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Glass Sponge Reefs in the Strait of Georgia and Howe Sound: Status Assessment and Ecological Monitoring Advice

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Glass sponge reefs are structured biogenic habitats unique to the North East Pacific that support diverse biological communities and provide high levels of bentho-pelagic coupling. Over the past 15 years, nine glass sponge reef complexes have been mapped by the Canadian Hydrographic Service and the Geological Survey of Canada, in the Strait of Georgia and Howe Sound, using remote sensing techniques. In 2015 DFO protected these complexes via formal bottom-contact fishing closures extending 150 m beyond the reef footprints. In order to monitor the established fishing closures, characterization of the reef status and a monitoring plan must be developed. Glass sponge reefs largely occur beyond diving limits, restricting survey methods to remote visual survey platforms which can be resource-intensive and logistically challenging. The reefs require a monitoring program that uses relevant quantitative metrics at appropriate spatial and temporal scales and provides well-resolved time series data.

This paper is based on the results of two remotely operated vehicle surveys of nine sponge reef complexes and associated communities in the Strait of Georgia and Howe Sound completed in 2012 and 2013 prior to bottom-contact fishing closure implementation. We used an empirical, quantitative approach to assess the distinct and unique characteristics of the glass sponge reef ecosystem. First, we applied a suite of both novel and previously published quantitative indices for assessing biogenic habitats to a subset of reef imagery data. Indices were evaluated based on consistency, ability to distinguish between reefs of qualitatively different status, and data processing effort involved. Indices that demonstrated the most potential – characterizing sponge cover, condition, and distribution, as well as associated community structure and indicator taxa abundance – were subsequently applied to the full imagery dataset. Standardized summaries characterizing reef complexes were developed from a compilation of the most informative indices to serve as best available pre-closure reference of reef status for monitoring.

To support the development of a reef monitoring program, considerations for survey design, sampling methods, and data analyses are provided. A range of monitoring indices and associated sampling methods are collated to provide options for comparing reef status over time and space. We recommend that management decisions are based on trend analysis and consider proposed indices in combination, rather than in isolation. A diagnostic decision tree is presented to guide reef monitoring and inform adaptive management.

The methods developed in this paper can be applied to other reefs in the Strait of Georgia and Howe Sound and adapted for assessment of glass sponge reefs in other areas such as Hecate Strait and Chatham Sound.

1. INTRODUCTION

1.1 GLASS SPONGE REEFS: UNIQUE HABITATS

Glass sponges (phylum Porifera, class Hexactinellida) are marine sponges with spicules of nearly pure glass. Dictyonine glass sponges (order Hexactinosa) fuse their spicules into a rigid three-dimensional silica framework (Leys et al. 2007). Some of these species are capable of forming reefs, or bioherms, through the attachment of larval sponges to exposed skeletons of dead sponges and by baffling and trapping of fine organic-rich sediments entrained in bottom currents (Leys et al. 2004, Krautter et al. 2006) leading to biohermal growth. Over time, the reefs accumulate to reach heights up to 25 meters and widths up to several kilometers. The bulk of the reef consists of dead sponges buried by sediments, with only the most recent generation of sponges growing 1 to 2 m above the surface (Conway et al. 2001) (Fig. 1).

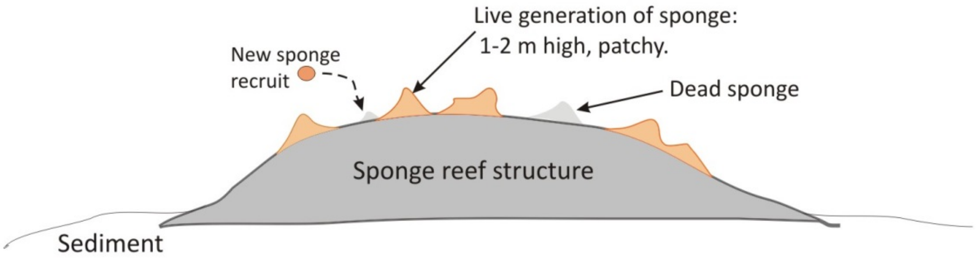

Figure 1. Diagram of glass sponge reef structure.

Glass sponge reefs were only known from fossil records where they occurred in mid-Jurassic to early-Cretaceous seas (Ghiold 1991, Wiedenmayer 1994), until analogous live hexactinellid reefs were discovered on the western Canadian continental shelf in the 1980s. The first glass sponge reefs discovered discontinuously cover over 700 km^2 of the continental shelf in Queen Charlotte Sound and Hecate Strait (Conway et al. 2001, Krautter et al. 2001). More recently, glass sponge reefs were found in the Strait of Georgia and Howe Sound (Conway et al. 2004, Conway et al. 2005, Conway et al. 2007) in British Columbia, in Portland Canal on the international boundary between Southeast Alaska and British Columbia (Stone et al. 2014), as well as Chatham Sound (Kim Conway, Natural Resources Canada, Sidney, BC, pers. comm.) and Desolation Sound (John Shaw, Geological Survey of Canada, Dartmouth, NS, pers. comm.) in British Columbia. In Hecate Strait, the reefs are formed by three species of dictyonine sponges: *Aphrocallistes vastus*, *Heterochone calyx,* and *Farrea occa* (Conway et al. 2001, Krautter et al. 2001), whereas only two of these species – *A. vastus* and *H. calyx* – are found in the Strait of Georgia and Howe Sound (Leys et al. 2004).

The reefs may have developed preferentially in the Pacific due to the rich Hexactinosa sponge fauna in this region and relatively high silicate levels which do not normally occur at shelf depths elsewhere. It is thought that reefs developed in response to ambient seabed currents and the availability of glacial substrate (reviewed in Maldonado et al. 2016). Reef initiation is only possible on non-depositional (exposed) seabed and requires a finely balanced system where sediment is needed to provide the reef matrix, but too much sediment would smother the filter feeding sponges (Conway 1999). Other factors that correlate with dense aggregations of glass sponges in shallow waters are low temperatures (below 12°C), low light, and high food availability (Leys et al. 2004, reviewed in Maldonado et al. 2016).

1.2 ECOSYSTEM ROLE OF GLASS SPONGE REEFS

Glass sponge reefs form unique benthic habitats with historical, ecological, and economic value. They represent a modern analogue of extinct reefs and can thus be used for better understanding of fossil records (Conway et al. 2001, Krautter et al. 2001). The reefs provide a link between benthic and pelagic environments and play an important role in carbon and nitrogen processing (Chu and Leys 2010*a*, Kahn et al. 2015). Through filtration, the sponges clear the equivalent of the entire water column above them of all bacteria daily, while new bacteria are supplied by prevailing currents (Kahn et al. 2015, Maldonado et al. 2016). Sponges were predicted to be on the “winners” side of climate change scenarios (Bell et al. 2013, Dayton et al. 2013) and may act as one of the buffers against climate change by sequestering carbon into sponge tissue (Kahn et al. 2015). The sponges also take up a significant amount of silicate from the water column to form their skeletal framework (Chu et al. 2011, Tréguer and De La Rocha 2013). In the living portion of three reefs in the Strait of Georgia, the standing stock of biogenic silica was estimated to be 7–12 kg m^2 (Chu et al. 2011). Since up to 25 m of reef structure may lie below the sediment surface, considerably more silica is locked below ground, and therefore sponge reefs form a regionally important silicon sink. The reefs also contribute to the productivity of benthic ecosystems by supporting diverse communities of invertebrates and fish including those of economic importance (Cook et al. 2008, Marliave et al. 2009, Chu and Leys 2010*a*, Dunham et al. 2015).

Reef-forming glass sponges are long-lived, but slow growing and exceptionally fragile. Radiocarbon dating suggests that the Queen Charlotte Sound reefs began forming 9000 years ago (Conway et al. 1991). While there is no data on the longevity of individual reef-building sponges, data on related Rosselid species suggest that they may be among the longest living animals in the world, with life spans greater than 220 years (Leys and Lauzon 1998). Reef-building glass sponge growth rates are estimated at 1-9 cm per year (Dunham et al. 2015, Kahn et al. 2016), and, as a result, the reefs are known to have low recovery rates from disturbances. Mechanical injuries, such as crushing, damage the framework of the reef and its ability to grow; Kahn et al. (2016) observed no evidence of recovery from large scale damage impacting the underlying skeletal structure even after a three year period. Intact old skeletons provide the framework for the vertical growth of the reef. As macerated (bare) skeletons are often the only available substrate for the attachment of new sponges on the reef, recruitment is expected to be inhibited by fragmentation of the exposed skeletons (Conway et al. 2001) and by silt accumulation over the dead reef structure. In addition, increased sedimentation can impact the living portion of the reef. Glass sponges are unique in possessing syncytial rather than cellular tissues, which allows them to communicate with electrical impulses even though they lack nerves. Consequently, the whole organism ceases feeding in response to excessive siltation (Leys and Mackie 1997, Leys et al. 1999). Direct injuries and disturbance to the surrounding sediment may thus have acute and cumulative impact on glass sponge condition.

1.3 STRAIT OF GEORGIA AND HOWE SOUND REEF PROTECTION INITIATIVE

Over the past 15 years, nine glass sponge reef complexes have been mapped by the Canadian Hydrographic Service and the Geological Survey of Canada in the Strait of Georgia and Howe Sound (Fig. 2) using remote sensing - multibeam swath bathymetry imagery (Conway et al. 2004, Conway et al. 2005, Conway et al. 2007; Kim Conway, Natural Resources Canada, Sidney, BC, pers. comm). This remote sensing technique readily identifies glass sponge reefs as they are much less acoustically reflective than the surrounding and underlying substrates: the sponge-rich clay sediments and the siliceous skeletons of the sponges absorb acoustic energy (Conway et al. 2005). However, this and other acoustical techniques available to date cannot differentiate between live, dead, and dead and buried patches of glass sponges within a reef. Therefore, while these techniques assist in locating and delineating glass sponge reef structure (indicated by the grey area in Fig. 1), they cannot provide information on current reef extent or character. In the past, some reefs were surveyed for presence and abundance of live glass sponges and associated community structure using Remotely Operated Vehicles (ROVs) (Conway et al. 2004, Conway et al. 2007, Cook et al. 2008, Chu and Leys 2010*a*), while others remained unexplored. Furthermore, no quantitative metrics of sponge condition or sponge reef status have been developed and applied.

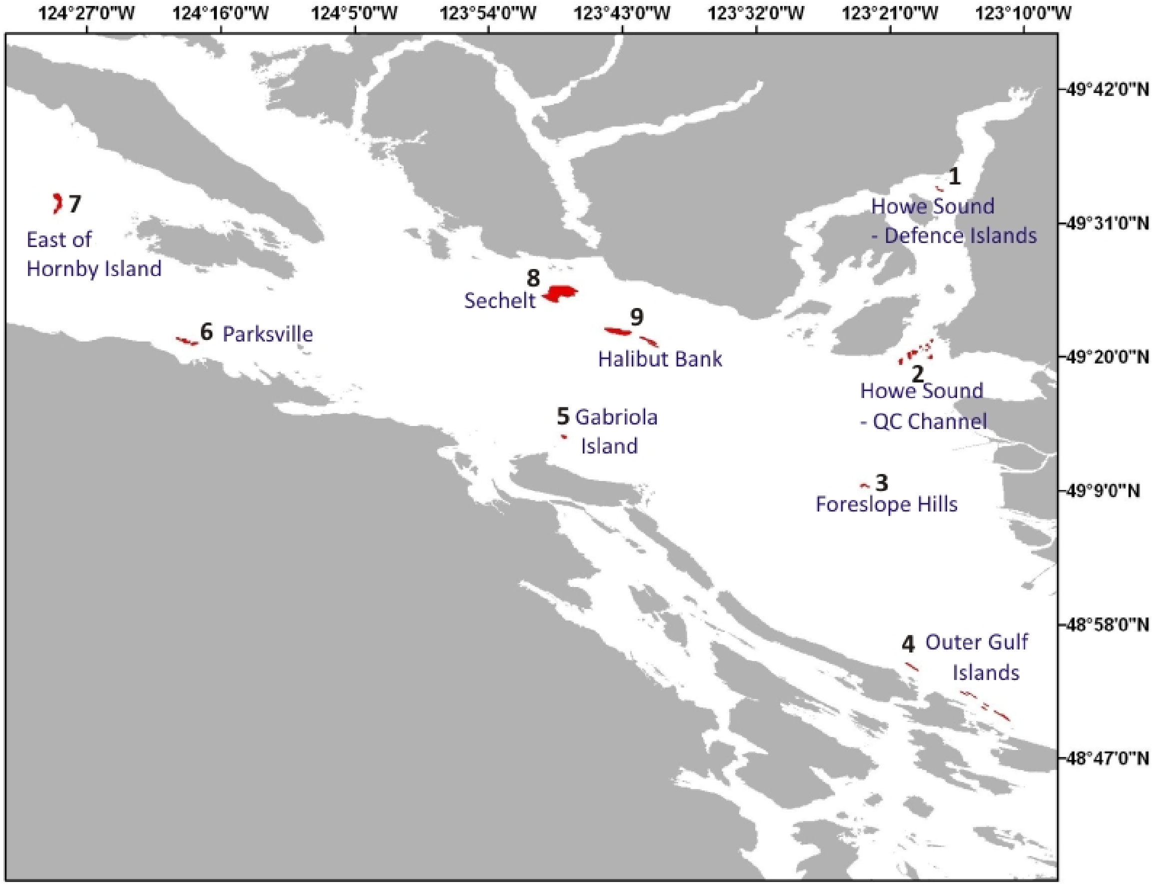

Figure 2. Glass sponge reefs in the Strait of Georgia and Howe Sound (shown in red) mapped by the Canadian Hydrographic Service and the Geological Survey of Canada using multibeam swath bathymetry imagery in 2002-2010.(Note that this technique cannot differentiate between live, dead, and dead-and-buried glass sponges within a reef). GIS shape files were provided by the Pacific Geoscience Centre, Natural Resources Canada. Reef names and numbers are consistent with bottom-contact fishing closures (DFO Fishery Notice FN0415).

In 2012 and 2013, nine glass sponge reefs in the Strait of Georgia and Howe Sound previously delineated with remote sensing techniques were surveyed by DFO Science using an ROV and a standardized survey design to document live glass sponges and megafaunal communities associated with them. These surveys confirmed the presence of live reef-building glass sponge species (*A. vastus* and/or *H. calyx*) at all nine reefs. In 2014, DFO requested that fisheries using bottom-contact gear (prawn trap, crab trap, shrimp trawl, groundfish trawl and hook-and-line) voluntarily avoid these nine glass sponge reef areas while DFO consulted on formal protection measures. After consultations with First Nations, commercial and recreational fisheries representatives, and conservation organizations, DFO proceeded with formal bottom-contact fishing closures to protect nine glass sponge reef complexes in the Strait of Georgia and Howe Sound, effective June 12, 2015 (Appendix 1) under the DFO Policy to Manage the Impacts of Fishing on Sensitive Benthic Habitats. All commercial and recreational bottom-contact fishing activities for prawn, shrimp, crab and groundfish (including halibut) were prohibited within closures that extend 150 m beyond the reef footprint (DFO Fishery Notice FN0415). Beginning April 1, 2016, the closures also apply to First Nations Food, Social, and Ceremonial fisheries that use bottom-contact fishing activities for prawn, shrimp, crab, and groundfish (DFO Fishery Notice FN0415). The protection of sponge reefs is a key component to a number of national initiatives and international commitments such as those made through the United Nations Convention on Biological Diversity and the United Nations Food and Agriculture Organization Code of Conduct for Responsible Fisheries.

During the consultation process, multiple stakeholders inquired about DFO's plans to monitor effectiveness of glass sponge reef protection measures and about DFO's vision for applying the adaptive management approach to reef ecosystems – key steps of the Policy to Manage the Impacts of Fishing on Sensitive Benthic Habitats. DFO Fisheries Management has thus requested DFO Science Branch to provide an evaluation of the current health and status of the reefs within nine closures, along with science advice for how these reefs could be monitored on an ongoing basis.

1.4 PREVIOUSLY KNOWN REEF STATUS AND NOMENCLATURE

Published articles and reports (e.g. Conway et al. 2004, Conway et al. 2007, Cook et al. 2008, Chu and Leys 2010*a*; DFO Fishery Notice FN0415) use different naming and numbering systems for the reef complexes in the Strait of Georgia and Howe Sound. Order of discovery, name(s) from previous literature, first published descriptions, and previous status assessment (if available) for each reef complex are summarized in Table 1. Detailed descriptions of each reef from published literature are provided in Appendix 2.

Table 1. Summary of previously known status of nine glass sponge reef complexes in the Strait of Georgia and Howe Sound protected by bottom-contact fishing closures.

Fishing closure[1]	**Order of reef discovery**	**Reef name (from literature)**	**First published description**	**Previous status assessment and date**[2]	**Sponge distribution maps (Chu and Leys 2010*a*)**[3]
Howe Sound - Defence Islands (1)	15	Howe Sound - Defence Islands	Marliave et al. 2009	Not available	No
Howe Sound - Queen Charlotte Channel (2)	14	Howe Sound - Passage Island	Cook et al. 2008, Marliave et al. 2009	Not available	Yes
Foreslope Hills (3)	1	Fraser Ridge	Conway et al. 2004	Healthy, undamaged (July 2002)	Yes
Outer Gulf Islands (4)	7	Active Pass North or Galiano Ridge	Conway et al. 2007	Healthy, undamaged (Oct 2005)	Yes
	8-12	Active Pass South	Conway et al. 2007	One reef largely dead, damaged (Oct 2005). Other 4 reefs: n/a.	No

Fishing closure[1]	Order of reef discovery	Reef name (from literature)	First published description	Previous status assessment and date[2]	Sponge distribution maps (Chu and Leys 2010*a*)[3]
Gabriola Island (5)	6	Nanaimo	Conway et al. 2007	Largely dead, damaged, possibly recovering (Nov 2004)	No
Parksville (6)	5	Parksville	Conway et al. 2007	Not available	No
East of Hornby Island (7)	16	Achilles (Ajax) Bank	K. Conway, Natural Resources Canada, unpubl.	Not available	No
Sechelt (8)	4	McCall Bank North	Conway et al. 2005	Healthy, undamaged (Oct 2003)	No
Halibut Bank (9)	3	McCall Bank South	Conway et al. 2005	Largely dead, damaged(Oct 2003)	No
	2	McCall Bank South	Conway et al. 2005	Not available	No

[1] For fishing closure locations see Fig. 2.

[2] Based on video transect data collected by the Pacific Geoscience Centre in 2002-2006 and described in Conway et al. (2005, 2007) and Cook et al. (2008). Status was assigned using qualitative descriptions. Conway et al. (2007) defined status using qualitative descriptions in which a healthy reef was not defined and Cook et al. (2008) defined status based on visual assessment of the condition of reef-building sponges, estimates of percent alive and dead sponge, and whether dead sponge was classed as standing, fragmented or broken; status was assigned based on the assessment of the majority (>50%) of a transect.

[3] "Yes" indicates availability of sponge distribution maps and associated megafaunal densities based on ROV data collected in 2007-2009 and described in Chu and Leys (2010*a*).

1.5 FOCUS OF THIS PAPER

The primary goals of this paper are:

1. To provide a status assessment of the nine glass sponge reef complexes in the Strait of Georgia and Howe Sound prior to bottom-contact fishing closures coming into effect (monitoring baseline); and
2. To provide recommendations for ecological monitoring (monitoring advice).

This work focuses on the nine glass sponge reef complexes included in the bottom-contact fishing closure initiative as of 2016; other sponge reefs that may be found in the area and sponge aggregations (such as sponge gardens) are out of scope of this assessment, although the approaches developed in this paper may be applied to them in the future.

The assessment and advice arising from this paper will be used to inform management decisions regarding adaptive management and monitoring of the sponge reefs in the Strait of Georgia and Howe Sound and to respond to stakeholder requests for scientific information on the sponge reefs. It will support the Department's implementation of the Policy to Manage the Impacts of Fishing on Sensitive Benthic Areas. The results may be relevant to other sponge reefs, such as those in Hecate Strait and Chatham Sound.

2. MATERIALS AND METHODS

2.1 STUDY LOCATION

The Strait of Georgia separates the British Columbia mainland from Vancouver Island on the Pacific coast of Canada and is approximately 222 km long by 28 km wide, with an average depth of 155 m (Thomson 1981). The deep subtidal habitats (50 to 380 m) comprise approximately 71% of the benthic surface area of the Strait; the seafloor is predominantly covered with fine-grained depositional sediments (mud) where benthic communities are generally dominated by burrowing megafauna such as echinoderms, bivalves, and polychaetes (Levings et al. 1983). Howe Sound is a high runoff fjord open on its southeast towards the Strait of Georgia just north of Vancouver and extending 42 kilometers to Squamish. Maximum depth of Howe Sound is 285 m; sill depth is 73 m located near Defence Islands. In the Strait of Georgia and Howe Sound glass sponge reefs are found on elevated bedrock features, such as mounds and ridges.

2.2 TERMINOLOGY

The following operational definitions are used throughout this paper:

- **Bioherm**: Ancient organic reef of mound-like form built by a variety of marine invertebrates and calcareous algae (Bioherm, 1998).
- **Sponge bioherm**: Bioherm formed by sponges (phylum *Porifera*); also known as reef mound (Conway et al. 1991). Modern sponge bioherms are found in the Antarctic and in tropical waters.
- **Glass sponge reef**: Sponge bioherm formed by hexactinellid glass sponges with sub-surface and above surface structure sufficient to produce contiguous multibeam sonar signature. Modern glass sponge reefs are unique to the Northeast Pacific Ocean.
- **Glass sponge reef footprint**: Area covered by individual glass sponge reefs mapped by the Canadian Hydrographic Service and the Geological Survey of Canada using multibeam swath bathymetry imagery in 2002-2010 (Fig. 3).
- **On-reef**: Occurring within the glass sponge reef footprint.
- **Off-reef:** Occurring outside of the glass sponge reef footprint.
- **Glass sponge reef fishing closure**: Area within bottom-contact fishing closure boundary currently extending 150 m beyond the reef footprint (Fig. 3).
- **Glass sponge reef fishing closure buffer zone**: area surrounding reef footprint which forms part of the glass sponge reef bottom-contact fishing closures (Fig. 3).
- **Glass sponge reef complex**: A group of glass sponge reefs located within a single designated bottom-contact fishing closure (Fig. 3).
- **Sponge garden**: Aggregation, or assemblage, of sponges at a notably higher density than in surrounding areas, without evidence of bioherm formation; also known as ‘glass sponge grounds’ and ‘glass sponge concentrations’. Sponge gardens are found in many parts of the world, at virtually all depths. Sponge gardens are beyond the scope of this paper.
- **Reef-building glass sponge:** Individual specimen of *Aphrocallistes vastus* or *Heterochone calyx*.
- **Sponge patch:** A visually contiguous area of reef-building glass sponge.

