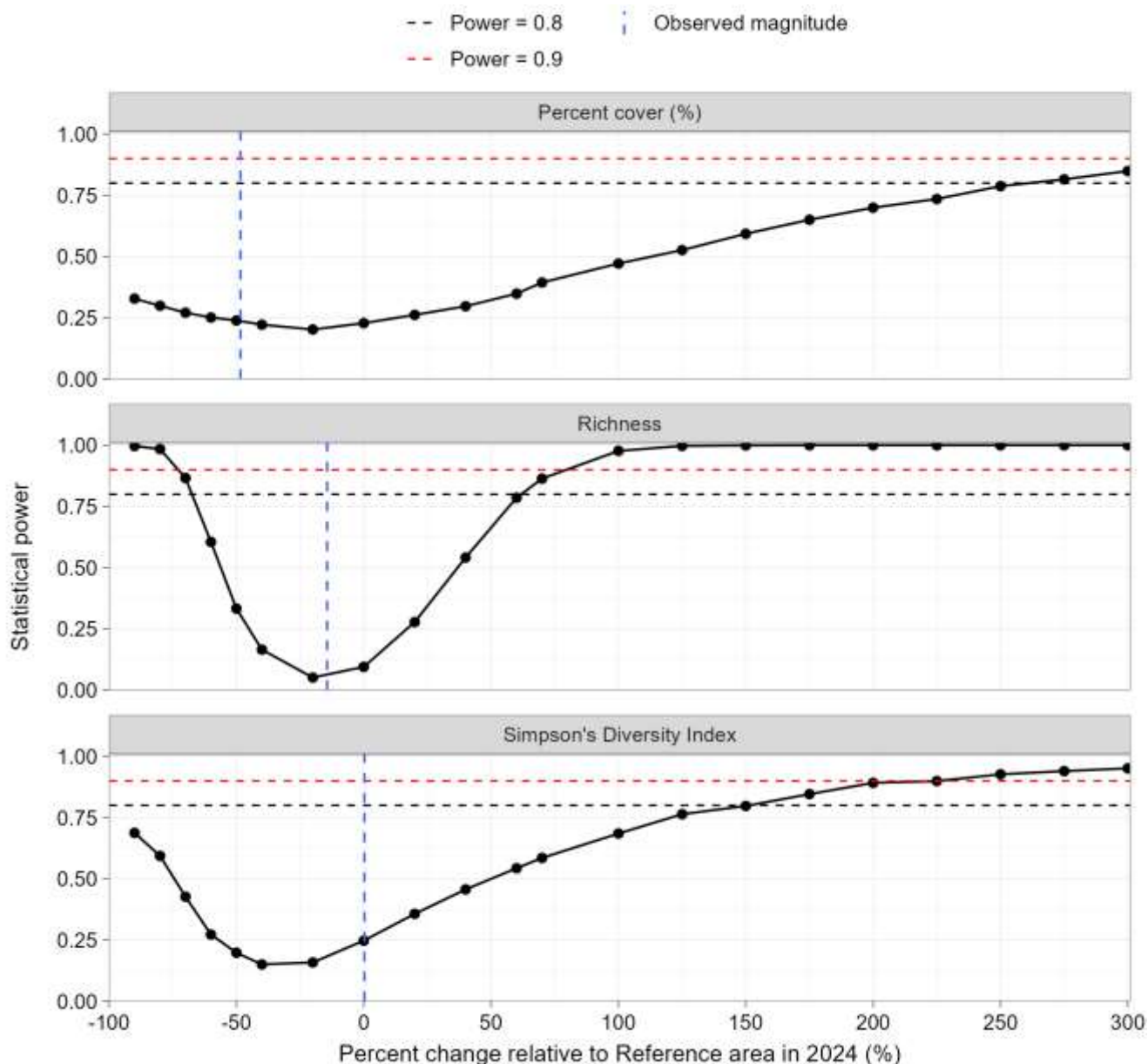


**Figure 2: Statistical Power of The Models of Benthic Macroalgae Data to Detect a Significant Interaction Between Year and Area Effects, Based on Quadrat Data Collected in 2021–2024.**