- **Reef function:** Biological, geochemical, and physical processes and their components that occur within a glass sponge reef.
- **Reef character:** Quality(ies) distinctive to glass sponge reefs.
- **Reef health:** Property(ies) of the nine glass sponge reef complexes DFO Fisheries Management initially requested Science Branch provide an assessment of in this paper. There is insufficient understanding of glass sponge reef ecology and function to comprehensively define and assess reef health at this time. Instead, an assessment of reef status (see below) is provided.
- **Sponge condition**: The term applied to *individual sponges* as a measure of state (*e.g.* signs of breakage).
- **Reef status**: The term applied to *the whole reef complex* as a quantitative measure of reef character based on best available knowledge to date.
- **Index:** A quantitative measure of a property related to individual sponge condition or whole reef status. Also referred to as metric.
- **Monitoring indices:** Indices assessed in this paper and recommended for monitoring glass sponge reefs. These indices are of reef status and/or sponge condition and should not be viewed as indicators of management measure effectiveness. Management effectiveness indicators can be developed after conservation objectives for the glass sponge reef complexes are established.

These definitions are consistent with the DFO Pacific Region Cold-Water Coral and Sponge Conservation Strategy (DFO 2010) and available scientific literature (e.g. Conway et al. 1991, Maldonado et al. 2016).

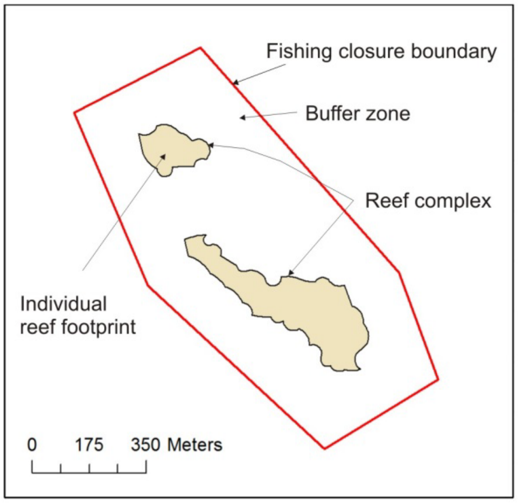

Figure 3. Glass sponge reef fishing closure (red boundary) around a reef complex consisting of two individual reefs (Howe Sound - Defence Islands reef complex used as an example).

We followed the reef naming and numbering system developed by DFO Fisheries Management during the Strait of Georgia glass sponge reef protection initiative (DFO Fishery Notice FN0415), to ensure consistency with bottom-contact fishing closures, and to streamline the use of resulting science advice for management applications. Identification letters were added to individual reefs within each closure (Fig. 4; also see Appendix 1: Table A1-1).

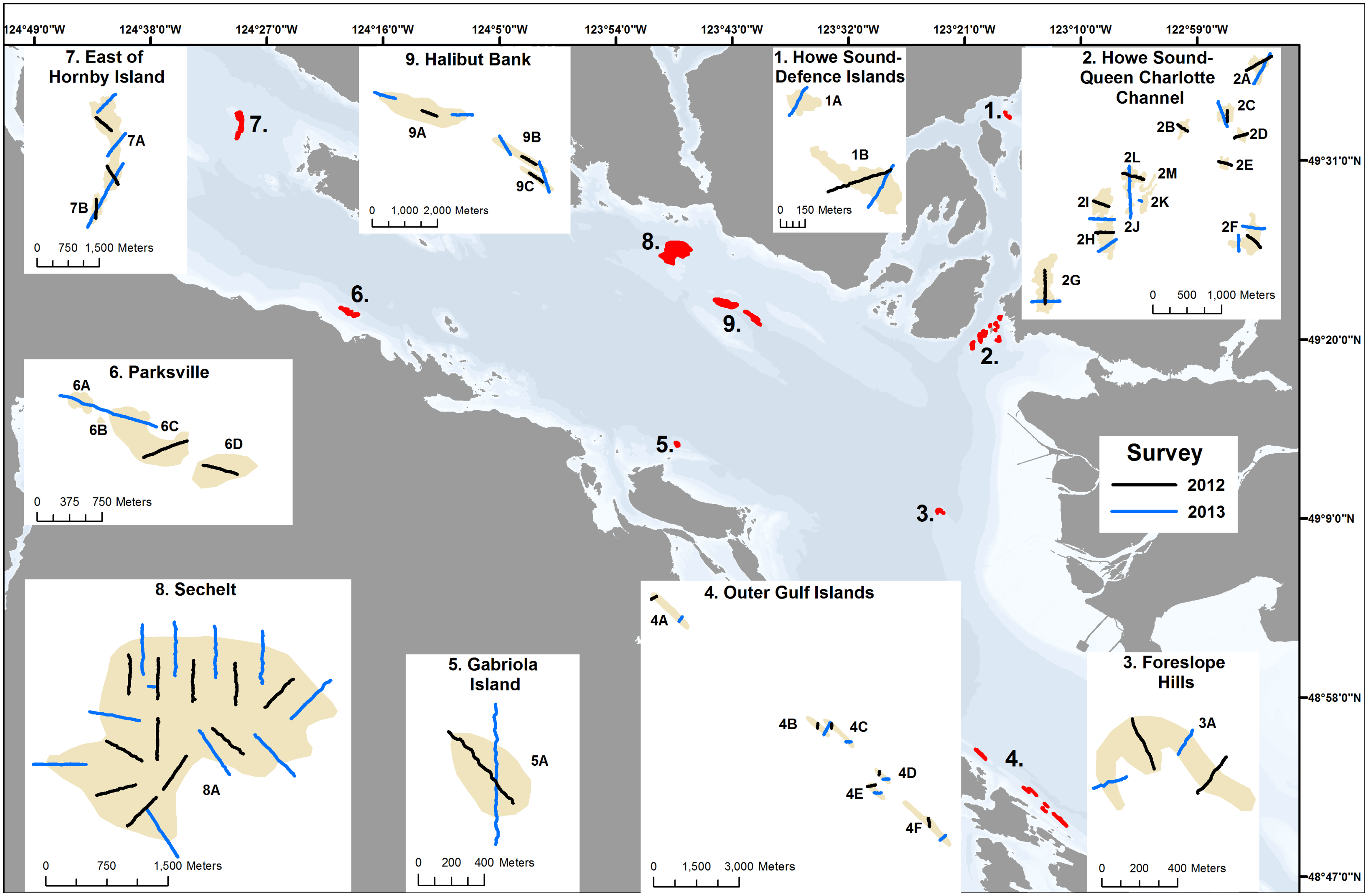

Figure 4. Maps of nine reef complexes showing individual reefs (identified by letters) and transects completed during the 2012 and 2013 surveys.

2.3 SURVEY DESIGN AND TECHNIQUES

To collect data on glass sponge reef status and associated community composition, surveys of nine reef complexes were conducted in September 2012 (Pac2012-068) and December 2013 (Pac2013-070) using Phantom ROV HD2+2 (Deep Ocean Engineering) aboard the CCGV Neocaligus. Video and still imagery were collected along predetermined line transects (Fig. 4). For each survey, transect locations were chosen independently using a stratified random design: each reef complex was visually divided into 2 to 11 sections, depending on its size, and one transect approximately 500 m long was plotted in each section from randomly selected waypoints. Due to its smaller area relative to other complexes, the Gabriola Island reef had only one transect per survey. In 2013, transects were planned in such a way that they ran perpendicular to the reef perimeter and began at least 50 m off reef. This was done to assess the accuracy of reef boundaries determined by remote sensing techniques and to determine sponge distribution and condition, as well as community composition, around reef edges. In total, 37 transects were completed in 2012 and 41 in 2013. Mean transect length was 473.6±271.69 m (mean±SD, *n*=78) and field of view was 1.31±0.305 m (mean±SD, *n*=13,989). A transect from a separate survey conducted in November 2011 (Pac2011-073) was incorporated into the 2012 dataset for the Outer Gulf Islands complex in order to provide additional spatial coverage of reef 4A. The resulting data set thus consisted of 79 transects crossing 31 out of 33 reefs within nine closures. Transects covered 0.24 to 0.78% of the total reef complex area (Table 2).

Table 2. Summary of spatial coverage of on- and off-reef areas using line transects.

Reef closure	Total reef area[1], m²	N of transects	On-reef transect length, m	Reef area surveyed, m²	Reef area surveyed, %	Off-reef transect length, m	Off-reef area surveyed, m²
Howe Sound - Defence Islands (1)	99,794	3	586	778	0.78	294	399
Howe Sound - Queen Charlotte Channel (2)	894,786	19	5,056	6,830	0.76	1,356	1,479
Foreslope Hills (3)	176,761	4	669	957	0.54	251	297
Outer Gulf Islands (4)	859,047	12	1,953	2,222	0.26	1,284	1,736
Gabriola Island (5)	168,114	2	1,048	1,314	0.78	476	485
Parksville (6)	614,240	3	1,744	3,065	0.50	436	775
East of Hornby Island (7)	1,097,695	6	3,944	5,036	0.46	789	744
Sechelt (8)	4,999,438	21	10,918	14,680	0.29	1,821	2,237
Halibut Bank (9)	2,004,966	9	3,803	4,893	0.24	987	1,336

[1] Calculated as a sum of areas of all reefs within closure (see Appendix 1).

The ROV was equipped with a forward-facing high-definition video camera (MiniZeus, Insite Pacific, 1080x1920 pixels resolution; oblique angle adjusted based on bottom slope) and downward-facing still camera (Cyclops, C-Map Systems Inc., 3264 x 2448 pixels resolution). Component HD video was converted subsea to HD-SDI and transmitted to the surface via fiber optics where a Proteus II video overlay (Videologix) was used to overlay time, date, and dive number before recording video as high bit rate (~50Mbps) mpeg2 files using StreamZ encoding system (Digital Rapids Corporation). The ROV surveyed the total length of each transect approximately 1 m above the benthos and captured video (continuously) and still images (every 15 sec). Images and video footage had laser projection dots 10 cm apart from a pair of parallel lasers, for scale. Conductivity, temperature, and depth (CTD) sensors were mounted onto the ROV frame during both surveys to collect environmental data (Falmouth Scientific Inc. CTD in 2012, 1/sec; Seabird SBE049 CTD in 2013, 16/sec).

During all ROV dives, Hypack navigation software (2014) continuously recorded date, time, depth, and ROV positional data in a Microsoft Access database. All spatial analyses were completed in ArcGIS 10.2.2 (ESRI 2014) using the WGS84 projection.

2.4 ANALYTICAL METHODS

2.4.1 Geo-referencing and spatial analyses

ROV tracks for all transects (*n*=79) were plotted using ArcMap 10.2.2 (ESRI 2014). A complete navigation dataset with positional data (x,y) for each timestamp was generated through interpolation and plotted as a point shapefile using R v3.2.3 (R Core Team 2016) in order to link video annotation and navigation datasets. On- and off-reef timestamps were reviewed to determine and verify on- and off-reef portions of each transect. Transects were processed in ArcMap to remove any loops in the ROV tracks greater than 7 m; these loops represent occasions where the ROV was pulled off the bottom by currents or experienced tracking errors. Timestamps with no corresponding positional data - navigation software errors - were also removed.

On-reef and total transect lengths were calculated in ArcMap by converting point shapefiles to line shapefiles using the point to line tool. The extent of on- and off-reef areas surveyed (Table 2) were calculated by multiplying respective transect section lengths by mean field of view for each transect. Footprint areas of all reefs within each closure (Table 2; also see Appendix 1: Table A1-1) were calculated in ArcMap using the sponge reef footprint shapefile provided by the Pacific Geoscience Centre, Natural Resources Canada.

2.4.2 Video annotation and quality control

Video clips of 79 transects were reviewed and observations were recorded, using VideoMiner V3.0 (DFO custom software), into a Microsoft Access database. The annotators recorded observations for each 10-second interval of the video (hereinafter referred to as ‘video bin’; Fig. 5).

Figure 5. Schematic representation of video bins along a transect. Each bin can be viewed as a rectangle with width equal to the video camera’s mean field of view (FOV; mean: 1.3 m) and length equal to distance ROV travelled over the 10-second interval (mean: 2.6 m).

Observations were linked to positional data through timestamps. Geological and biological features recorded were: relief, dominant and subdominant substrate types, all megafauna observations with counts and/or abundance estimates, anthropogenic objects encountered, and any relevant comments. Field of view was calculated by measuring the screen width and calibrating using the known 10 cm distance between laser projection dots. In this study, megafaunal organisms were defined as any organism larger than approximately 4 cm which could be clearly seen on video. Megafaunal organisms were identified to the lowest taxonomic level possible. When counting megafaunal organisms was not practical, the ACFOR relative abundance scale was used (Emmett et al. 2007; for cut-offs see Appendix 3).

Observations of live reef-building glass sponges (*A. vastus* and *H. calyx*) were combined into 'reef-building glass sponges' since the two reef-building species cannot be accurately distinguished using visual survey methods. Relative abundance of sponges was recorded for each video bin using the ACFOR scale (Emmett et al. 2007; for cut-offs see Appendix 3) as it was not always possible to count individual sponges in a dense patch of sponges. Therefore, live reef-building glass sponges were transcribed from the video in three ways: as species records, as relative abundance scores, and as dominant or subdominant substrate observations (whenever applicable). Dead reef-building glass sponges, if present, were recorded as dominant or subdominant substrate. The complete video review protocol can be found in Appendix 3.

Video clips were processed using a standard review protocol, but for logistical reasons the 2012 and 2013 datasets were processed separately by two different annotators. Annotators were experienced in the detection and classification of a broad range of megafauna in seabed video and imagery, rather than being specialists in particular taxonomic groups. To increase consistency in video interpretation and to assess annotator variability, annotators processed a subset of the same 5 transects (QA/QC dataset; total of 149 minutes of video). Resulting QA/QC databases were compared to determine instances where one annotator observed a megafaunal organism and the other did not; corresponding video bins were re-reviewed and changes were made on a case-by-case basis. A taxonomic list of all megafaunal observations made by both annotators was compiled and percent agreement between annotators was calculated for all taxonomic groups using two methods: direct match-to-bin (comparing exact bins only) and fuzzy match-to-bin (including two adjacent bins in the comparison). For each taxonomic group with poor annotator agreement, at least 10 randomly selected bins from 2012 and 2013 datasets where this megafaunal organism was present were re-reviewed by a taxonomic group expert; if agreement level between the annotator and the expert was less than 75%, the records were adjusted to reflect the expert's identification. In cases where annotators disagreed consistently on a lower taxonomic level but agreed at a higher taxonomic level, observations were rolled up to a higher taxonomic level.

Based on the above, the QA/QC dataset and the complete 2012 and 2013 datasets were post-processed to reflect the QA/QC adjustments. To characterize detection and annotation accuracy, two indices were calculated following Durden et al. (2016) and applying 'exact match-to-bin' and 'fuzzy match-to-bin' methods described above to the post-processed QA/QC dataset:

- Detection Rate Index (for each annotator, %): number of specimens detected by the annotator as a fraction of the total number of specimens detected by at least one annotator.
- Annotation Success Index (overall, %): number of specimens that were both detected and identically classified by both annotators as a fraction of the number of specimens detected by at least one annotator.

All subsequent video-based analyses were run on post-processed 2012 and 2013 datasets.

2.4.3 Still image processing

Mean live reef-building sponge area was determined for each reef complex using a set of randomly selected still images; 10 images with reef-building glass sponges present were processed from each reef complex. Each image was calibrated using ROV laser points and all discrete reef-building glass sponges captured in the image were identified. If a still image contained more than 10 reef-building sponges, 10 sponges were chosen at random for measurements. Each measured sponge was assigned a geometric shape it most closely resembled (circle, oval, triangle, sector, rectangle, trapezium, or rhombus) and corresponding area calculations were applied using ImageJ (Rasband 2016). As a result, 10-100 sponge area estimates per reef complex were obtained.

All still images from four randomly selected transects (# 3, 4, 19, and 23) of the 2012 dataset were processed to test different methods of estimating sponge abundance (protocol details and illustrations can be found in Appendix 4). Each still image was first calibrated in Image J using laser projection dots located 10 cm apart. Next, the following methods of estimating sponge abundance were applied:

- Grid method: A 10 x 10 cm cell grid was overlaid onto the image in GIMP v2.6 (GNU Image Processing Software) and dominant benthic cover (occupying ≥50% of the cell) was recorded for each cell. Benthic cover types included sediment types (*e.g*. bedrock, sand, mud), biogenic habitats (live and dead reef-building sponge, boot sponges, other sponges), non-sponge sessile biota (*e.g*., coral), mobile biota (where obscuring view of the benthos), and anthropogenic objects (for full list see Appendix 4).
- Oscula method: All live reef-building sponge oscula were counted.
- Outline method: Live and dead reef-building glass sponges, *Rhabdocalyptus dawsoni*, and other sponges were outlined using a digital photo editing tablet (Wacom Intuos Photo Pen and Touch) in GIMP v2.6; area occupied by each type of sponge was measured in ImageJ.

The grid method of sponge assessment was subsequently applied to all images from two randomly selected transects per reef complex (*n*=1834; 204±74 images per reef complex; image area: 0.7±0.42 m^2; image spacing: 3.0±1.31 m, mean±SD). Still images from one randomly selected transect per reef complex from the 2012 dataset (total of 9 transects) were reviewed for the presence of broken reef-building sponges (yes or no) and for megafauna. All visible organisms were identified to the lowest taxonomic level possible, counted, and assigned an ID confidence score. All megafaunal records with a low or very low confidence score were subsequently reviewed by an expert and necessary changes were made on a case by case basis. For all unique taxonomic groups identified, 10 images were randomly selected for review (or all images if the taxonomic group was seen less than 10 times). If expert and annotator agreement was ≥75%, no further changes were made. In cases where expert and annotator disagreed in a consistent manner, the expert’s ID was applied to all occurrences of respective taxonomic group. If there was significant, but not consistent disagreement, a full review of the taxonomic group was done and changes were made on an image-by-image basis.

2.4.4 Reef-building glass sponge assessment

To address the request for evaluation of current reef health and status, a literature review was conducted, augmented by expert opinion - both indicate that there is insufficient understanding of glass reef ecology and ecosystem function to explicitly define and assess reef “health” at this time. Instead, we focused on developing empirically-derived, quantitative indices characterizing distinct properties of the glass sponge reefs, unique and discrete ecosystems. These indices are based on empirical data collected using visual survey methods and reflect best available knowledge to date.

No specific conservation objectives have been developed for the glass sponge reefs in the Strait of Georgia and Howe Sound to date. Thus, this paper focused on developing indices that relate to the state of the glass sponge reefs rather than pressure (or stressor-specific) indicators.

2.4.4.1 Sponge-based indices

No standard quantitative metrics for sponge condition or sponge reef status have been developed and applied prior to this study. Primary literature on glass sponge reefs, coral reefs, and coral and sponge grounds, as well as general literature on landscape and seascape ecology metrics, were consulted. Studies assessing structure and function of biogenic habitats typically utilize indices related to habitat-forming (foundation) species cover and/or density, as well as condition. For example, live coral cover, coral mortality index, and coral damage index are used for describing coral reefs (reviewed in Díaz-Pérez et al. 2016). Percent cover and density of live oysters have been used to assess oyster reef habitats (e.g., Bergquist et al. 2006). Because the approach of using indices related to habitat-forming species is widely accepted and has theoretical basis, we focused on developing methods to effectively and efficiently quantify reef-building sponge abundance.

Four suites of indices were selected to test for applicability to the glass sponge reef system.

Suite I: To estimate live reef-building sponge abundance:

1. **Live abundance, bin method**: Calculated as the number of 10 second video bins where live reef-building sponges were recorded as species records divided by the total number of video bins (similar to Huvenne et al. 2016). This index was calculated for on-reef sections of transects and results in an estimate of sponge abundance per transect.
2. **Live abundance, oscula method** (as in Chu and Leys 2010*a*)**:** The number of live reef-building sponge oscula per m^2 of area surveyed. This index results in an estimate of sponge abundance per image.
3. **Live % cover, Monte Carlo method:** Each live reef-building sponge recorded in each video bin was multiplied by a randomly selected sponge area from the measured sponges for the respective reef complex. This process was repeated 1,000 times in a Monte Carlo simulation; the mean sponge area of all simulations was used as an estimate of sponge cover. Calculated using a subset of images and the full video dataset for on-reef sections of transects; results in an estimate of total sponge area and, when combined with total area surveyed, an estimate of sponge percent cover per transect.
4. **Live % cover, outline method:** Mean percent cover of live reef-building sponges measured from still images using the outline method (see Section 2.4.3). This index results in an estimate of percent cover per image.
5. **Live % cover, grid method:** Mean percent cover of live reef-building sponges estimated from still images using the grid method (see Section 2.4.3). Calculated as the number of cells assigned to benthic cover of 'live reef-building sponge' divided by the total number of cells; results in an estimate of percent cover per image.

Suite II: To assess visible (exposed) dead sponge structure:

1. **Dead abundance, video bin method:** Calculated as the number of 10 second video bins where dead reef-building sponges were recorded as dominant or subdominant substrate divided by the total number of video bins. Calculated using full video dataset for on-reef sections of transects and results in an estimate of dead sponge structure per transect.
2. **Dead % cover, grid method:** Mean percent cover of dead reef-building sponges estimated from still images using the grid method (see Section 2.4.3). Calculated as the number of cells assigned to benthic cover of 'dead reef-building sponge' divided by the total number of cells; results in percent cover estimate per image.

Suite III: To characterize sponge distribution:

1. **Live Sponge Clumpiness Index:** a normalized index depicting patch type's deviation from random distribution (McGarigal and Marks 1995); ranges from -1 when the patch type is maximally disaggregated (*i.e.* there are no like patch type adjacencies) to 1 when it is maximally clumped (and returns 0 for a random distribution). Calculated for live reef-building sponges using still images from two transects per reef complex. Benthic cover types assigned to grid cells (see Section 2.4.3) were used to compute the index using CLUMPY function in FRAGSTATS v3 (McGarigal et al. 2002).

Suite IV: To assess sponge condition:

1. **Percent of images with broken sponges:** Calculated as percentage of images with visibly broken live reef-building glass sponges relative to the total number of images reviewed that contained reef-building sponges. Calculated using all still images from one randomly selected transect per reef complex from the 2012 survey.
2. **Percent of images with intact sponges:** Calculated as percentage of images without visibly broken live reef-building glass sponges relative to the total number of images reviewed that contained reef-building sponges. Calculated using all still images from one randomly selected transect per reef complex from the 2012 survey. This index is the inverse of the previous index.

All sponge-based indices tested, with corresponding input data and sample sizes, are summarized in Table 3.

Table 3. Sponge-based indices tested, with corresponding input data and sample sizes.

Suite of indices	**Index**	**Input data**	**Sample size**
Live sponge abundance	Live abundance, bin	Video	All 79 transects (2012+2013)
	Live abundance, oscula	Still images	9 transects (1 per reef complex, 2012)
	Live % cover, Monte Carlo	Video + still images	All 79 transects (2012+2013) + input data gathered from 10 randomly selected images per reef complex (2012+2013)
	Live % cover, outline	Still images	4 transects (2012)
	Live % cover, grid	Still images	18 transects (2 per reef complex, 2012+2013)
Dead sponge structure	Dead abundance, bin	Video	All 79 transects (2012 + 2013)
	Dead % cover, bin	Still images	18 transects (2 per reef complex, 2012+2013)
Sponge distribution	Clumpiness index	Still images	18 transects (2 per reef complex , 2012+2013
Sponge condition	% images with broken sponge	Still images	9 transects (1 per reef complex, 2012)
	% images with intact sponge	Still images	9 transects (1 per reef complex, 2012)

Following the classification of aquatic ecosystem functions developed by Giller et al. (2004), all of the indices address the functions of physical structuring and biomass production. Live abundance, oscula method index also addresses elemental cycling function and organic matter transformation function (filtration capacity).

2.4.4.2 Habitat categories

A habitat category approach was developed to integrate the three types of reef-building sponge records described in Section 2.4.2: counts under species observations, ACFOR scale relative abundance scores, and dominant and subdominant substrate records.

Each 10 second video bin (on- and off-reef sections of all transects) was assigned one of five habitat categories: ‘dense live reef’, ‘live reef’, ‘mixed reef’, ‘dead reef’, or ‘no visible reef’. ‘Dead reef’ habitat had visible dead sponge skeletons, whereas ‘no visible reef’ represents habitat with no visible live or dead sponges (although sponge structure may be buried under the sediment); the distinction between ‘dead reef’ and ‘no visible reef’ was introduced because these areas differ in their recovery potential. Habitat categories were assigned using a combination of the three ways reef-building glass sponges were recorded; each bin received three category scores based on these three data types (Table 4) and the highest score was used as the final habitat category. For example, a video bin with 20 counts of live reef-building sponges, ‘occasional’ relative abundance, and dead sponge as subdominant substrate would receive a final score of ‘live reef’.

Table 4. Habitat category matrix applied to the video dataset.

Habitat category	**Species observations, percentile (count)**	**Relative abundance**	**Substrate type**
Dense live reef	≥90th percentile (24)	Abundant	Live sponge - dominant
Live reef	75-89.99th percentile (16-23)	Common or Frequent	Live sponge - subdominant
Mixed reef	10-74.99th percentile (2-15)	Occasional or Rare	Dead sponge – dominant or subdominant
Dead reef	0-9.99th percentile (0-1)	Not applicable	Dead sponge – dominant or subdominant
No visible reef	0	Not applicable	No live or dead sponges – dominant or subdominant

A similar approach was applied to the still image dataset (all images from 2 transects per reef complex). Each image received three category scores based on three data types (Table 5): percent cover of live and dead reef-building sponges (determined using the grid method described above) and oscula counts. The highest score was used as the final habitat category. For example, an image with 10% cover of live reef-building sponges, 20% cover of dead reef-building sponges, and oscula count of 1 would be scored as “mixed reef”.

Table 5. Habitat category matrix applied to the still image dataset.

Habitat category	Percent cover of live reef-building sponges, percentile (% cover)	Percent cover of dead reef-building sponges	Live reef-building sponges oscula counts
Dense live reef	>90th percentile (>35.38%)	Not used	>1
Live reef	75-90th percentile (17.07-35.38%)	Not used	>1
Mixed reef	10-74.99th percentile (0.94-17.06%)	>0	>1
Dead reef	0-9.99th percentile (0-0.93%)	>0	1
No visible reef	0	0	0

2.4.4.3 Sponge distribution maps

Distribution of live reef-building sponges along each transect was plotted based on habitat categories assigned to video bins. Point shapefiles of transect positional data were created in R to facilitate the creation of polygon-based heat maps in ArcMap 10.2.2 for individual transects and point-based heat maps for reef complexes. Positional data (X1 and Y1) at the start of each consecutive time bin were extracted to act as the end coordinates for the previous bin (X2 and Y2) for polygon-based transect heat maps. The centroid of each time bin was extracted for point-based reef complex heat maps. Point-based heat maps used a weighted display scale such that dense, live habitat categories were weighted as highest and no data habitat categories as lowest. Mean field of view (m) for each transect was used as the measurement of bin width (buffer distance) during polygon creation and for bin area calculations (see Appendix 5). A mean bin width of 4 m was applied to polygon-based transect heat maps to facilitate visual interpretation of polygons.

2.4.5 Megafaunal community analyses

Univariate and multivariate methods were used to compare megafaunal communities among the reef complexes. The following community indices were calculated for each transect and analyzed using univariate statistics:

- Total megafaunal density (total number of organisms excluding reef-building glass sponges), ind/m^2;
- Species richness, *S*
- Shannon-Wiener diversity index $H' = -\sum P_i \log_e(P_i)$ (Shannon and Weaver 1949);
- Pielou evenness index $J' = H'/\mathrm{Ln}(S)$ (Pielou 1975), where P_i is the relative abundance of the *i*th taxon in a sample containing S taxa.

Because of the differences in the numbers of transects per reef complex (3 to 21), community indices were compared among reef complexes using Kruskal-Wallis test. Post hoc comparisons were done using the Dunn test for multiple comparisons (Dunn 1964).

For multivariate analyses, a zero-adjusted Bray-Curtis dissimilarity matrix was created using square root-transformed megafaunal abundance data (Clarke et al. 2006). To test for differences in community structure, PerMANOVA ($P<0.05$) was run and data were visualized using non-metric multi-dimensional scaling (NMDS). These analyses were conducted in R with the “vegan” package (Adonis and metaMDS, respectively). Pairwise comparisons between reef complexes were performed with PerMANOVA post hoc tests ($\alpha = 0.05$; $P \leq 0.0375$ after applying

Benjamini and Hochberg (1995) correction for multiple comparisons). Taxonomic groups accounting for dissimilarity between the complexes were determined using similarity percentage (SIMPER) analysis in R with the “vegan” package.

2.4.6 Community-habitat associations

Total megafaunal density, species richness, diversity, and evenness indices were compared between on- and off-reef sections of transects from the 2013 video dataset using a paired t-test ($P<0.05$).

Species-habitat associations within reef complexes were explored by running indicator species analysis following Dufrêne and Legendre (1997). The Dufrêne-Legendre Indicator Species Analysis combines taxonomic group’s relative abundance with its relative frequency of occurrence in a given habitat. Indicator Value Index reaches a maximum of 1 when all individuals of the taxonomic group are found in one habitat category only (100% specificity) and are present in all sites of this habitat category (100% fidelity). Indicator species analysis was performed by linking megafaunal records from video (all transects) and still images (1 randomly selected transect per reef complex) with habitat categories assigned to respective video bin or image. The indicator value for each species was calculated using “multipatt” function from the “indicspecies” package in R (available as a companion package to De Cáceres and Legendre 2009). Indicator lists from video and still images were compared to determine taxonomic groups identified by both methods.

2.4.7 Reef complex summary cards

Summaries by reef complex (“reef status cards”) were assembled in Corel Draw X4. Aster plots demonstrating sponge-based indices scores were created using the ‘aster’ plot function in R available through the GitHub repository.

3. RESULTS AND DISCUSSION

3.1 APPROACHES DEVELOPED

3.1.1 Dataset adjustments

A number of adjustments were made to the video datasets due to video annotator effects. Taxonomic group with lowest annotator agreement was subphylum Vertebrata (fish; Annotation Success Index 0.11). Expert re-reviews of 10 bins for each taxonomic group under subphylum Vertebrata (total of 401 records) resulted in adjustments to 19 types of megafaunal records to reflect the expert’s identification. In all instances where annotator and expert disagreed consistently on a lower taxonomic level but agreed at a higher taxonomic level, observations were rolled up to a higher taxonomic level. These adjustments resulted in a 0.19 Annotation Success Index for fish.

Detection success was similar among annotators (Table 6), with mean detection rate of 65 and 69% (exact and fuzzy match-to-bin methods, respectively). These detection rates are comparable with the 77% value reported by Durden et al. (2016) for a study where multiple investigators annotated seabed imagery.

Overall annotation success was 31% using exact match and 36% using fuzzy match-to-bin. These values are somewhat lower than identification accuracy values reported in other studies. For example, Ninio et al. (2003) reported accuracy of identification of benthic organisms from video transects ranging from 41 to 100%, depending on the taxonomic group. Beijbom et al.

(2015) used multiple annotators for coral reef imagery and reported an identification accuracy range (measured as Cohen's kappa) for corals from 59 to 84% (Carletta 1996). Mabrouk et al. (2014) described 53% observer agreement in total epifaunal species counts from remote video collected under aquaculture farms. Lower annotation success in this study may be due partially to annotator bias and partially to procedural problems: for example, annotators reported difficulties in consistently applying the 10 second bin method, making comparisons between annotator records based on bins problematic. Our values fall in the 21-40% range considered 'fair agreement' by Landis and Koch (1977)[1]. The uncertainty introduced by the lower annotation success in our dataset is partially offset by the fact that each annotator reviewed transects from each reef complex, thus balancing data processing design.

Table 6. Detection Rate and Annotation Success Indices for post-processed video datasets.

Video annotator	**Exact match-to-bin method**		**Fuzzy match-to-bin method**	
	Detection Rate Index, % (±SE, *n*=4)	**Annotation Success Index, %** (±SE, *n*=4)	**Detection Rate Index, %** (±SE, *n*=4)	**Annotation Success Index, %** (±SE, *n*=4)
#1	64± 3.7	31±5.6	66±4.4	36±5.9
#2	68±3.9		70±0.03.8	

After adjustments to the megafaunal records were completed for the full 2012 and 2013 datasets, the following decisions were made for subsequent video-based data analyses:

- Class Polychaeta and Subphylum Tunicata were to be excluded from community analyses due to one annotator being much more likely to detect these taxa.
- Habitat categories were to be used for exploring species-habitat associations. This approach facilitated greater consistency between datasets generated by the two annotators (one annotator was more likely to default to relative abundance scores for reef-building sponges than the other) and enabled differentiating five habitat categories to which megafaunal observations could be related.

3.1.2 Sponge-based indices selection

The results of the five methods for estimating live reef-building glass sponge abundance –bin, Monte Carlo, grid, outline, and oscula – could not be directly compared because the methods themselves created differences in the scale of sponge abundance data. In general, all five methods produced similar relative assessments (Table 7, Fig. 6).

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[1] Ranges in Landis and Koch (1977) are for Cohen's kappa method of assessing annotator agreement.

Table 7. Comparison of five methods for estimating live reef-building glass sponge abundance applied to a subset of four transects.

Transect	**Video-based method**	**Video- and still image-based method**	**Still image-based methods**		
	Live abundance, bin method (% bins with sponge)	**Live % cover, Monte Carlo method** (% cover)	**Live % cover, grid method** (% cover)	**Live % cover, outline method** (% cover)	**Live abundance, oscula method** (count/m^2)
3	2.05	0.0003	0.08	0.18	0.18
4	63.73	1.0050	5.26	2.63	2.83
19	6.05	0.0005	0	0	0
23	61.79	3.1822	10.11	10.59	12.5

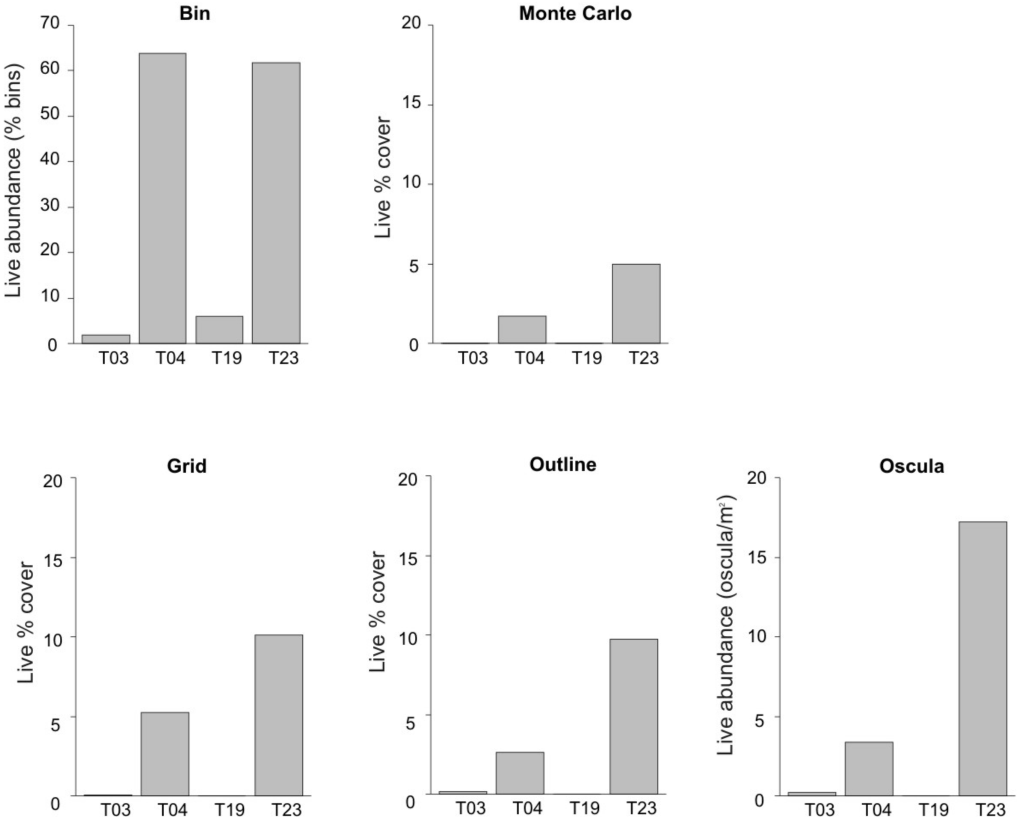

Figure 6. Five methods of assessing abundance of live reef-building glass sponges: comparison of results for four randomly selected transects.

All methods had positive and negative aspects and differed in terms of effort and resources required. The video bin method has a number of advantages: it can be applied to a large video dataset representing a long swathe of benthos, thus efficiently building a synoptic view of a larger area. Reviewing the video and recording presence-absence of live sponges in each 10 second video bin can be accomplished as quickly as 1:1 viewing-to-annotation ratio and requires less taxonomic training for annotators than other methods. A similar index has been used in monitoring deep-sea cold-water corals (Huvenne et al. 2016). However, in the case of glass sponge reefs, the video bin method does not take into account percent cover of live sponges within video bins; as such, a video bin with 100% cover and one with 1% cover would contribute equally to the overall assessment. Therefore, while the video bin method offers an efficient method of assessment, it does not represent an accurate measure of abundance of glass sponges and cannot be used as a proxy for percent cover.

The Monte Carlo method also has the advantage of efficiency: once the mean sponge size per reef complex is determined, this method can be fairly quickly applied to a large video dataset by counting live sponges in each 10 second video bin. The drawback lies in the fact that sponge size varies considerably within each reef. In addition, it is often difficult to distinguish individual sponges in dense sponge aggregations, which can result in inaccurate counts of sponges and therefore estimates of mean sponge area per reef complex.

All three still image-based methods – grid, outline, and oscula – resulted in the same transect ranking order with respect to sponge abundance: T23, T04, T03, and T19, from high to low. The outline method was the most time-consuming: on average, it took 15 minutes per image. In comparison, the grid method required 5 minute 30 seconds per image and resulted in very similar percent cover estimates (Fig. 6). The drawback of the grid method is apparent in situations when sponges not large enough to cover 50% of the 10 x 10 cm grid cell (*e.g.*, new recruits) are present. In these cases, the grid method returns 0% live sponge cover, but oscula counts indicate presence of live sponges (Table 8). East of Hornby Island and Parksville reefs had the largest proportions of images assigned 0% cover but with live oscula recorded (39% and 22%, respectively); Parksville reef had the highest proportion (10%) of images with ≥7 oscula (Table 8).

Table 8. Percentage of images where the grid method of analysis returned 0% live sponge cover, but live sponge oscula were recorded, per reef complex.

Reef complex	**Total number of images with 0% live sponge cover**	**% of images with and without oscula counts**		
		No oscula	**1 to 6 oscula**	**7 to 14* oscula**
Howe Sound - Defence Islands (1)	61	72	26	2
Howe Sound- Queen Charlotte Channel (2)	41	93	7	0
Foreslope Hills (3)	51	84	16	0
Outer Gulf Islands (4)	125	95	5	0
Gabriola Island (5)	80	75	23	3
Parksville (6)*	94	79	12	10
East of Hornby Island (7)	86	60	36	3
Sechelt (8)	83	94	6	0
Halibut Bank (9)	144	100	0	0

*One image from the Parksville reef had an oscula count of 42 due to high abundance of very small sponges.

Oscula counts offer an efficient method of estimating sponge abundance from still images. However, sizes of individual sponges differ considerably between reefs (Fig. 7). Chu and Leys (2010*a*) also found that the sizes of oscula and numbers of oscula per area of dense reef differed significantly between the three reefs studied (Howe Sound – Queen Charlotte Channel reef 2F [Howe Sound reef], Outer Gulf Islands reef 4A [Galiano Ridge reef], and Foreslope Hills [Fraser Ridge reef]). Thus, the oscula count method offers a way to assess live reef-building sponge abundance (reflecting filtration capacity), but cannot be used as a proxy for live sponge cover.

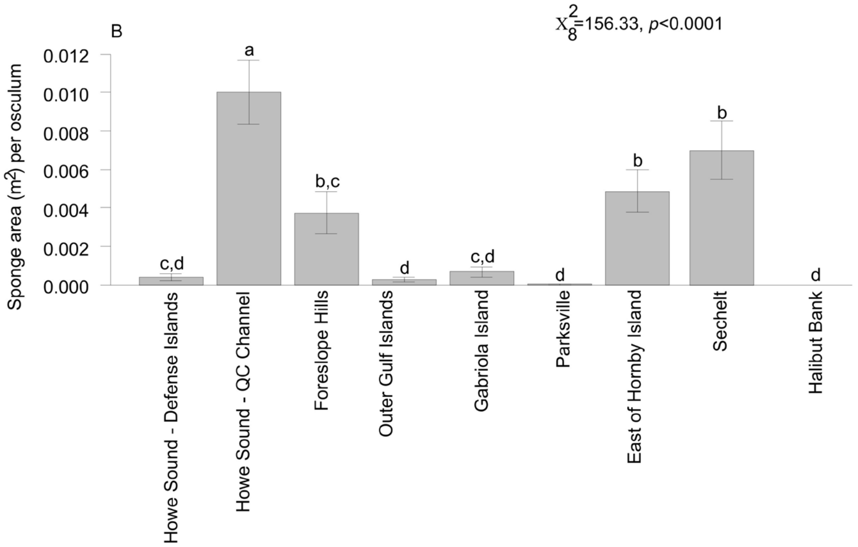

Figure 7. Live reef-building sponge area per osculum. Significance of differences between reef complexes was determined by a Kruskal-Wallis test. Treatments denoted by different letters differ significantly (P<0.05, Dunn test for multiple comparisons).

Upon reviewing the results described in this section, a decision was made to apply the video bin, grid, and oscula count methods to the complete 2012 and 2013 datasets.

3.2 REEF CHARACTER ASSESSMENT

3.2.1 Environmental conditions

Surveyed glass sponge reefs in the Strait of Georgia and Howe Sound encounter a fairly narrow range of temperature and salinity (Table 9, Fig. 8). Overall, the temperature range recorded in September 2012 and December 2013 approximately 1 m above the reefs was 7.92 to 9.73°C. Mean temperature per reef complex ranged between 8.04°C (Foreslope Hills in December 2013) to 9.58°C (Howe Sound - Queen Charlotte Sound in September). The overall salinity range recorded was 29.40 to 31.13 PSU. Mean salinity recorded per reef complex ranged between 29.76 (Parksville in September 2012) and 31.08 PSU (Foreslope Hills in September 2012). The depth range covered during our surveys was 30 to 230 m.

Comparing fall and winter temperature ranges (Fig. 8A and C) shows that overall temperature range shifted slightly lower in December. Salinity range became narrower in December by shifting toward the top of its September range (Fig. 8B and D). Halibut Bank reef complex exhibited the tightest temperature range and greatest consistency between seasons in both temperature and salinity.

Table 9. Temperature, salinity, and depth recorded approximately 1 m above glass sponge reef surface in 2012 and 2013.