In the analysis of sessile epifauna percent cover, there was sufficient power ( $\geq 0.8$ ) to detect an effect of +275%, but none of the negative effect sizes (Figure 3). That is, the 2024 percent cover of sessile epifauna at the exposure area had to be 275% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into percent cover, a 275% increase means that the sessile percent cover in the exposure area would need to be 60% (compared to the 29% value estimated in the 2024 reference area). In comparison, the observed effect size was -48%. Statistical power to estimate the observed effects was  $< 0.3$ .

In the analysis of sessile epifauna richness, there was sufficient power ( $\geq 0.8$ ) to detect an effect of -68% or +62% (Figure 3). That is, the 2024 sessile richness at the exposure area had to be 62% lower or 80% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into richness values, this means that in the exposure area, there would need to be an average of 1.8 sessile species or an average of 9.3 sessile species (compared to the 5.7 value estimated in the 2024 reference area). In comparison, the observed effect size was -14%. Statistical power to estimate the observed effects was  $< 0.1$ .

In the analysis of sessile SDI, there was sufficient power ( $\geq 0.8$ ) to detect an effect of +155%, but none of the negative effect sizes (Figure 3). That is, the 2024 sessile SDI at the exposure area had to be 155% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into SDI values, this means that in the exposure area, sessile SDI would need to be an average of 0.810 (compared to the 0.626 value estimated in the 2024 reference area). In comparison, the observed effect size was 0.3%. Statistical power to estimate the observed effects was  $< 0.3$ .



**Figure 3: Statistical power of the models of benthic sessile epifauna data to detect a significant interaction between year and area effects, based on quadrat data collected in 2021–2024.**

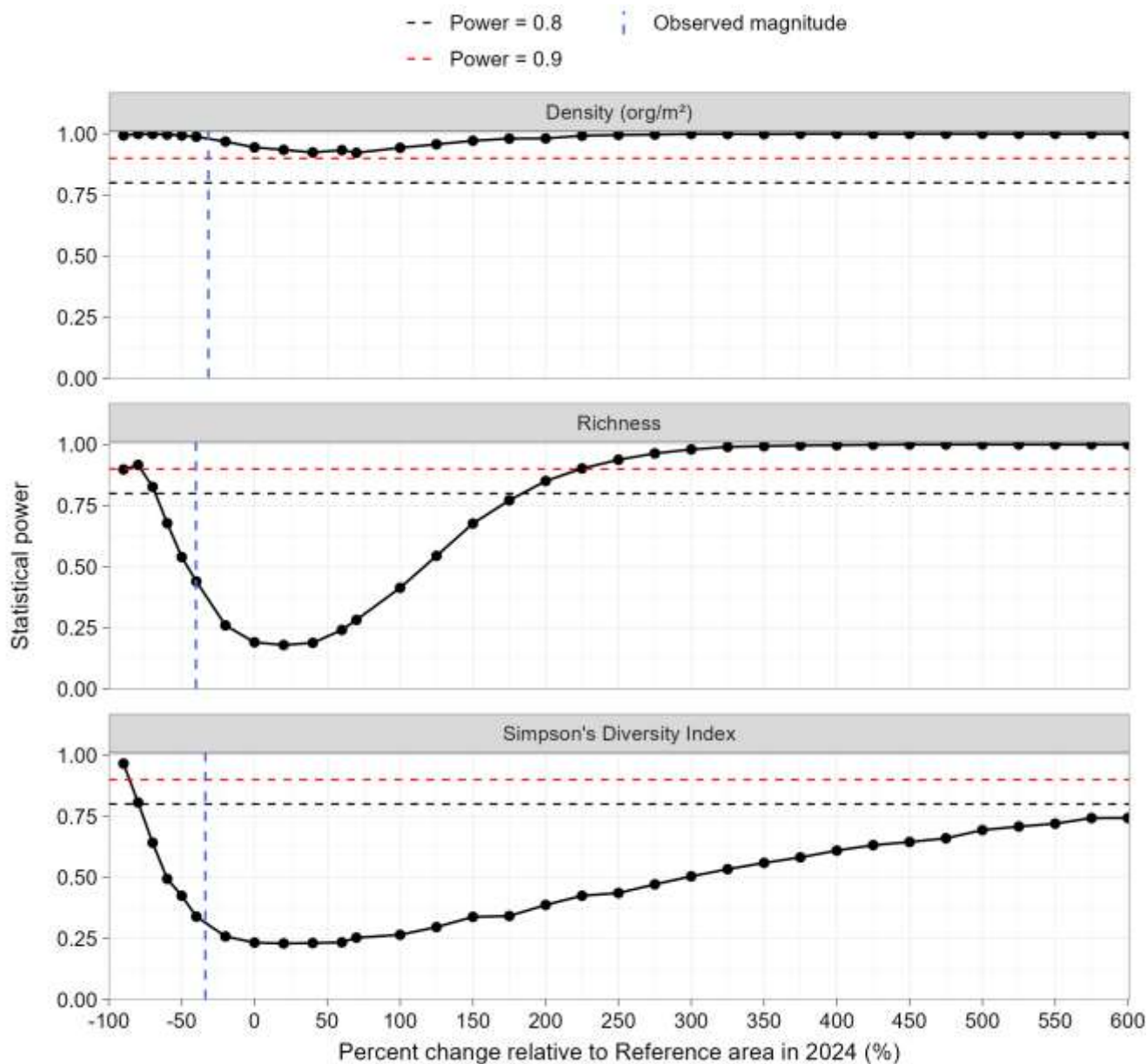
In the analysis of motile epifauna density, there was sufficient power ( $\geq 0.8$ ) to detect any of the examined effects (Figure 4). This was due to the interannual differences in motile density differing between reference and exposure areas prior to 2024. That is, the interaction term of the model was significant regardless of the simulated 2024 effects.