September 2012

Reef complex	**Temperature, °C**			**Salinity, PSU**			**Depth, m**	
	Min	Max	Mean	Min	Max	Mean	Min	Max
Howe Sound - Defence Islands (1)	8.42	8.88	8.69	29.91	30.29	30.10	57	96
Howe Sound - Queen Charlotte Channel (2)	9.45	9.71	9.58	29.40	30.76	30.45	30	128
Foreslope Hills (3)	9.45	9.47	9.46	31.06	31.09	31.08	148	191
Outer Gulf Islands (4)	9.27	9.73	9.50	30.33	30.9	30.56	80	156
Gabriola Island (5)	8.69	8.99	8.81	30.43	30.78	30.61	116	151
Parksville (6)	8.78	8.92	8.85	29.66	29.85	29.76	55	80
East of Hornby Island (7)	8.57	8.97	8.63	29.53	30.70	30.34	58	141
Sechelt (8)	8.76	9.32	8.98	30.09	30.88	30.65	71	185
Halibut Bank (9)	9.03	9.07	9.05	30.84	31.07	30.98	167	221

December 2013

Reef complex	**Temperature, °C**			**Salinity, PSU**			**Depth, m**	
	Min	Max	Mean	Min	Max	Mean	Min	Max
Howe Sound - Defence Islands (1)	9.31	9.42	9.36	30.44	30.70	30.62	58	100
Howe Sound - Queen Charlotte Channel (2)	8.44	9.25	8.58	30.53	30.70	30.64	47	127
Foreslope Hills (3)	7.92	8.22	8.04	30.63	30.76	30.67	165	190
Outer Gulf Islands (4)	8.23	9.31	8.64	30.18	30.89	30.67	72	158
Gabriola Island (5)	9.25	9.46	9.34	30.59	30.87	30.78	108	142
Parksville (6)	9.31	9.40	9.34	30.18	30.31	30.23	70	77
East of Hornby Island (7)	9.26	9.44	9.34	30.27	30.91	30.74	69	146
Sechelt (8)	9.19	9.44	9.29	30.65	31.01	30.88	93	198
Halibut Bank (9)	8.99	9.08	9.03	30.94	31.13	31.01	166	230

Temperatures observed during both surveys for all nine reef complexes are within ranges of reported oceanographic observations. Riche et al. (2014) reported seasonal ranges at the Nanoose Bay station of <7 to > 20°C for the surface (0-50 m) layer, <8 to 10 for the mid-depth (50-200 m) layer, and 8 to 10°C for the deep (>200 m) layer. Salinities observed are also generally consistent with ranges summarized by Riche et al. (2014) for the Nanoose Bay station: 30 to 31 PSU for mid-water salinity and 31±0.2 PSU for bottom salinity. Slightly lower minimum observations of 29.91 and 29.40 PSU were observed in September 2012 in Howe Sound - Defence Islands and Howe Sound - Queen Charlotte Channel reef complexes, respectively, which may be due to the freshwater input from the Fraser River.

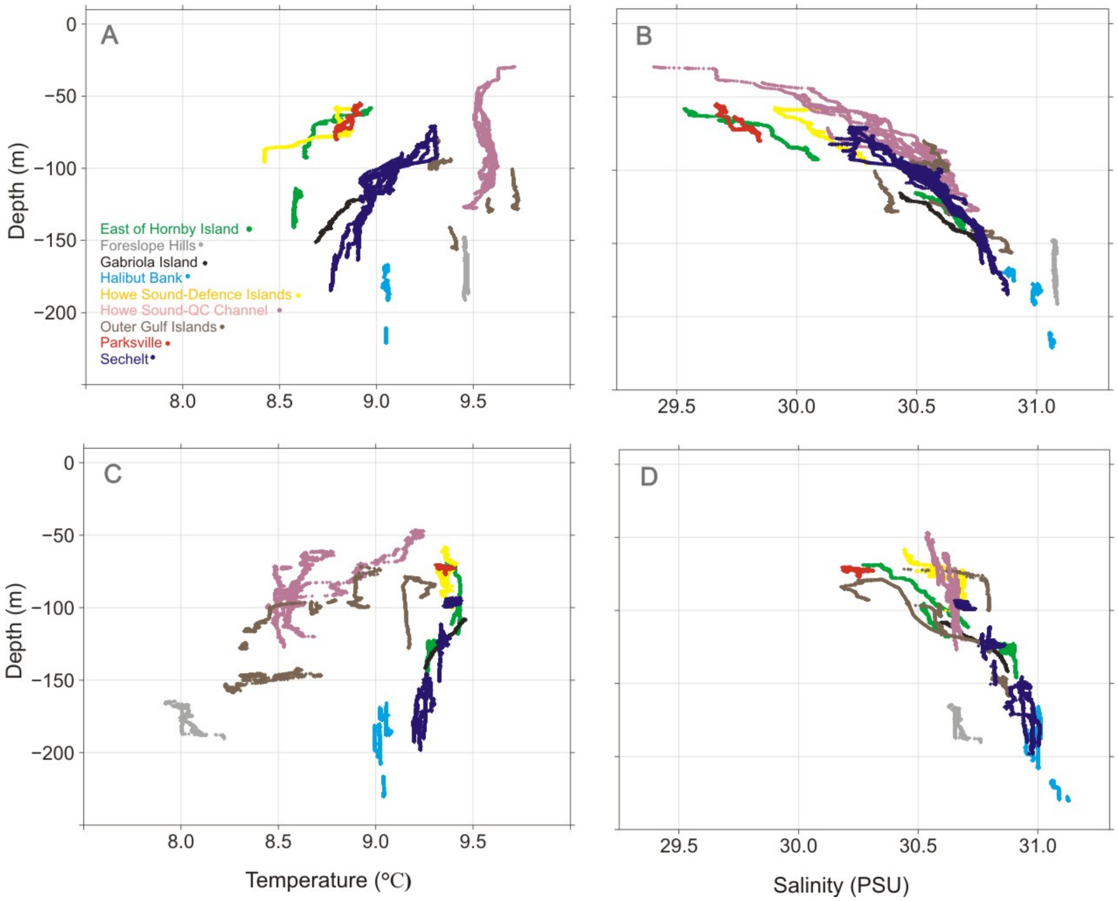

Figure 8. Temperature and salinity recorded 1 m above the benthos at nine reef complexes, in relation to depth: (A) temperature in September 2012, (B) salinity in September 2012, (C) temperature in December 2013, and (D) salinity in December 2013.

3.2.2 Glass sponge assessment

3.2.2.1 Sponge-based indices

Indices of live reef-building sponge abundance calculated using the bin, grid, and oscula methods are summarized in Table 10.

Table 10. Indices of live reef-building sponge abundance for nine reef complexes.

Reef complex	**Video-based method**	**Still image-based methods**	
	Live abundance, bin[1] (mean±95%CI)	**Live % cover, grid**[2] (mean±SE)	**Live abundance, oscula**[3] (mean±SE)
Howe Sound - Defence Islands (1)	53±20.6	0.65±0.20	2.22±0.592
Howe Sound - Queen Charlotte Channel (2)	65±8.0	9.84±1.49	7.43±1.559
Foreslope Hills (3)	39±28.4	6.93±1.61	3.84±1.143
Outer Gulf Islands (4)	26±8.8	0.22±0.12	0.39±0.134
Gabriola Island (5)	70±31.9	0.30±0.12	0.69±0.159
Parksville (6)	7±12.4	0.09±0.02	2.52±0.611
East of Hornby Island (7)	83±10.3	3.76±0.52	3.42±0.437
Sechelt (8)	26±6.4	2.03±0.42	2.14±0.409
Halibut Bank (9)	23±11.2	0.01±0.01	0

[1] Based on complete 2012 and 2013 datasets.

[2] Based on still images from 2 transects per reef complex (one from 2012 and one from 2013 dataset).

[3] Based on still images from 1 transects per reef complex (2012 dataset).

Grid and oscula methods resulted in similar relative ranking of the reef complexes, with the highest abundance of live reef-building glass sponges observed at Howe Sound – Queen Charlotte Sound, Foreslope Hills, and East of Hornby Island (Fig. 9). A notable difference occurred in the ranking of the Parksville reef: it ranked 8th using the grid method, but 4th using the oscula count method. Noting small sponge area per osculum recorded for this reef (see Fig. 7) and a high number of images where grid method of analysis returned 0% live sponge cover, but live sponge oscula were recorded (see Table 7), this disparity can be attributed to the high abundance of small sponges, possibly new recruits, in this reef complex.

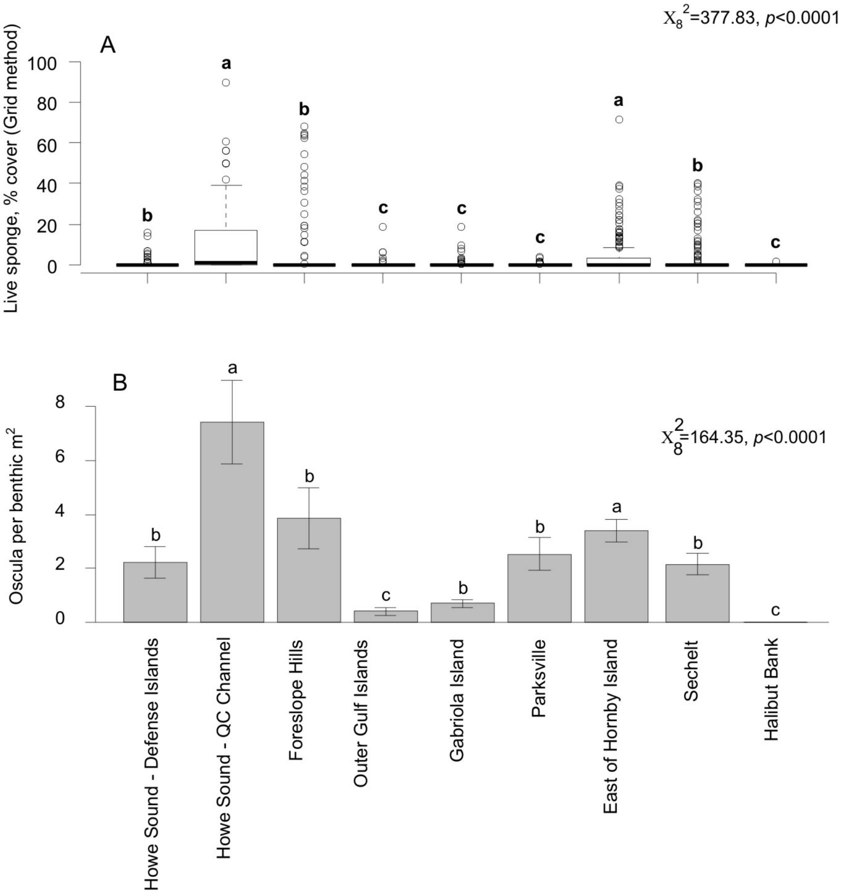

Figure 9. Indices of live reef-building sponge abundance for nine reef complexes: (A) Percent cover estimated via Grid method, by still image, (B) Density of oscula relative to area surveyed for each reef, by still image. Error bars indicate SE (n =64 to 144). Overall significance of differences between reefs was determined by a Kruskal-Wallis test. Treatments denoted by different letters differ significantly (P<0.05, Dunn test for multiple comparisons).

Indices estimating abundance of dead reef-building sponges are presented in Table 11. The video bin method returned much higher estimates of dead sponge for all reef complexes than the grid method. This is due to the fact that the video bin method incorporates all video bins where dead sponge was recorded as dominant or sub-dominant substrate, regardless of percent cover. The grid method provides an estimate of the true percent cover of dead sponge (as seen in two-dimensional imagery, top view).

Table 11. Indices of dead reef-building sponge structure for nine reef complexes.

Reef complex	**Video-based method**	**Still image-based method**
	Dead abundance, bin method[1] (mean±95%CI)	**Dead % cover, grid method**[2] (mean±SE)
Howe Sound - Defence Islands (1)	88±19.7	1.53±0.49
Howe Sound - Queen Charlotte Channel (2)	80±7.8	7.65±1.11
Foreslope Hills (3)	61±41.4	4.68±1.11
Outer Gulf Islands (4)	61±20.7	1.21±0.51
Gabriola Island (5)	93±12.5	0.36±0.09
Parksville (6)	31±56.7	0.05±0.01
East of Hornby Island (7)	81±12.5	9.05±1.25
Sechelt (8)	63±11.5	3.94±0.72
Halibut Bank (9)	64±20.4	0.05±0.03

[1] Based on complete 2012 and 2013 datasets.

[2] Based on still images from 2 transects per reef complex (one from 2012 and 2013 each).

Clumpiness index was greater than 0 for all reef complexes indicating positive aggregation of live sponge patches and ranged from 0.05 (Parksville, 2013) to 0.71 (Foreslope Hills, 2013) (Table 12).

Table 12. Live sponge distribution: Clumpiness index for nine reef complexes (based on still images from two transects per reef complex, one from 2012 and one from 2013 datasets).

Reef complex	**Live Sponge Clumpiness Index**	
	2012	**2013**
Howe Sound - Defence Islands (1)	0.16	0.34
Howe Sound - Queen Charlotte Channel (2)	0.61	0.66
Foreslope Hills (3)	0.68	0.71
Outer Gulf Islands (4)	0.28	0.50
Gabriola Island (5)	0.35	0.49
Parksville (6)	0.15	0.05
East of Hornby Island (7)	0.55	0.28
Sechelt (8)	0.56	n/a[1]
Halibut Bank (9)	n/a[1]	n/a[1]

[1] Values not available due to the absence of sufficient number of live sponge patches in still image subset used.

The proportion of still images containing visibly broken live reef-building sponges ranged from 16.2 to 75.0% per reef complex (Table 12). Visual survey methods alone do not allow reliably attributing observed damage to specific anthropogenic activities, objects, or natural causes. Table 13 thus presents a snapshot of overall visible damage to sponges.

Table 13. Sponge condition: percent of images with broken sponges recorded from one randomly selected transect per reef.

Reef complex	**N of images reviewed**	**N of images with at least one live reef-building glass sponge**	**N of images with broken sponges**	**% of images with broken sponges**
Howe Sound - Defence Islands (1)	82	32	6	18.8
Howe Sound - Queen Charlotte Channel (2)	84	43	21	48.8
Foreslope Hills (3)	64	21	5	23.8
Outer Gulf Islands (4)	140	12	9	75.0
Gabriola Island (5)	89	29	4	13.8
Parksville (6)	100	26	13	50.0
East of Hornby Island (7)	132	81	20	24.7
Sechelt (8)	118	37	6	16.2
Halibut Bank (9)	144	0	0	n/a

Sponge morphology, colour, and growth form, differed between reef complexes (Table 14, Fig. 10).

Table 14. Qualitative observations of reef composition, status, and sponge morphology of surveyed areas of each reef complex.

Reef complex	**Description**
Howe Sound - Defence Islands (1)	Small patches and isolated small-to-medium *A. vastus / H. calyx* growing on dead sponge; mostly white/cream in colour. Signs of recovery.
Howe Sound - Queen Charlotte Channel (2)	Large patches of large *A. vastus / H. calyx* of white, yellow, and orange* colour. Wide range of sponge morphology: short and wide to very tall and thin. Healthy reef appearance.
Foreslope Hills (3)	Large, wide patches and mounds of *A. vastus / H. calyx*, with a mix of white/cream, yellow, and orange[1] sponges. Many surveyed areas have healthy reef appearance.
Outer Gulf Islands (4)	Northern (4A) and Southern (4B-E) reefs differ considerably. Reef 4A has large, wide mounds/patches of *A. vastus / H. calyx*; mix of white/cream, yellow, and orange* sponges; healthy reef appearance. Reefs 4B-E contain large areas of dead and broken reef structure.
Gabriola Island (5)	Small *A. vastus / H. calyx,* mostly white/cream in colour. Large areas of visible (exposed) dead reef structure. Signs of recovery.

Reef complex	Description
Parksville (6)	Many tiny *A. vastus / H. calyx*. Very high abundance of *Rhabdocalyptus dawsoni*.
East of Hornby Island (7)	Small, medium, and large *A. vastus / H. calyx*, mostly white/cream in colour.
Sechelt (8)	Large, wide mounds/patches of *A. vastus / H. calyx*, mostly white/cream in colour. Healthy reef appearance.
Halibut Bank (9)	Very few sponges: isolated patches of small, occasionally medium *A. vastus / H. calyx*, mostly white/cream in colour. Large areas of dead and broken reef structure.

[1] Orange colour of reef-building glass sponges may be due to the presence of a symbiotic hydroid found in *H. calyx* (Schuchert and Reiswig 2006) or due to their food particle uptake (Sally Leys, University of Alberta, Edmonton, Alberta, pers. comm.).

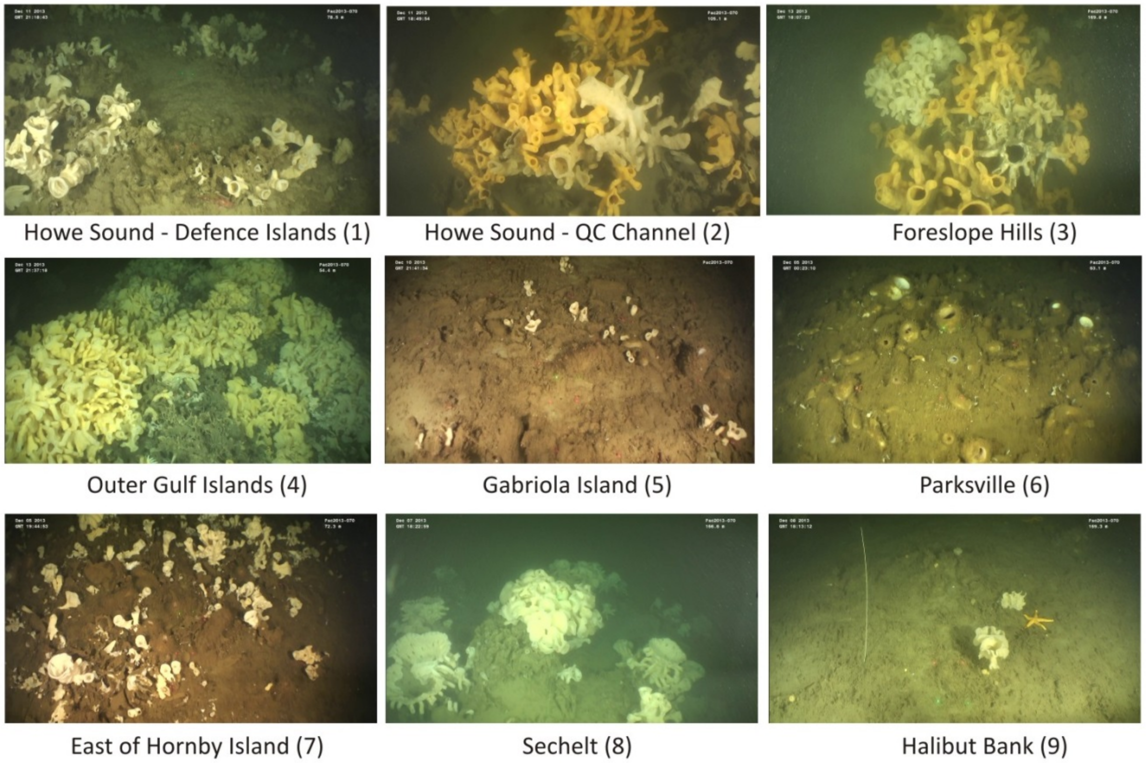
Howe Sound - Defence Islands (1)
Howe Sound - QC Channel (2)
Foreslope Hills (3)
Outer Gulf Islands (4)
Gabriola Island (5)
Parksville (6)
East of Hornby Island (7)
Sechelt (8)
Halibut Bank (9)

Figure 10. Examples of sponge morphology, colour, and growth forms at the nine reef complexes.

3.2.2.2 Habitat categories

The frequency of occurrence of the five habitat categories varied between reef complexes (Fig. 11). The surveyed areas within Gabriola, Parksville, and Halibut Bank reefs did not have any ‘dense live reef’ segments. All complexes had at least some areas designated as ‘live reef’: from 0.2% at Parksville to 24.3% at Howe Sound – Queen Charlotte Channel. ‘Dense live reef’, ‘live reef’ and ‘mixed reef’ habitat categories combined (*i.e.* areas with ≥2 live reef-building sponges per video bin) ranged from 8.4% (Parksville) to 81.9% (East of Hornby Island); these values closely matched sponge abundance estimates resulting from the video bin method (see Table 9). Areas with any visible reef structure designation (*i.e.* all except ‘no visible reef’ habitat category) accounted for 38.2% (Parksville) to 96.9% (East of Hornby Island) of the surveyed area.

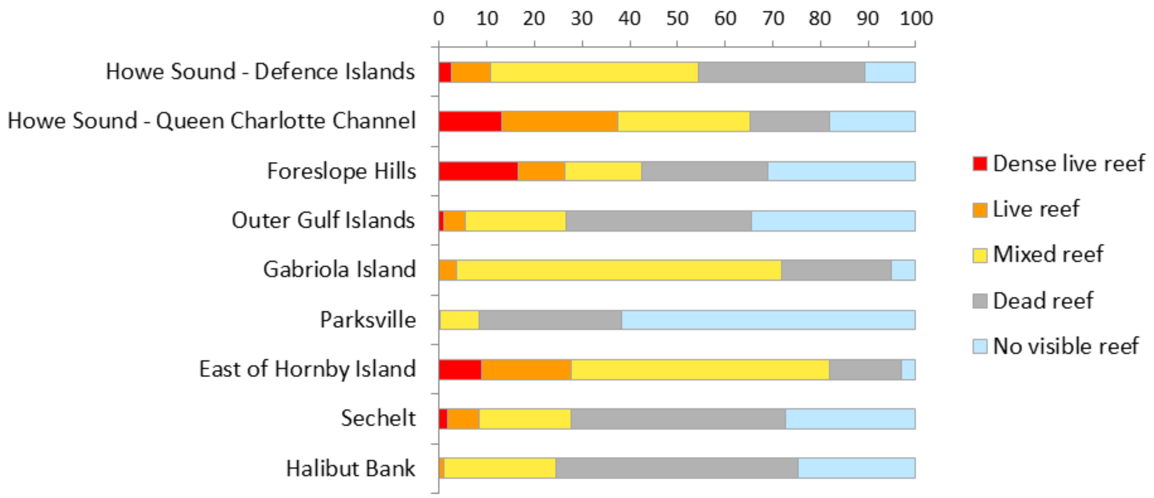

Figure 11. Frequencies of occurrence of habitat categories within nine reef complexes (based on video dataset).

Frequencies of occurrence of all habitat categories within and adjacent to each reef complex can be found in Appendix 6. An example of habitat category distribution along a single transect is shown in Fig. 12. Summary maps by reef complex can be found in Section 3.4. Detailed maps by transect are available in Appendix 7.

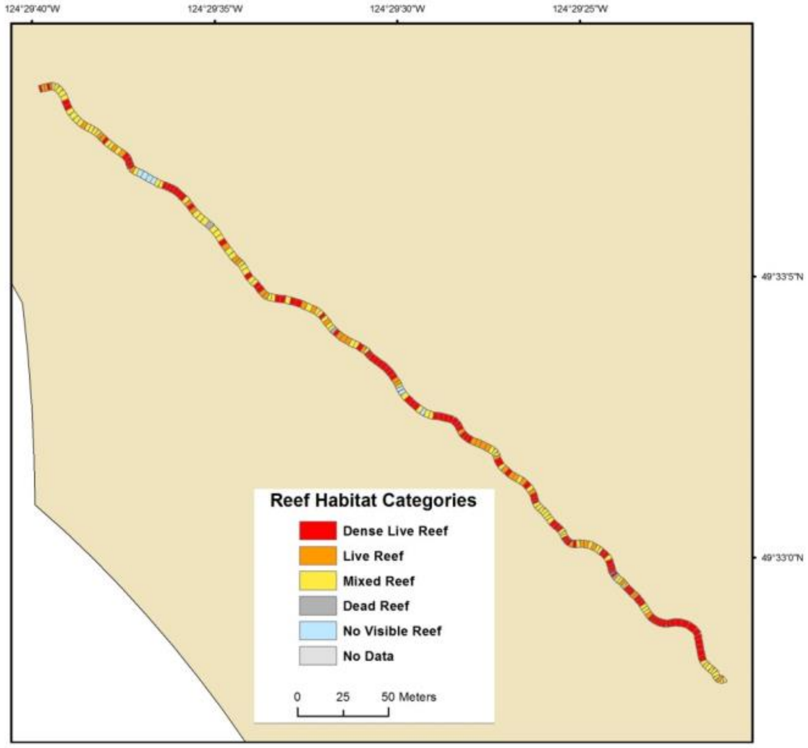

Figure 12. Habitat categories distribution within the East of Hornby Island reef complex (transect 6, 2012).

A notable difference in habitat category composition was observed between the Northern and Southern groups of reefs in the Outer Gulf Islands complex (see Appendix 7); reef 4A (referred to as Galiano Ridge reef in the primary literature) displayed much greater frequency of occurrence of video bins classified as ‘dense live reef’ and ‘live reef’ than reefs 4B to 4F. Therefore, it may be beneficial to treat the Northern and Southern reefs as separate sub-complexes during future monitoring surveys.

3.2.2.3 Composite Index of reef status

We developed and applied the following composite index of reef status (S):

$S = (0.8 \times L + 0.2 \times D) \times (1.25 - B/100)$, where

- L = Live % cover (grid method)
- D = Dead % cover (grid method)
- B = % images with visibly broken sponges. If B value is unavailable, the multiplier ($1.25 - B/100$) is removed from the equation.

This index incorporates live sponge cover, dead sponge cover (used as an estimate of area available for recruitment), and sponge condition (breakage). It combines weighted averages with a weighted geometric average to increase the influence of a “bad” score (Andreasen et al. 2001; Rice and Rochet 2005). It employs different weighting for live sponge cover (80%, or 0.8) and dead sponge cover (20% or 0.2). The value of 1.25 (as opposed to 1) in the multiplier was chosen to accommodate hypothetical cases where all sponges in a reef are visibly broken, *i.e.* B=100%; such reefs would still receive S>0.

For example, a hypothetical reef with 100% live sponge cover and no visible signs of sponge breakage would receive S = 100. A reef with 100% live sponge cover and visible signs of sponge breakage in 100% of images would receive S = 20. A reef with 10% live sponge cover, 50% dead sponge cover, and visible signs of sponge breakage in 50% of images would receive S = 13.5.

In this study, composite index of reef status ranged from 0.02 (Halibut Bank) to 7.16 (Howe Sound – Queen Charlotte Sound) (Table 15, Fig. 13).

Table 15. Composite indices of reef status [S = (0.8 x L + 0.2 x D) x (1.25 – B/100)], where L = Live % cover, grid method, D = Dead % cover, grid method; B = % images with visibly broken sponges) for nine reef complexes.

Reef complex	L	D	B	S
Howe Sound - Defence Islands (1)	0.65	1.53	18.8	0.88
Howe Sound - Queen Charlotte Channel (2)	9.84	7.65	48.8	7.16
Foreslope Hills (3)	6.93	4.68	23.8	6.56
Outer Gulf Islands (4)	0.22	1.21	75.0	0.21
Gabriola Island (5)	0.30	0.36	13.8	0.35
Parksville (6)	0.09	0.05	50.0	0.06
East of Hornby Island (7)	3.76	9.05	24.7	4.83
Sechelt (8)	2.03	3.94	16.2	2.62
Halibut Bank (9)	0.01	0.05	n/a	0.02

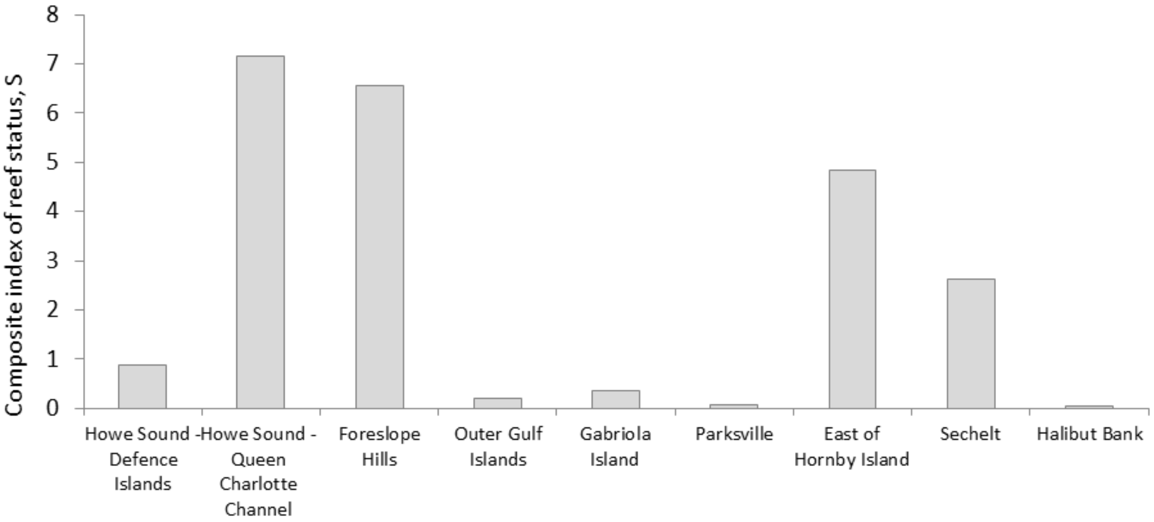

Figure 13. Composite indices of reef status [S = (0.8 x L + 0.2 x D) x (1.25 – B/100)], where L = Live % cover, grid method, D = Dead % cover, grid method; B = % images with visibly broken sponges) for nine reef complexes.

On a scale from 0 to 100, all these values appear quite low. However, they should not necessarily be interpreted as very poor reef status. Areas designated as ‘visible reef’ habitat categories occupied a significant proportion of each reef’s area: from 38.2 to 96.9% (Fig. 11). It is important to note that *S* value of 100 (a reef with 100% live sponge cover with no sponge damage) represents an upper value limit, but is likely not attainable in reality for a number of reasons. The reefs appear to be naturally patchy. Dead sponges provides important structural habitat for the settlement of new sponge recruits. Death of sponges is a natural process within the reefs: as a sponge grows, the base may die with the flow regime changes (S. Leys, pers. comm.). The optimal ratio or range of live-to-dead sponge within a reef remains unknown. Sponges likely experience a small degree of breakage due to natural causes. For example, dorid nudibranchs *Peltodoris lentiginosa* and *Archidoris odhneri* have been shown to prey on reef-building glass sponges (Chu and Leys 2012). Chu and Leys (2010a) also observed the longhorn decorator crab *Chorilia longipes* inside *Aphrocallistes vastus* oscula decorating itself with live sponge tissue. Fish have been seen knocking off small pieces of sponge *in situ* (J. Pegg, pers. comm.). As more glass sponge reefs are described and assessed and as time series are developed through monitoring, the range of observed *S* values will likely expand. Although the true upper limit of reef character may remain unknown, the composite index allows the status of individual reef complexes to be tracked (or followed) over time and facilitates relative comparison between complexes.

The relative scoring of reef complexes aligns with the previous qualitative status assessment described in Cook et al. (2008) (see Table 1). The Foreslope Hills (Fraser Ridge reef) and Sechelt complexes - which are among the four that scored relatively high on the composite index - were characterized as “healthy, undamaged” in 2002 and 2003, respectively. Halibut Bank that scored the lowest in our assessment was described as “largely dead” in 2003; Gabriola Island reef, which was the fourth lowest scoring reef in our assessment, was described as “largely dead, possibly recovering” (Cook et al. 2008). One of the Howe Sound – Queen Charlotte Channel reefs, although not assessed by Cook et al. (2008), was described by Marliave et al. (2009) and (Chu and Leys 2010*a*) as having abundant sponges and a diverse megafaunal community, which correlates well with it receiving the highest score in our assessment.

The Outer Gulf Islands complex scored low in our assessment, but the reef 4A from this complex (Galiano Ridge reef) was described in Chu and Leys (2010*a*) as one with high live sponge cover. This can be explained by the fact that neither of the two transects used for calculating the composite index for the Outer Gulf Islands complex crossed reef 4A; instead, both transects captured the Southern reefs of this complex. This supports the suggestion described in section 3.2.2.1 above to treat the Northern and Southern reefs as separate sub-complexes during monitoring surveys. The composite index of 0.21 assigned to the Outer Gulf Islands complex in this paper may be considered the monitoring baseline value for the Southern sub-complex.

The remaining three reef complexes - Howe Sound - Defence Islands, Parksville, and East of Hornby Island – were not assessed either quantitatively and qualitatively prior to this assessment.

Overall, the composite index of reef status (S) appears to perform well in distinguishing between reefs of qualitatively different status. However, there currently is uncertainty around the relative importance of live and dead sponge cover for reef function; as our understanding of the importance of live and dead cover improves, the multipliers in the composite index may need to be adjusted. In addition, the composite index incorporates only a small number of potential metrics related to reef-building glass sponges and thus should not be used as the main index of reef status. A number of additional metrics may be incorporated into the composite index of reef status in the future as our knowledge of glass sponge reefs and associated communities, as well as our understanding of function and diversity in biogenic habitats in general, expands. For example, a biodiversity component may be incorporated into the composite index once a better understanding of the functional linkages between various trophic levels in glass sponge reef ecosystem is achieved. Seascape ecology metrics can be incorporated as well, after autocorrelation in sponge distribution and associations between habitat distribution metrics and indicator taxa are explored further. Finally, recovery potential component of the composite index can be refined and strengthened through a better understanding of sponge larval ecology and recruitment, as well as resilience and recovery of individual sponges and sponge reefs as a whole.

3.2.2.4 Reef boundaries

Live reef-building sponges were found outside (off-reef) of all reef footprints delineated by multibeam bathymetry (Table 16). Gabriola Island and East of Hornby Island had the highest frequency of occurrence of sponge habitat categories outside of reef footprints. As much as 20.8 and 37.1% of the area surveyed outside of these reef complexes, respectively, was classified as 'dense live' or 'live' reef. Moreover, 97.4 and 100% of the area surveyed outside of these reef complexes, respectively, contained visible reef structure (Table 16, Appendix 7; note, however, that areas outside of reef complexes were not surveyed extensively).

Table 16. Comparison of frequencies of occurrence of various habitat categories within and outside of reef footprints[1]. Denominators in on- and off-reef calculations were total numbers of on- and off-reef video bins, respectively).

Reef complex	**Dense live reef, % occurrence**		**Live reef, % occurrence**		**Live sponges present (dense live, live, and mixed combined), % occurrence**		**Visible reef structure (all except 'no visible reef' combined), % occurrence**	
	On-reef	**Off-reef**	**On-reef**	**Off-reef**	**On-reef**	**Off-reef**	**On-reef**	**Off-reef**
Howe Sound - Defence Islands (1)	2.6	0	8.2	1.0	54.3	12.4	89.2	27.6
Howe Sound - Queen Charlotte Channel (2)	13.1	0.9	24.3	2.1	65.3	7.6	81.9	10.3
Foreslope Hills (3)	16.5	0	9.8	1.0	42.5	3.0	68.9	16.8
Outer Gulf Islands (4)	1.0	0.8	4.5	4.8	26.8	23.3	65.4	28.8
Gabriola Island (5)	0	**3.9**	3.6	**16.9**	71.8	**82.5**	94.8	**97.4**
Parksville (6)	1.8	0	6.8	0	27.6	8.8	72.5	27.7
East of Hornby Island (7)	9.0	**2.8**	18.6	**34.3**	81.9	**96.8**	96.9	**100.0**
Sechelt (8)	0	0	0.2	0	8.4	0.7	38.2	7.5
Halibut Bank (9)	0	0	1.0	0.3	24.4	4.1	75.4	9.6

[1] Based on 2013 video dataset only; transects 18 and 23 were excluded due to off-reef portions being removed during quality control.

In three reef complexes our survey transects extended slightly (approximately 10 m) beyond closure boundaries (note that surveys were planned and conducted before the boundaries were established). In two of these cases (Halibut Bank and Sechelt reefs) no visible signs of live or dead reef were noted outside the boundaries. However, dense live and live reef areas were noted outside of the closure boundary at the Gabriola complex (Fig. 14). The presence of live sponges outside of the multibeam-based footprint of the Outer Gulf Islands complex reef 4A

(Galiano Ridge reef) has been noted during earlier studies (Jackson Chu, Fisheries and Oceans Canada, Sidney, Canada, pers. comm; Anya Dunham, pers. comm.).

The presence of live sponges outside of the reef footprints may signify reef expansion, but may also be capturing the presence of sponge gardens (without sub-surface and above surface structure sufficient to produce multibeam sonar signature) adjacent to the reefs.

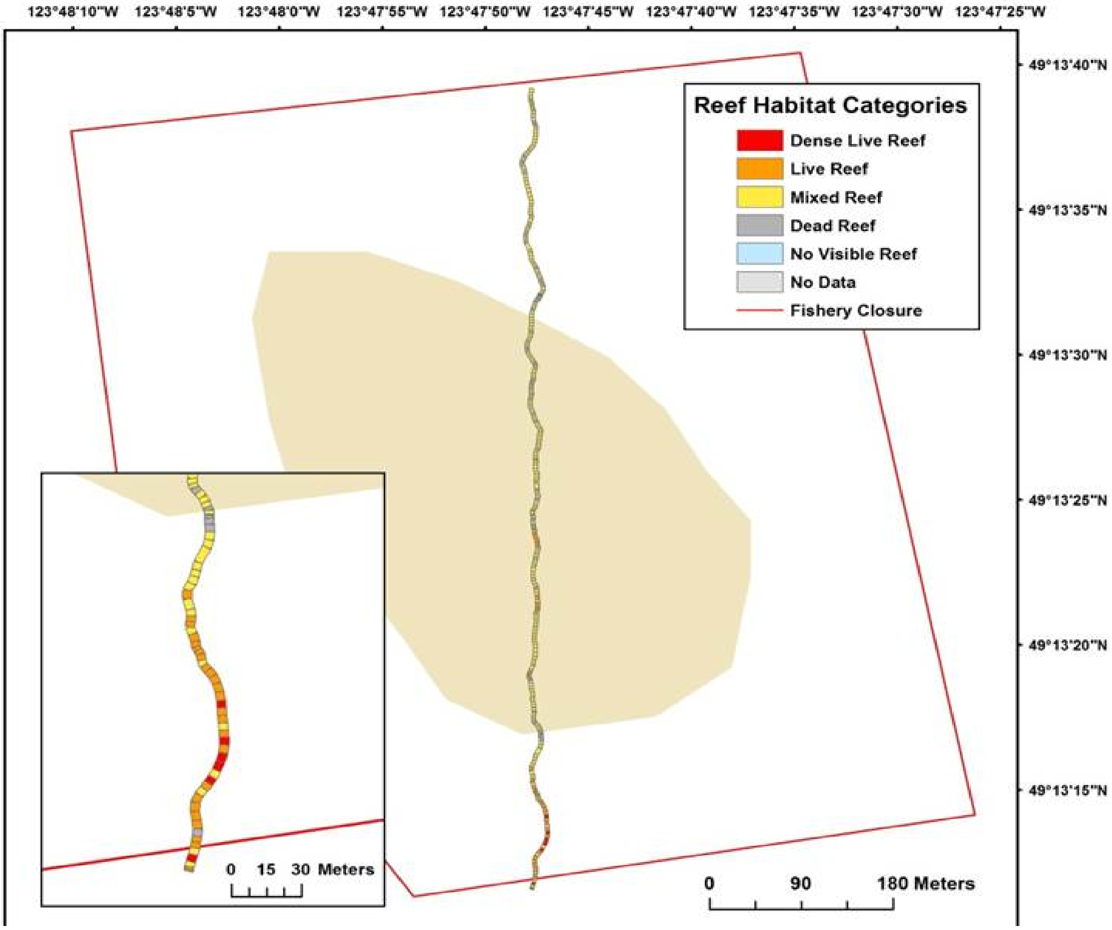

Figure 14. Distribution of habitat categories in and adjacent to the Gabriola Island reef (transect 24, 2013). Note dense, live, and mixed reef areas noted not only outside of reef footprint, but also outside of the closure boundary (see inset).

3.2.3 Megafaunal community

Diverse megafaunal communities - 9 phyla, 101 unique taxonomic groups - were observed in association with the glass sponge reefs (Table 17).

The taxonomic list generally agrees with and expands the lists developed in earlier studies. Cook et al. (2008) surveyed 7 reefs and observed 31 unique taxa. Chu and Leys (2010*a*) described a diverse assemblage of animals representing 7 phyla and 14 classes in Howe Sound – Queen Charlotte Channel reef 2F [Howe Sound reef], Outer Gulf Island reef 4A [Galiano Ridge reef], and the Foreslope Hills complex [Fraser Ridge reef]. Dunham et al. (2015) observed macrofauna from 7 phyla and 14 classes in the Outer Gulf Island reef 4A.

Table 17. Fish and invertebrate taxa observed on- and off-reef (i.e. within and outside of the glass sponge reef footprint). Identifications were made to the lowest taxonomic level possible. ”x” denotes presence, “-“ denotes absence.