In the analysis of motile epifauna richness, there was sufficient power ( $\geq 0.8$ ) to detect an effect of -69% or +190% (Figure 4). That is, the 2024 motile richness at the exposure area had to be 69% lower or 190% higher than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated



into richness values, this means that in the exposure area, there would need to be an average of 0.67 motile species or an average of 6.3 motile species (compared to the 2.2 value estimated in the 2024 reference area). In comparison, the observed effect size was -40%. Statistical power to estimate the observed effects was 0.4.

In the analysis of motile epifauna SDI, there was sufficient power ( $\geq 0.8$ ) to detect an effect of -80%, but none of the assessed positive effect sizes (Figure 4). That is, the 2024 motile SDI at the exposure area had to be 80% lower than the 2024 reference values for the model to have sufficient power to detect a significant interaction. When translated into SDI values, this means that in the exposure area, motile SDI would need to be an average of 0.09 (compared to the 0.33 value estimated in the 2024 reference area). In comparison, the observed effect size was -34%. Statistical power to estimate the observed effects was  $< 0.3$ .



**Figure 4: Statistical Power of The Models of Benthic Motile Epifauna Data to Detect a Significant Interaction Between Year and Area Effects, Based on Quadrat Data Collected in 2021–2024.**

## SUMMARY

Overall, statistical power to detect an interaction between year and area was low for effect sizes less than 50% in absolute value. This outcome is due to several factors, as follows:

- 1) High variability in data, as seen for macroalgae percent cover and richness, sessile richness and SDI, and motile SDI.
- 2) Low or high values for data that are bound between 0 and 1 (or 100%), as seen for soft substrate and sessile percent cover.
- 3) The increasing number of sampling years – since the effect size is only being applied to the latest year (i.e., 2024), the existence of previous three years without a strong indication for an interaction would mean that the 2024 effect would need to be large for the overall interaction to be significant.

Given the low statistical power, going forward, it is recommended differences between areas should be assessed using effect sizes rather than a strict adherence to statistical significance.

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