Phylum **Class** **Order** **Family**	**Species**[1]	**Common name**	**Reef closure**																	
			1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
Porifera Hexactinellida	*Aphrocallistes vastus* or *Heterochone calyx*	Cloud or Goiter Sponges (reef-building species)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Rhabdocalyptus dawsoni	Boot Sponge	x	x	x	x	-	-	x	x	x	x	x	x	x	x	x	x	x	x
Demospongiae	Unidentified species	Demosponges	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x
	Stylinos spp.	Puff Ball Sponge	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Bryozoa	Unidentified species	Bryozoans	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
Cnidaria Anthozoa Subclass Ceriantharia Actiniaria	Unidentified species	Anemones	-	-	x	x	x	x	x	x	-	-	-	-	-	-	x	x	x	-
	Cribrinopsis fernaldi	Crimson Anemone	x	-	x	x	x	x	x	x	-	-	-	-	-	-	-	-	x	x
	Metridium spp.	Plumose anemones	x	-	x	x	x	x	x	x	-	-	-	-	-	-	-	-	x	-
Spirularia	*Pachycerianthus fimbriatus*	Tube-dwelling Anemone	x	x	x	x	x	x	x	x	x	x	-	x	-	-	x	x	x	x
Subclass Hexacorallia Zoantharia	Unidentified species	Zoanthid Cnidarians	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-

Phylum Class Order Family	Species[1]	Common name	Reef closure																	
			1		2		3		4		5		6		7		8		9	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
Subclass Octocorallia Alcyonacea	*Paragorgia* spp.	Gorgonian corals	-	-	-	-	-	-	x	x	-	-	-	-	-	x	-	-	-	-
	Swiftia spp.	Gorgonian corals	-	-	-	-	x	x	-	x	x	-	-	-	x	x	x	x	x	-
Scleractinia	*Balanophyllia elegans*	Orange Cup Coral	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-	-
Pennatulacea	Unidentified species	Sea pens	x	x	x	x	x	x	x	-	x	x	x	x	-	-	x	x	x	x
	Ptilosarcus gurneyi	Orange Sea Pen	-	-	-	-	-	x	-	-	-	-	x	x	-	-	-	-	-	-
Hydrozoa	Unidentified species	Hydroids	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Anthoathecata	*Stylaster* spp.	Hydrocorals	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
Leptothecata	*Aequorea* spp.	Water jellies	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
Annelida Polychaeta	Unidentified species	Polychaete worms	-	-	x	-	x	x	x	x	x	-	x	-	x	-	x	x	x	x
Brachiopoda	Unidentified species	Lamp shell worms	x	-	x	-	x	x	x	x	-	-	-	-	x	-	x	x	x	x
Mollusca Bivalvia	Unidentified species	Bivalve mollusks	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
Pectinida	*Chlamys hastata*	Swimming Scallop	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
Cephalopoda Octopoda	Unidentified species	Octopod Mollusks	-	-	-	-	-	-	-	-	x	-	-	-	-	x	-	-	-	-

Phylum **Class** **Order** **Family**	**Species**[1]	**Common name**	**Reef closure**																	
			1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
	Enteroctopus dofleini	Giant Pacific Octopus	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-
	Octopus sp.	Octopus	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
Teuthida	Unidentified species	Squids	x	-	-	-	x	-	x	-	-	-	x	-	-	-	x	-	x	-
Gastropoda	Unidentified species	Gastropod mollusks	-	-	-	-	-	-	x	x	x	-	-	-	x	-	x	-	-	-
Littorinimorpha	*Fusitriton oregonensis*	Oregon Hairy Triton	-	-	-	-	x	-	x	x	x	x	-	-	x	x	x	x	x	x
Nudibranchia	Unidentified species	Sea slugs	-	-	x	x	-	-	x	x	-	x	-	-	x	-	x	-	x	-
	Tochuina tetraquerta	Orange Peel Nudibranch	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arthropoda Infraclass Cirripedia	Unidentified species	Barnacles	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-
Decapoda	Unidentified species	Decapod crustaceans	-	-	x	-	-	-	x	x	-	-	-	-	-	-	x	x	-	-
Cancridae	*Metacarcinus magister*	Dungeness Crab	-	-	x	x	x	x	-	-	-	-		-	-	-	x	x	-	-
	Glebocarcinus oregonensis	Pygmy Rock Crab	-	-	x	x	x	x	-	-	-	-	-	-	-	-	x	x	-	-
S/o Dendrobranchiata	Unidentified species	Shrimps and prawns	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Epialtidae	*Chorilia longipes*	Longhorn Decorator Crab	x	-	x	x	x	-	x	x	-	-	x	-	x	x	x	x	x	-
Galatheidae	*Munida quadrispina*	Squat Lobster	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hapalogastridae	*Acantholithodes hispidus*	Spiny Lithode Crab	x	-	x	x	x	-	x	x	-	-	-	-	x	-	x	-	-	-

Phylum **Class** **Order** **Family**	**Species**[1]	**Common name**	**Reef closure**																	
			1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
Lithodidae	Unidentified species	Lithodid crabs	-	-	-	-	-	-	x	-	-	-	-	-	x	-	x	x	-	-
	Lopholithodes sp.	Box crabs	-	-	x	-	-	-	x	-	x	-	-	x	x	-	x	-	x	x
Majidae	Unidentified species	Spider crabs	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oregoniidae	*Chionoecetes* spp.	Tanner crabs	-	-	x	x	x	-	x	-	-	-	-	-	-	-	x	x	x	-
Pandalidae	*Pandalus platyceros*	Spot Prawn	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Paguridae	Unidentified species	Hermit Crabs	-	-	-	-	x	x	x	x	x	x	-	x	x	x	x	x	x	x
Echinodermata Asteroidea	Unidentified species	Sea stars	x	-	-	-	-	x	x	x	x	x	x	-	x	x	x	x	x	-
	Ceramaster patagonicus	Cookie Star	x	x	-	-	-	x	x	x	x	x	x	-	x	x	x	x	x	x
	Crossaster papposus	Common Sun Star	-	-	-	-	-	-	x	x	-	-	x	-	-	-	-	-	-	-
	Gephyreaster swifti	Gunpowder Star	x	x	-	-	-	-	x	x	x	x	-	-	x	x	x	x	x	-
	Henricia spp.	Blood stars	x	x	x	-	x	x	x	x	x	-	x	-	-	-	x	x	x	x
	Luidia foliolata	Sand Star	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
	Mediaster aequalis	Vermillion Star	-	-	x	-	-	x	x	x	x	x	x	-	x	x	x	x	x	x
	Pteraster militaris	Wrinkled Star	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
	Pteraster tesselatus	Cushion Star	x	-	-	-	x	-	x	x	x	x	x	-	-	x	x	x	x	-
	Pycnopodia helianthoides	Sunflower Star	-	-	-	-	x	-	x	-	-	-	x	-	-	-	x	-	-	-

Phylum **Class** **Order** **Family**	**Species**[1]	**Common name**	**Reef closure**																	
			1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
	Solaster spp.	Sun stars	-	-	-	-	-	-	x	x	-	-	x	-	-	-	-	-	-	-
	Stylasterias forreri	Velcro Star	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
Crinoidea	*Florometra serratissima*	Common Feather Star	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-
Echinoidea	*Strongylocentrotus* spp.	Sea urchins	-	-	-	-	x		x	x	-	-	-	-	-	-	-	-	-	-
Holothuroidea	Unidentified species	Sea cucumbers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
	Apostichopus californicus	California Sea Cucumber	-	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
	Psolus chitonoides	Armoured Sea Cucumber	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
	Psolus squamatus	Scaly Sea Cucumber	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-
Ophiuroidea	Unidentified species	Brittle stars	-	-	-	-	-	-	x	x			x	-	-	-	-	-	x	-
	Gorgonocephalus eucnemis	Basket Star	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chordata **Subphylum Tunicata**	Unidentified species	Tunicates	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-
	Halocynthia spp.	Sea Peaches	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
Subphylum Vertebrata (Fish) Actinopteri	Unidentified species	Ray-finned fishes	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x
Batrachoidiformes	*Porichthys notatus*	Plainfin Midshipman	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Phylum Class Order Family	Species[1]	Common name	Reef closure																	
			1		2		3		4		5		6		7		8		9	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
Gadiformes Gadidae	Unidentified species	Codfishes	x	x	x	x	x	-	x	x	x	-	x	-	x	-	x	x	x	x
Osmeriformes Osmeridae	Unidentified species	Smelts	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perciformes Bathymasteridae	*Ronquilus jordani*	Northern Ronquil	-	-	x	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-
Embiotocidae	*Rhacochilus vacca*	Pile Perch	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pholidae	*Pholis clemensi*	Longfin Gunnel	-	-	x		-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stichaeidae	Unidentified species	Pricklebacks	x	x	x	x	-	-	-	-	-	-	x	x	-	-	x	x	-	-
	Lumpenus sagitta	Snake Prickleback	-	-	x		-	-	-	-	-	-	x	-	-	-	-	-	-	-
Zoarcidae	*Lycenchelys* spp.	Eelpouts	-	-	x	x	-	-	-	-	-	-	x	x	-	-	-	-	-	-
	Lycodes pacificus	Blackbelly Eelpout	-	-	x	x	-	-	-	-	-	-	-	-	-	x	-	-	-	-
Pleuronectiformes Pleuronectidae	Unidentified species	Flatfishes	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x
	Hippoglossoides elassodon	Flathead Sole	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
	Lepidopsetta bilineata	Rock Sole	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Phylum Class Order Family	Species[1]	Common name	Reef closure																	
			1		2		3		4		5		6		7		8		9	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
	Lyopsetta exilis	Slender Sole	x	x	x	x	-	-	-	-	x	x	x	x	x	-	x	x	-	-
	Microstomus pacificus	Dover Sole	-	-	-	-	-	-	-	-	x	-	x		x	-	-	-	-	-
Scorpaeniformes	Unidentified species	n/a	x	-	-	x	-	-	x	-	-	-	x	x	-	-	-	-	-	-
Agonidae	Unidentified species	Poachers	-	-	x	x	-	-	x	x	-	-	x	x	x	-	x	-	-	-
	Agonopsis vulsa	Northern Spearnose Poacher	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cottidae	Unidentified species	Codfishes	x	-	x	x	x	-	x	-	x	-	x	-	x	-	x	x	-	-
	Artedius spp.	Sculpins	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
Hexagrammidae	*Hexagrammos decagrammus*	Kelp Greenling	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
	Ophiodon elongatus	Lingcod	-	-	-	-	-	-	x	x	x	-	x	-	x	-	x	-	-	-
Liparidae	Unidentified species	Snailfishes	x	x	x	x	x	-	x	-	-	-	x	-	x	-	x	-	x	-
Sebastidae	Unidentified species	Rockfishes and thornyheads	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	-	x	x
	Sebastes diploproa	Splitnose Rockfish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x
	Sebastes elongatus	Greenstriped Rockfish	-	-	x	x	x	-	x	x	x	x	x	-	x	x	x	x	-	-
	Sebastes maliger	Quillback Rockfish	-	x	x	x	x	-	x	x	-	-	x	-	x	x	x	-	-	-
	Sebastes ruberrimus	Yelloweye Rockfish	-	-	x	-	-	-	x	-	-	-	x	-	x	x	x	-	x	x

Phylum **Class** **Order** **Family**	**Species**[1]	**Common name**	**Reef closure**																	
			1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**	
			on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off	on	off
	Sebastolobus spp.	Thornyheads	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-
Elasmobranchii																				
Squaliformes	*Squalus suckleyi*	Pacific Spiny Dogfish	-	-	x	x	x	-	-	x	-	-	-	-	x	-	x	x	-	-
Rajiformes	Unidentified species	Skates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	x
	Raja binoculata	Big Skate	-	-	x	-	-	-	-	-	-	x	-	-	-	-	x	-	-	x
	Raja rhina	Longnose Skate	-	-	x	x	-	-	-	-	x	-	-	-	-	-	x	-	x	x
Holocephali	*Hydrolagus colliei*	Spotted Ratfish	-	-	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x

[1] "spp." is used when number of species is unknown (≥1)

Nine taxa were found in all reef complexes: class Demospongiae, order Pennatulacea, suborder Dendrobranchiata, *Munida quadrispina*, *Pandalus platyceros*, class Actinopteri, and families Gadidae, Sebastidae, and Pleuronectidae (Table 16). For each reef complex, a number of dominant taxonomic groups were present, with the rest of the community being less abundant. The identity of the dominant taxonomic groups differed between complexes (Fig. 15).

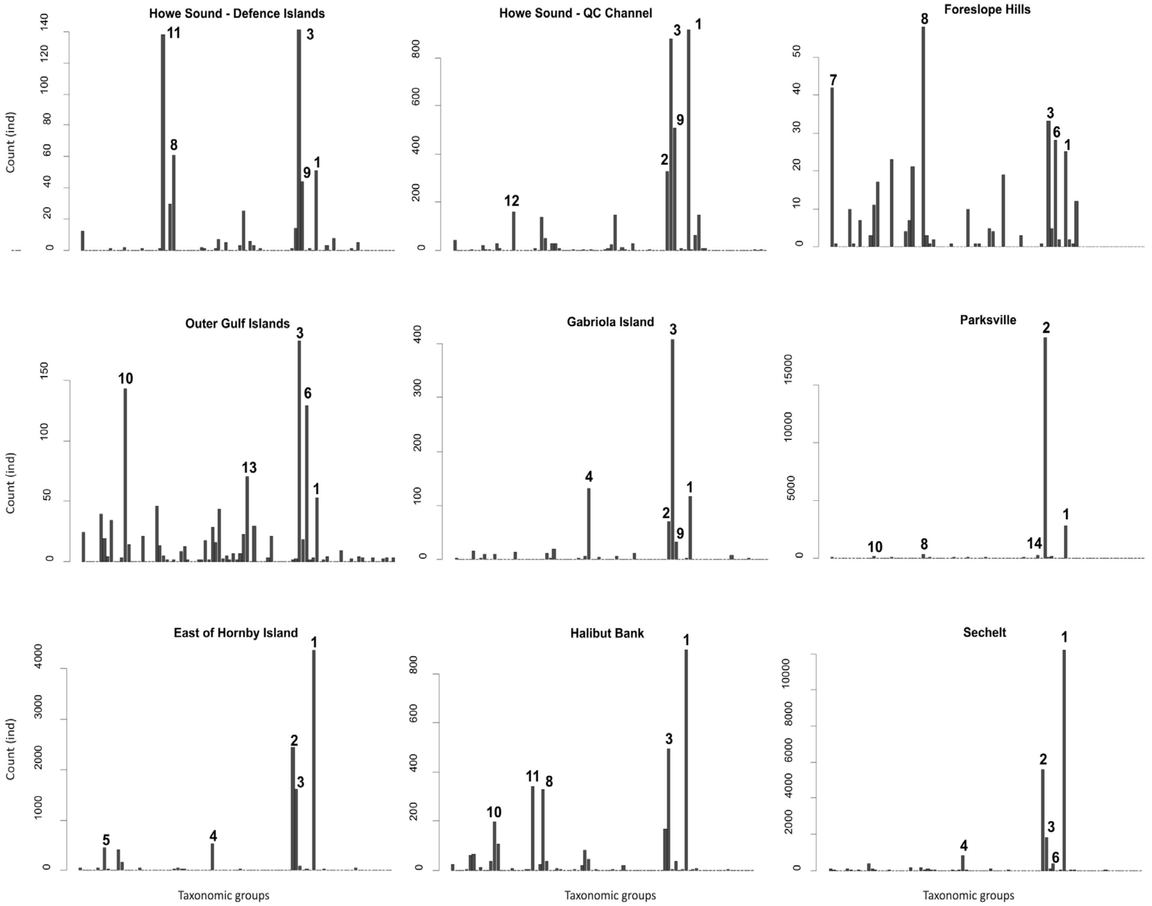

Figure 15. Abundance of taxonomic groups within reef complexes. Top five most abundant taxa in each reef complex are marked with numbers (1-14): 1 – Munida quadrispina (Squat Lobster), 2 - Rhabdocalyptus dawsoni (Boot Sponge), 3 - S/o Dendrobranchiata (Shrimp), 4 - Ceramaster patagonicus (Cookie Star), 5 - Class Gastropoda (Gastropod Molluscs), 6 – Fam. Paguridae (Hermit Crabs), 7 – Class Actinopteri (Ray-Finned Fishes), 8 – Order Pennatulacea (Sea Pens), 9 - Pandalus platyceros (Spot Prawn), 10 – Demospongiae (Demosponges), 11 - Pachycerianthus fimbriatus (Tube-dwelling Anemone), 12 – Fam. Sebastidae (Rockfishes and Thornyheads), 13 – Class Ophiuroidea (Brittle Stars), 14 - Halocynthia spp. (Sea Peaches).

Total megafaunal density ranged from 0.38 to 7.56 individuals per m^2. Mean megafaunal density assessments in sponge reefs vary greatly between published literature (e.g. Chu and Leys 2010*a*, Dunham et al. 2015). Megafaunal density in glass sponge reefs was found to largely be driven by crustaceans, in particular *Munida quadrispina* (Chu and Leys 2010*a*), the detectability of which strongly depends on the method of assessment, equipment, and the resolution of the imagery. This underscores the importance of monitoring surveys using compatible sampling platforms and data processing hardware to enable valid comparisons over space and time.

Reef complexes with the highest megafaunal density – Parksville, East of Hornby Island, and Sechelt – had the lowest diversity and evenness indices. Overall species richness ranged from 28 (Howe Sound - Defence Islands) to 53 (Outer Gulf Islands). Species richness is presented for the total area surveyed per reef complex in Table 18 and by transect in Figure 16.

Table 18. Univariate indices of community composition for nine reef complexes (calculated using all video bins in each reef complex combined).

Reef complex	**Total megafaunal density, ind/m^2**	**Species richness**	**Shannon-Wiener diversity index**	**Pielou evenness index**
Howe Sound - Defence Islands (1)	0.49	28	2.28	0.68
Howe Sound - Queen Charlotte Channel (2)	0.54	47	2.31	0.60
Foreslope Hills (3)	0.38	33	2.90	0.83
Outer Gulf Islands (4)	0.49	53	3.04	0.77
Gabriola Island (5)	0.69	34	1.97	0.56
Parksville (6)	7.56	42	0.69	0.18
East of Hornby Island (7)	2.10	41	1.78	0.48
Sechelt (8)	1.53	51	1.47	0.37
Halibut Bank (9)	0.63	40	2.39	0.65

Species richness, diversity and evenness indices, as well as total megafaunal density differed significantly between reef complexes (Kruskal-Wallis test, $P<0.05$; Fig. 16). Interestingly, Parksville reef complex, while having low live sponge cover (see Fig. 9A), exhibited the greatest species richness (Fig. 16A) and megafaunal density (Fig. 16D). High megafaunal density within this reef is largely driven by the high abundance of Boot Sponge *Rhabdocalyptus dawsoni* (see Fig. 14). High species richness may be due to Boot Sponge-dominated habitat supporting a rich megafaunal community; in addition, Boot Sponges are less morphologically complex compared to reef-building glass sponge species *A. vastus* and *H. calyx*, and thus megafaunal organisms have higher detection likelihood among Boot Sponges when visual survey methods are used.

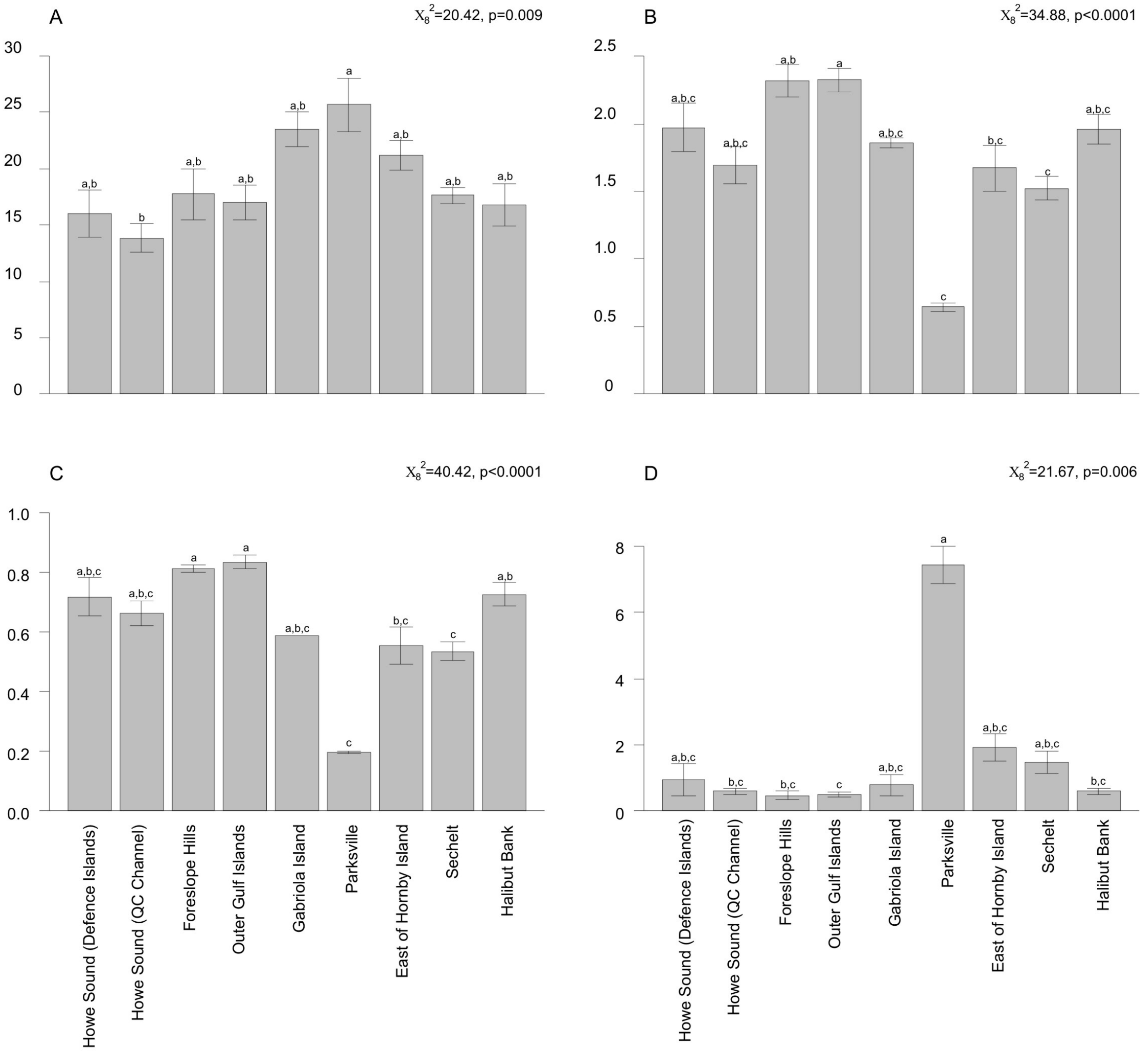

Figure 16. Univariate indices of community composition at nine reef complexes, per transect Species richness (A), Shannon-Wiener diversity index (B), Pielou evenness index (C), and total megafaunal density, ind/m2 (D). Error bars indicate SE (for n transects per reef see Table 2). Overall significance of differences between reefs was determined by Kruskal-Wallis test. Treatments denoted by different letters differ significantly (P<0.05, Dunn test for multiple comparisons).

Community structure was significantly different across reef complexes, with Howe Sound – Queen Charlotte Channel and Parksville complexes exhibiting the most distinct community structure (Fig. 17, Table 19).

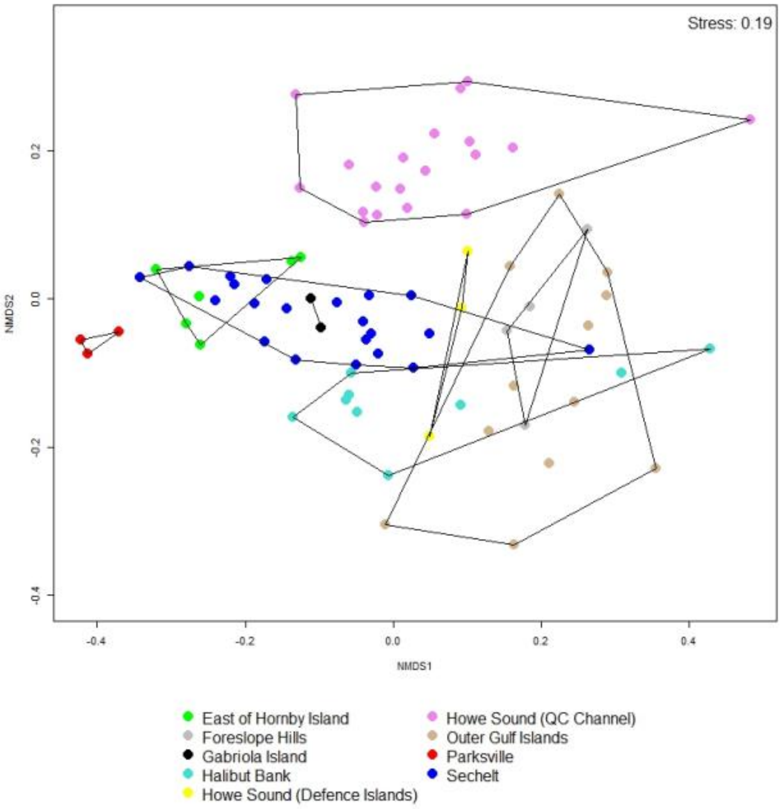

Figure 17. Two-dimensional non-metric multidimensional scaling (nMDS) ordination plot of megafaunal community structure across reef complexes. Zero-adjusted Bray-Curtis dissimilarity matrix of square root-transformed megafaunal abundance data was used.*Table 19. Results of perMANOVA analysis of the Bray-Curtis dissimilarities for megafaunal community structure in reef complexes. Bold face indicates statistical significance (P<0.05). P-value is based on 999 permutations.*

Model Terms	**df**	**SS**	**MS**	**Pseudo-F**	**R^2**	***P*(perm)**
Reef complex	8	7.47	0.928	6.38	0.422	**0.001**
Residual	70	10.19	0.146	-	0.578	-
Total	78	17.61	-	-	1	-

Pairwise comparisons between reef complexes (Table 20; for *F*, R^2, and *P* values see Appendix 7; *n*=36) revealed 29 pairs with statistically significant differences. Howe Sound – Queen Charlotte Channel and East of Hornby Island reefs had unique community structure (significantly different from each other and from all other reef complexes). Community structure at Outer Gulf Island was significantly different from that in all other complexes except Foreslope Hills. Sechelt was significantly different from all other complexes except Gabriola Island. Halibut Bank was significantly different from all other complexes except Howe Sound - Defence Islands.

Table 20. Reef complex groupings resulting from pairwise comparisons of community structure (PerMANOVA post hoc tests, α = 0.05, P≤0.0375). Groupings containing only one reef complex are bolded. Full pairwise comparisons table can be found in Appendix 8.

Reef complex	**Groupings**							
Howe Sound - Defence Islands (1)	-	B	-	-	-	-	-	H
Howe Sound - Queen Charlotte Channel (2)	-	-	**C**	-	-	-	-	-
Foreslope Hills (3)	-	-	-	D	-	-	G	-
Outer Gulf Islands (4)	-	-	-	D	-	-	-	-
Gabriola Island (5)	-	-	-	-	E	F	G	H
Parksville (6)	-	-	-	-	-	F	-	H
East of Hornby Island (7)	**A**	-	-	-	-	-	-	-
Sechelt (8)	-	-	-	-	E	-	-	-
Halibut Bank (9)	-	B	-	-	-	-	-	-

The twenty-one taxonomic groups that contributed to 50% dissimilarity between reef complexes are listed in Table 21. Four taxonomic groups that contributed to the highest number of significant comparisons were *Munida quadrispina* (Squat Lobster), *Rhabdocalyptus dawsoni* (Boot Sponge), suborder Dendrobranchiata (Shrimps), and *Ceramaster patagonicus* (Cookie Star). Densities of these taxonomic groups by reef complex are shown in Fig. 18.

Table 21. Taxonomic groups that contributed to 50% dissimilarity between reef complexes (SIMPER on zero-adjusted, square root-transformed Bray-Curtis community structure dissimilarity matrix).

Taxonomic group	**Number of significant comparisons (out of *n*=29)**
Gastropoda - Class	1
Actinopteri - Class	1
Fusitriton oregonensis	3
Demospongiae - Class	5
Brachiopoda - Phylum	2
Sebastidae - Family	4
Pachycerianthus fimbriatus	12
Pennatulacea - Order	13
Sebastes elongatus	3
Ceramaster patagonicus	**18**

Taxonomic group	**Number of significant comparisons (out of *n*=29)**
Henricia spp.	1
Pleuronectidae - Family	3
Lyopsetta exilis	2
Hydrolagus colliei	1
Rhabdocalyptus dawsoni	**29**
Dendrobranchiata - Suborder	**24**
Pandalus platyceros	9
Paguroidea - superfamily	8
Munida quadrispina	**29**
Chorilia longipes	2

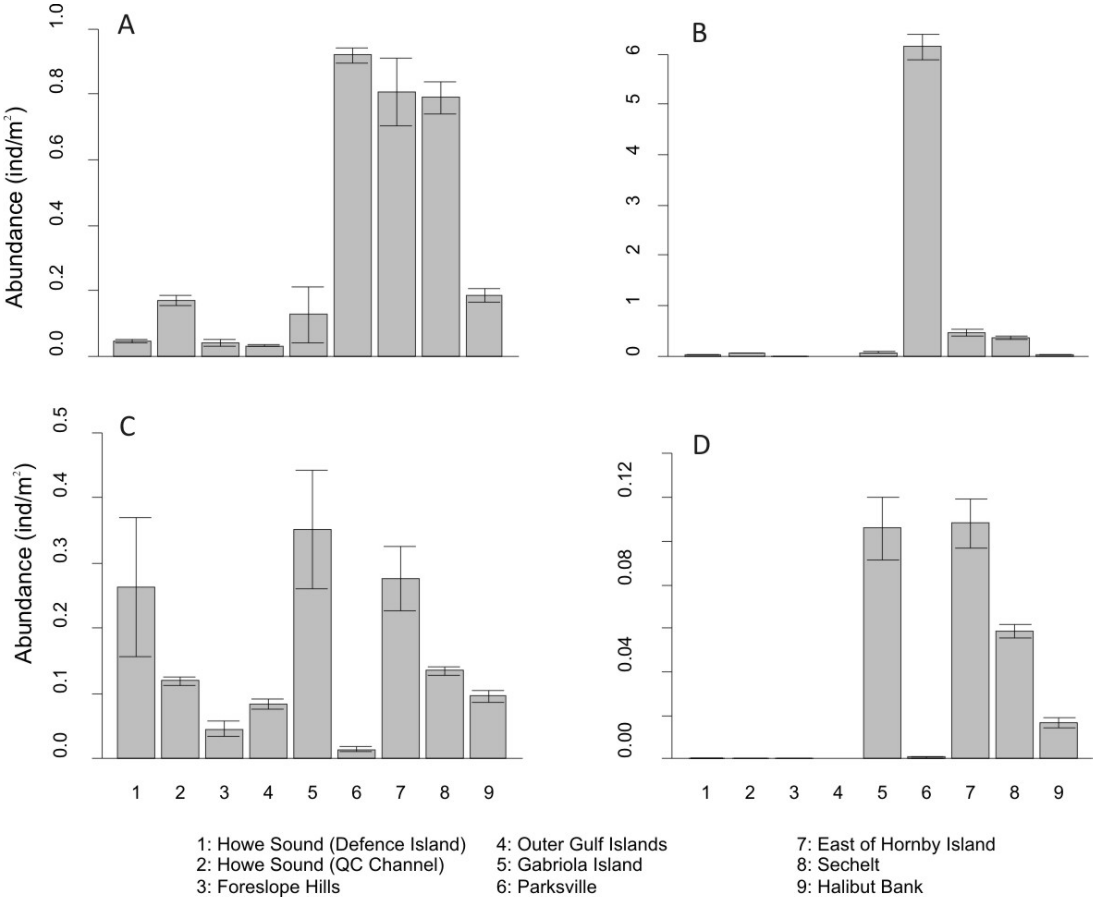

Figure 18. Densities of (A) Munida quadrispina (Squat Lobster), (B) Rhabdocalyptus dawsoni (Boot Sponge), (C) suborder Dendrobranchiata (Shrimps), and (D) Ceramaster patagonicus (Cookie Star) in nine reef complexes (mean±SE; for numbers of transects per reef see Table 2).

3.2.4 Community-habitat associations

3.2.4.1 Community comparison within and outside of reef complexes

Species richness and diversity was significantly higher within reef boundaries compared to surrounding areas. Pielou's evenness and total megafaunal density were slightly higher within reef boundaries, but the differences were not statistically significant (Table 22).

Table 22. Comparison of univariate community composition indices within and outside of reef complexes (paired t-test, P<0.05).

Univariate community index	**On-reef mean**	**Off-reef mean**	**df***	**t**	***P***
Species richness	18.31	13.77	38	4.73	**<0.0001**
Shannon-Wiener diversity index	1.95	1.72	38	3.27	**0.002**
Pielou evenness index	0.69	0.67	38	0.86	0.39
Total megafaunal density	1.06	0.98	38	0.62	0.54

*based on 2013 dataset only; transects 18 and 23 were excluded due to off-reef portions being removed during quality control

Species richness counts are highly sensitive to the number of individuals sampled and to the number, size, and spatial arrangement of samples (Gotelli and Colwell 2011). Thus, Shannon-Wiener diversity index is a more appropriate community composition-based index for monitoring glass sponge reefs.

3.2.4.2 Indicator species analysis

A number of significant associations of taxa with habitat categories within reef complexes were identified using the video dataset (Table 23). The top 6 strongest associations, in descending order, were: *Munida quadrispina* (dense live, live, mixed, and dead reef), *Ceramaster patagonicus* (dense live, live, and mixed reef), *fam. Pennatulacea* (no visible reef), family Sebastidae (dense, live, and mixed reef), *Pandalus platyceros* (dense live, live, mixed, and dead reef), and *Chorilia longipes* (dense and live reef).

Table 23. Results of the Dufrêne-Legendre Indicator Species Analysis based on video dataset. Habitat categories and megafaunal observations were pooled across reef complexes.

Taxonomic group	**Habitat categories**	**Indicator value**[1]	***P* value**
Chorilia longipes	🟥 + 🟧 dense+live	0.244	0.005
Acantholithodes hispidus		0.112	0.005
Sebastes maliger		0.107	0.005
Family Sebastidae	🟥 + 🟧 + 🟨 dense+live+mixed	0.264	<0.001
Ceramaster patagonicus		0.357	0.005
Pteraster tesselatus		0.082	0.005
Gephyreaster swifti	🟧 + 🟨 live+mixed	0.109	0.005
Swiftia spp.	🟨 + ⬜ mixed+dead	0.09	0.005
Stylinos spp.		0.083	0.005
Class Hydrozoa		0.069	0.005
Munida quadrispina	🟥 + 🟧 + 🟨 + ⬜ dense+live+mixed+dead (i.e. all visible reef structure)	0.647	0.005
Pandalus platyceros		0.255	0.005
Order Pennatulacea	🟦 no visible reef	0.305	0.005
Chionoecetes spp.		0.132	0.005
Halocynthia spp.		0.102	0.005
Class Ophiuroidea		0.100	<0.001
Gorgonocephalus eucnemis		0.088	0.005
Family Stichaeidae		0.087	0.003
Ptilosarcus gurneyi		0.078	0.01
Apostichopus californicus		0.064	0.005

[1] Maximum possible value is 1.

Still image-based Indicator Species Analysis revealed fewer, but stronger associations of taxonomic groups and habitat (Table 24).

Table 24. Results of the Dufrêne-Legendre Indicator Species Analysis based on still image dataset. Habitat categories and megafaunal observations were pooled across reef complexes.

Taxonomic group	**Habitat categories**	**Indicator value**[1]	***P* value**
Order Decapoda	🟥 dense	0.300	0.035
Family Oregoniidae		0.258	0.016
Family Lithodidae		0.249	0.021
Sebastes maliger	🟧 live	0.236	0.04
Chorilia longipes	🟥 + 🟧 dense+live	0.276	0.042
Munida quadrispina	🟥 + 🟧 + 🟨 + ⬜ dense+live+mixed+dead (i.e. all visible reef structure)	0.845	0.001
Rhabdocalyptus dawsoni		0.535	0.002
Pandalus platyceros		0.418	0.012
Class Ophiuroidea	⬜ + 🟦 dead+no visible reef (i.e. no live reef-building glass sponges)	0.466	0.016

[1] Maximum possible value is 1.

Five taxonomic groups were present in both video- and still image-based indicator species lists. These taxonomic groups were found to be associated with the same[2] habitat categories:

- *Sebastes maliger*: associated with 'dense live reef' and 'live reef' (video) and 'live reef' (still images);
- *Chorilia longipes*: associated with 'dense live reef' and 'live reef' (both video and still images);
- *Munida quadrispina*: associated with visible reef structure, whether live or dead (both video and still images);
- *Pandalus platyceros*: associated with visible reef structure, whether live or dead (both video and still images).
- *Class Ophiuroidea*: associated with habitats without live reef-building glass sponges (both video and still images). Gorgonocephalus eucnemis, the most commonly observed representative of class Ophiuroidea, was associated with 'no visible reef' habitat category in video.

Combining the results of video- and still image-based indicator species analyses, we suggest seven indicator taxa for monitoring the status of glass sponge reefs in the Strait of Georgia and Howe Sound (Table 25). When Dufrêne-Legendre indicator species analysis was run for each reef complex separately, associations between these suggested indicator taxa and habitat categories were found significant for some, but not all of the reef complexes where indicator species and respective habitat categories were observed (Table 25; for full results see Appendix 9). This is not surprising as for several of the reef complexes the number of observations for certain individual indicator taxa was low.

Table 25. Suggested indicator taxa (determined by combining the results of video- and still image-based Dufrêne-Legendre Indicator Species Analyses), number of reef complexes each taxon was observed at, and number of reef complexes where the taxon exhibited statistically significant habitat association(s).

Taxon	**Common name**	**Associated with (indicator of)**	**N of reef complexes where taxon and habitat category combination was observed**[1]	**N of reef complexes where taxon was associated with habitat category (N of reef complexes where association was statistically significant)**[1]
Sebastes maliger	Quillback Rockfish	Dense live and live reef	5	5 (2)
Chorilia longipes	Longhorn Decorator Crab		7	6 (4)
Rhabdocalyptus dawsoni	Boot Sponge	Visible reef structure, live or dead	9	6 (4)
Pandalus platyceros	Spot Prawn		9	6 (1)
Munida quadrispina	Squat Lobster		9	8 (8)

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[2] Note that still image-based analysis suggested a more narrow association for *S. maliger.*

Taxon	Common name	Associated with (indicator of)	N of reef complexes where taxon and habitat category combination was observed[1]	N of reef complexes where taxon was associated with habitat category (N of reef complexes where association was statistically significant)[1]
Order Pennatulacea	Sea Pens	Lack of visible reef structure	8	8 (5)
Class Ophiuroidea	Brittle Stars		5	4 (2)

[1] For full results of Dufrêne-Legendre Indicator Species Analyses within individual reef complexes see Appendix 9.

3.2.5 Evidence of anthropogenic activities

This section provides a snapshot of signs of anthropogenic activities we observed using underwater video and imagery. It is not intended to describe any activities which may be occurring inside or outside of reef complexes and/or closures, or attribute observed sponge damage to any particular activity.

3.2.5.1 Anthropogenic objects

The following anthropogenic objects were noted within the reefs' boundaries: lost fishing gear (traps; Fig. 19A, B), netting (Fig. 19C), tire, pipe, cables, ropes, plastic and glass bottles, metal cans, and unidentified objects. Numbers of observations per closure are summarized in Table 26.

Although the number of anthropogenic objects observed during the two surveys may be viewed as fairly low, extrapolating the number of objects found per area of reef surveyed to the total area of the reefs reveals a considerable anthropogenic signature. For example, Howe Sound – Queen Charlotte Channel reef may contain up to 395 lost traps and 526 bottles. The types and quantities of the anthropogenic objects observed within reef footprints provide evidence to some of the anthropogenic stressors that may be affecting the reefs.

Table 26. Anthropogenic objects observed within reef footprints.

Reef complex	Total number of objects observed	Object types
Howe Sound - Defence Islands (1)	0	None observed
Howe Sound - Queen Charlotte Channel (2)	13	Bottle (4), log (1), pipe (1), trap (3), wood or metal object (1), unidentified object (3)
Foreslope Hills (3)	0	None observed
Outer Gulf Islands (4)	6	Buoy (1), garbage (1), metal cable (1), trap (1), unidentified object (2)
Gabriola Island (5)	9	Bottle (6), can (2), metal cable (1)
Parksville (6)	0	None observed
East of Hornby Island (7)	2	Bottle (1), metal cable (1)

Reef complex	Total number of objects observed	Object types
Sechelt (8)	4	Bottle (1), can (1), rope (1), log (1)
Halibut Bank (9)	6	Tire (1), unidentified object (5)

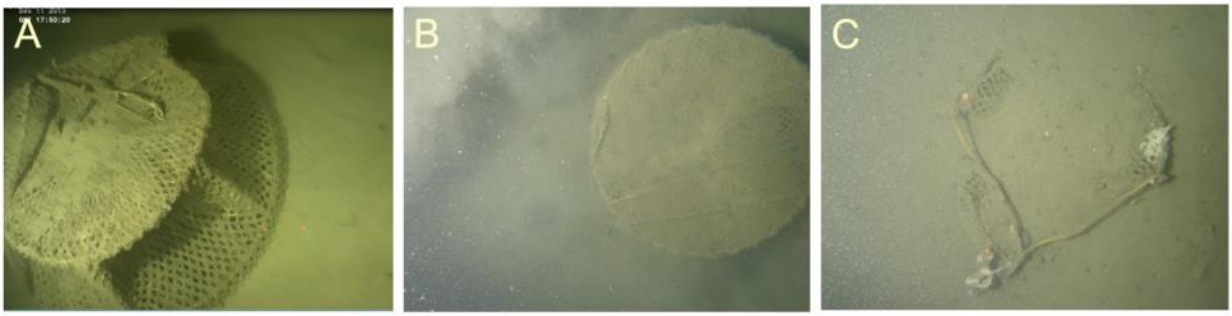


Figure 19. Lost fishing gear and netting observed within the reef footprints. Note sponges growing on the netting (C).

Reef 4A of the Outer Gulf Islands complex (Galiano Ridge reef) is traversed by submarine power transmission cables installed and operated by the BC Hydro to connect Vancouver Island to mainland British Columbia. The effects of these cables on glass sponge reefs are described in Dunham et al (2015).

3.2.5.2 Non-indigenous species

A number of specimens of a solitary ascidian resembling *Ciona intestinalis* (Vase Tunicate) were observed in one location in Howe Sound – Queen Charlotte Channel reef in 2013 at a depth of 149 m (Fig. 20); no samples were collected to confirm species identification. No other cases of suspected non-indigenous species were observed.

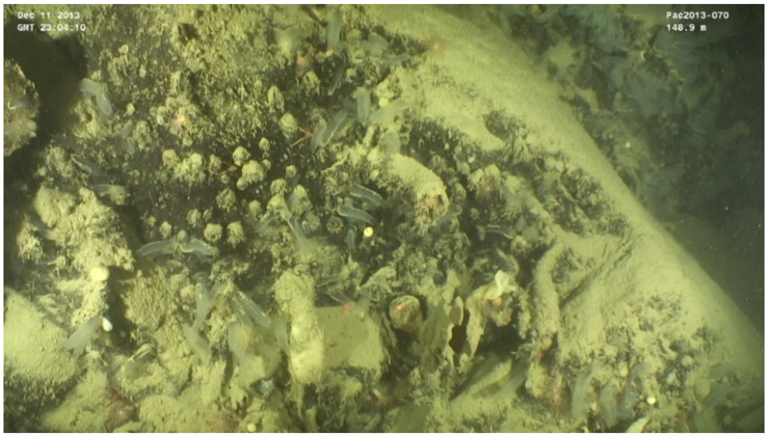


Figure 20. Solitary ascidians observed in Howe Sound – Queen Charlotte Channel reef in 2013.

3.3. ANALYSIS OF SAMPLING EFFORT

Analyses were undertaken to understand the sampling effort required to adequately characterize glass sponge reefs and associated communities.

Comparing the results of ‘leave-some-out’ analyses with analysis of the complete dataset (Table 27) suggests that using fewer images per transect (*e.g.* every 2nd image) would have little effect on the ability to detect significant trends and would lessen data processing time, but at the expense of statistical reliability. This finding is consistent with studies on sampling design in coral reefs (e.g. Molloy et al. 2013).

Table 27. Results of analyzing all still images within transects (images taken 15 seconds apart; image area: 0.7±0.42 m^2; image spacing: 3.0±1.31 m, mean±SD) compared to a subset of images (every 2nd, 3rd, 4th, and 5th image) using the grid method.

Reef complex	**Total N of images**	**Live % cover** (Mean ± 95% Confidence Interval)					**Total processing time** (min)				
		All	**Every 2nd**	**Every 3rd**	**Every 4th**	**Every 5th**	**All**	**Every 2nd**	**Every 3rd**	**Every 4th**	**Every 5th**
Howe Sound - Defence Islands (1)	126	0.65±0.39	0.31±0.28	0.34±0.28	0.48±0.56	0.47±0.63	693	347	231	171	138
Howe Sound - Queen Charlotte Channel (2)	121	9.84±2.95	9.75±3.79	8.11±5.89	11.63±6.38	7.53±5.71	666	330	220	165	132
Foreslope Hills (3)	109	6.93±3.20	5.47±4.14	6.47±5.97	5.56±6.74	9.92±9.52	600	297	198	149	116
Outer Gulf Islands (4)	179	0.22±0.23	0.09±0.10	0.16±0.24	0.16±0.20	0.11±0.16	985	490	325	242	193
Gabriola Island (5)	202	0.30±0.23	0.24±0.24	0.03±0.05	0.07±0.11	0.29±0.48	1111	556	369	275	220
Parksville (6)	272	0.09±0.05	0.13±0.08	0.10±0.09	0.11±0.13	0.04±0.06	1496	748	495	374	297
East of Hornby Island (7)	254	3.76±1.02	4.19±1.63	2.94±1.85	5.74±3.04	3.3 ±1.98	1397	699	462	347	275
Sechelt (8)	258	2.03±0.83	1.96±1.09	2.35±1.74	1.59±1.53	1.76±1.56	1419	710	473	352	281
Halibut Bank (9)	313	0.01±0.01	0	0	0	0.03±0.05	1722	858	572	429	341

To explore the minimum number of still images per reef complex required for adequate characterization of sponge cover using the grid method, we applied the Hewitt et al. (1992) modification of the Bros and Cowell (1987) randomization technique for optimizing sample size. This technique allowed us to estimate the standard error percent cover estimated from a given sample size of images. Figure 21 shows the results for 9 reef complexes.

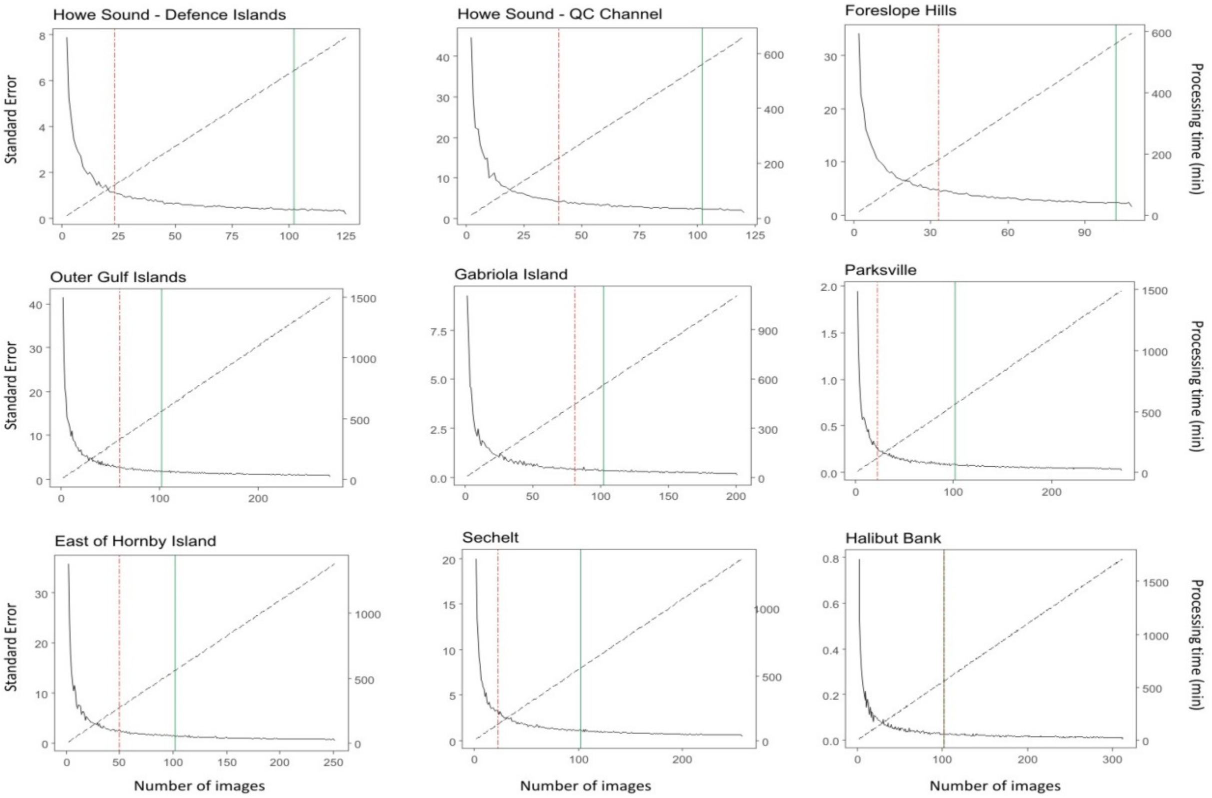


Figure 21. Standard error (95th percentile; solid black line) and image processing time (dashed black line) as a function of the number of still images analyzed, per reef complex, using the grid method, for 9 reef complexes. The cut-offs (shown as red dotted lines) were determined by applying Hewitt et al. (1992) modification of the Bros and Cowell (1987) randomization technique for optimizing sample size; for each reef complex, the cut-off was set as the point at which the slope of the curve was ≤1% of the initial slope. Maximum observed cut-off value of 102 images per reef complex– recommended as minimum number of images per reef complex to be analyzed - is shown as a green line.

The cut-offs (shown as red dotted lines in Fig. 21) can be viewed as the points where increasing the number of images provides a relatively small gain in the precision of your estimate of percent cover of live reef building sponges. After this point other considerations, such as the effort required and cost of analysis can be taken into account when determining the most appropriate sample size. The cut-off values ranged between 24 and 102 (Fig. 21; highest value of 102 was observed in Halibut bank). We therefore recommend 102 images as the minimum number of images per reef complex to be analyzed for adequate characterization of glass sponge cover using the grid method.

Next, the same randomization technique for optimizing sample size was applied at the individual transect level to determine the minimum number of still images per transect required for adequate characterization of sponge cover using the grid method. The results for 20 transects are shown in Appendix 10. The cut-off values were similar among analyzed transects, with the highest observed value of 38 (Fig. A10-1, panel PAC2013-070 6). We therefore suggest 38 images as the minimum number of images per transect to be analyzed for adequate characterization of glass sponge cover using the grid method. Thirty-eight images required 209 minutes to process in our study.

Video-based species accumulation curves demonstrate that for most reef complexes, at least 1000 video bins (Figure 22A) was the minimum effort required to approach expected species richness and adequately characterize megafaunal community structure. This is equivalent to approximately 167 minutes of ROV on-bottom transecting time which in our survey covered approximately 2,500 linear m (at ROV speed of 0.25 m/sec), or 3,250 m^2 (with mean FOV of 1.3 m). To characterize community structure from still images, more than 140 still images per reef complex (with mean image area of 0.7 m^2) would be required (Figure 22B).

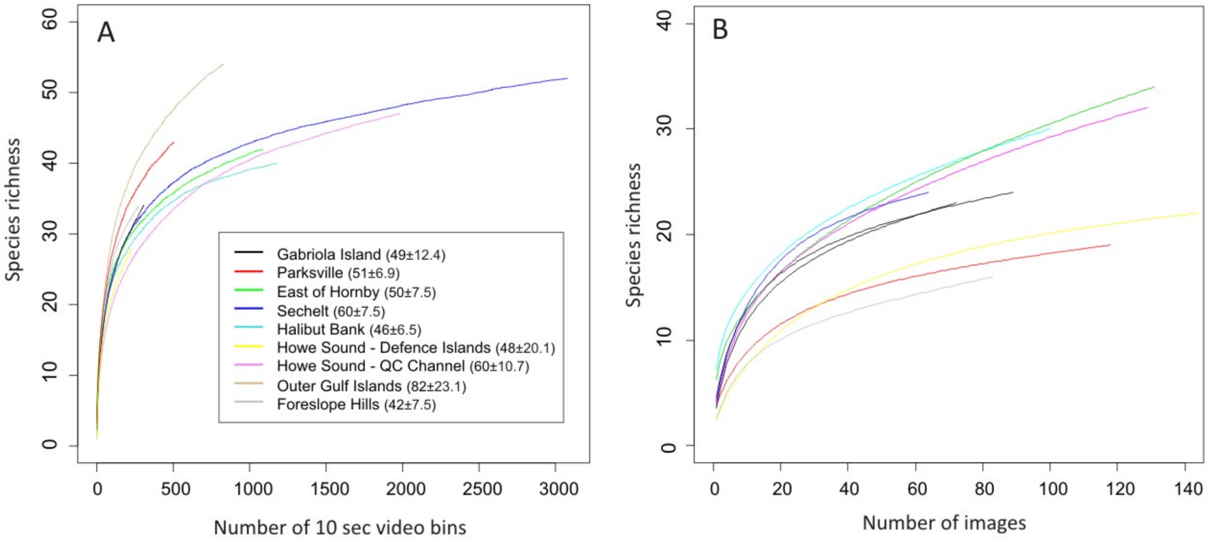


Figure 22. Species accumulation curves resulting from the analysis of (A) full video dataset and (B) still images (one transect per reef complex, 2012 dataset only). Values in the legend are expected species richness (mean±SD) calculated based on the video dataset following Chao (1987).

Taken together, the results of the sampling effort analyses suggest that for characterizing sponge cover using field techniques and grid method of analysis described in this paper, a minimum of 3 transects of approximately 500 m length per reef complex should be surveyed and a minimum of 38 images per transect should be analyzed. This would cover 1,500 linear m, or 1,950 m^2 of the reef complex area and result in approximately 100 minutes of video (33 minutes per transect) and 114 images. In our study, the still image cut-off was met for all 9 reef complexes (204±74, mean±SD). Over 1,500 m, or 1,950 m^2 of the reef area were surveyed in 6 out of 9 reef complexes (see Table 2).

For characterizing associated megafaunal community composition using field techniques and analytical methods described in this paper, a minimum of 5 transects of approximately 500 m length should be surveyed, with a minimum of 167 minutes of video and 140 images analyzed per reef complex. This would cover 2,500 linear m, or 3,250 m^2 of the reef complex. In our study, the cut-off of 140 still images was met for 6 out of 9 reef complexes (complexes 4 through 9). Over 2,500 m, or 3,250 m^2 of the reef area were surveyed in 4 out of 9 reef complexes (see Table 2).

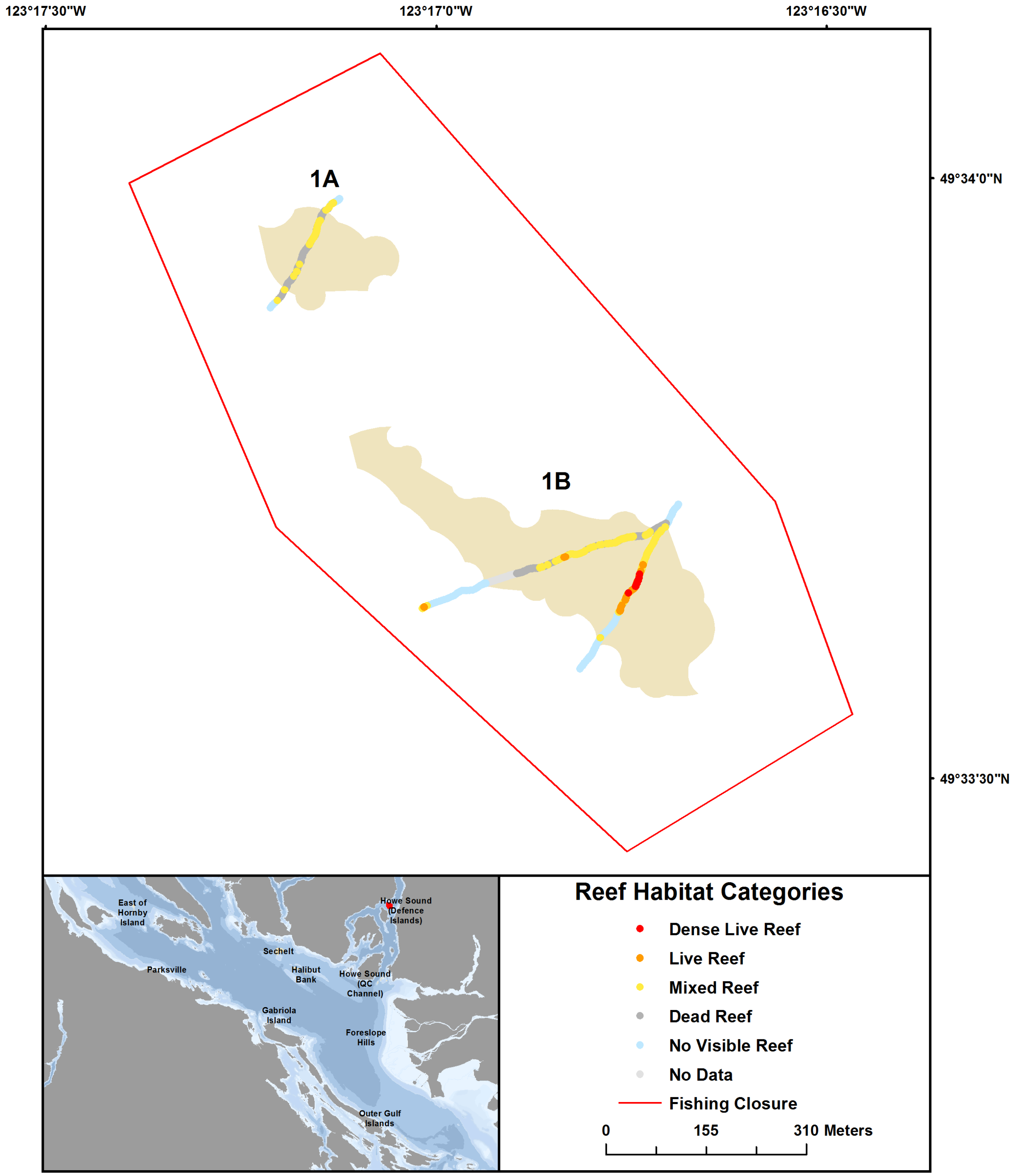
3.4 REEF STATUS SUMMARIES

This section contains reef complex status summaries. We included indices that showed most promise in characterizing the reef complexes in this study, based on best available knowledge to date. These summaries can be viewed as a reference for future monitoring, recognizing, however, that they represent before-closure reference points only for select indices covered in this paper.

Each status summary card covers one reef complex and contains the following elements organized on two pages:

- Map of reef complex showing location, fishing closure boundaries, individual reef(s), transects completed in this study, and point-based heat maps showing habitat categories distribution along each transect.
- Temperature, salinity, and depth ranges recorded in this study.
- Aster plots showing the values of six sponge-based indices found most relevant for reef characterization in this study (for details, see sections 2, 3.1, and 3.2). The scale for live % cover, dead % cover, % intact, and visible reef structure indices is 0 to 100%. The scale for Clumpiness index is 0 to 1; maximum value recorded per transect is presented. The oscula index is scaled from 0 to the maximum mean number of oscula per m2 observed in this study (7.4). If a comprehensive composite index of reef status is developed in the future, the values can be added to the centre portions of the aster plots to facilitate visual comparisons.
- Summary of the frequency of occurrence of five habitat categories within surveyed reef area.
- Representative images showing examples of ‘dense live’, ‘live’ and ‘mixed’ habitat categories.
- Densities of indicator taxa.
- In addition, the values of sponge- and community-based indices suggested for characterizing reef complexes are presented in Table 28 to facilitate side-by-side comparisons.

Howe Sound - Defence Islands



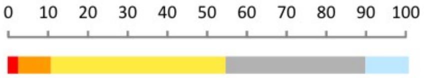
Complex 1: Howe Sound - Defence Islands

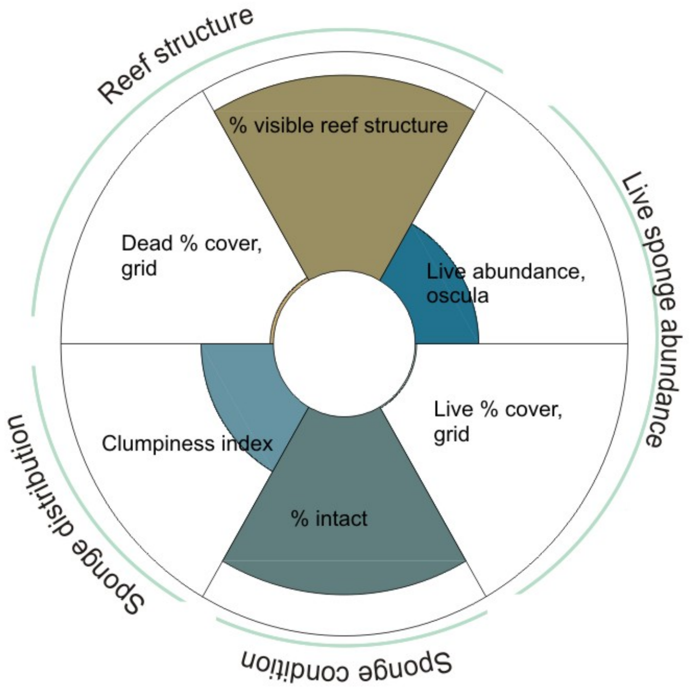
Temperature: 8.42 – 9.42°C

Salinity: 29.91 – 30.44 PSU

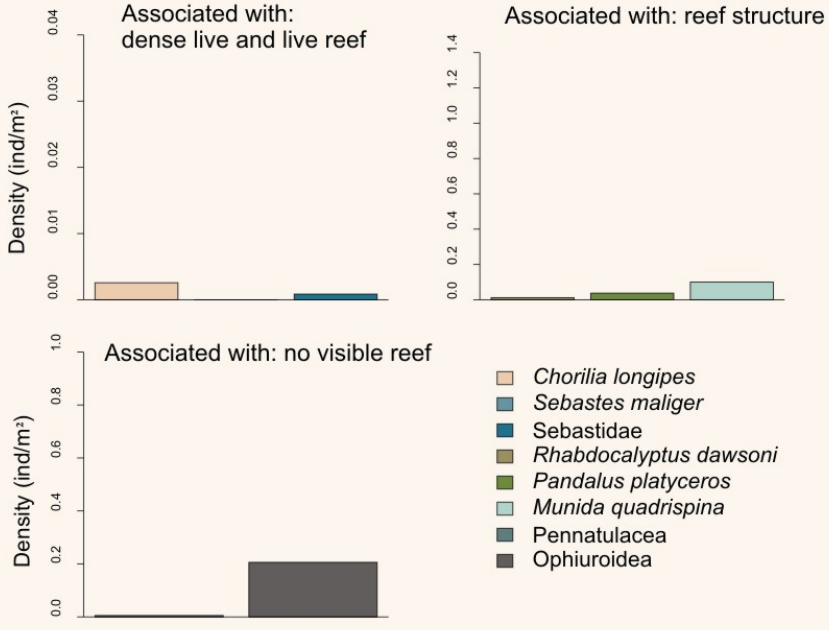
Depth surveyed: 57 – 100 m

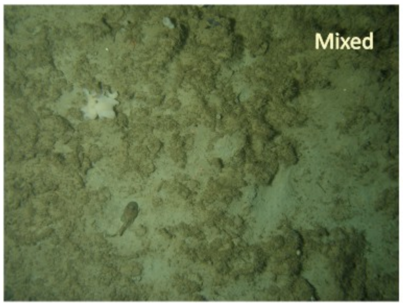
Habitat categories, %:



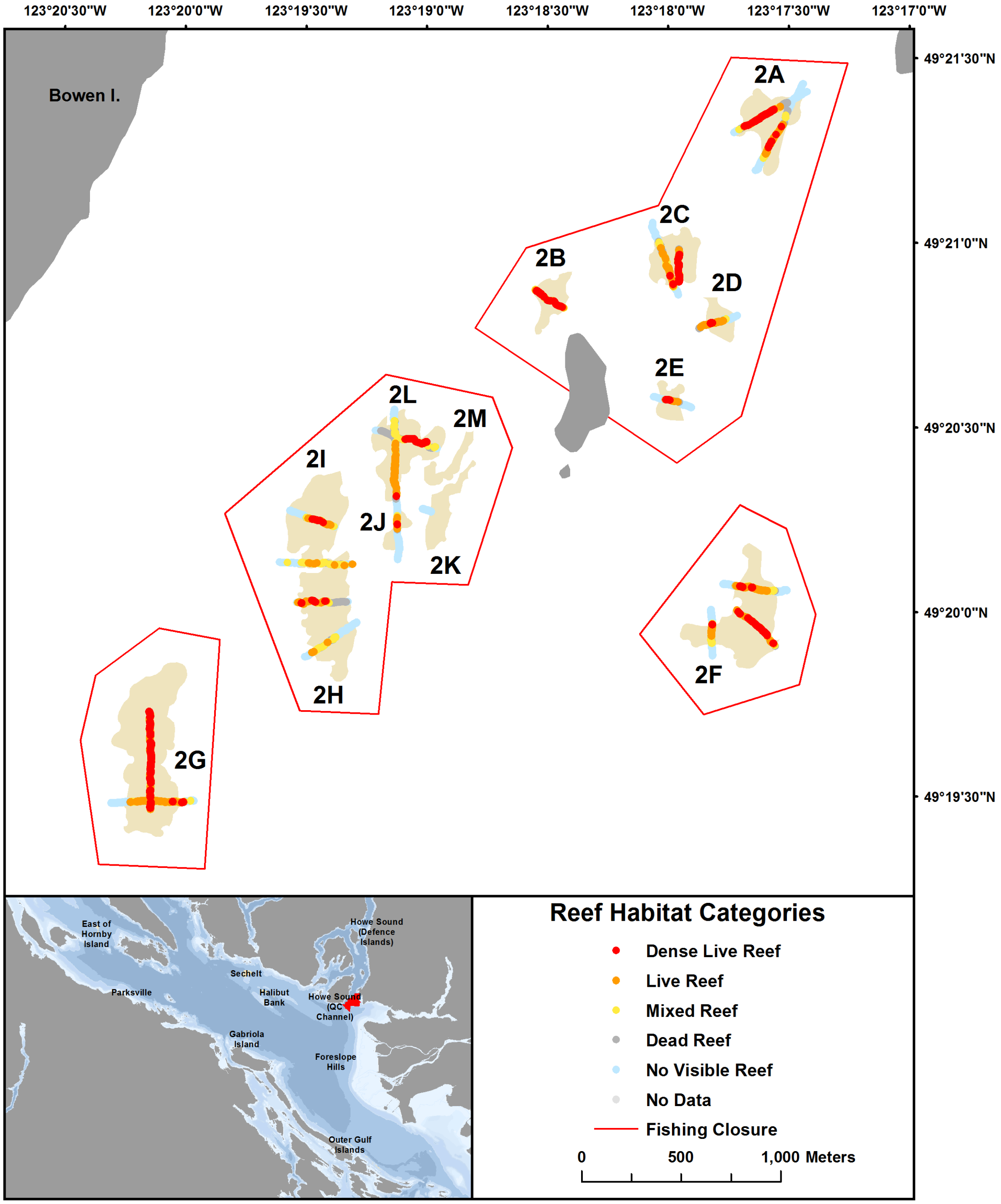


Shannon-Wiener diversity index: 2.28





Howe Sound - Queen Charlotte Channel



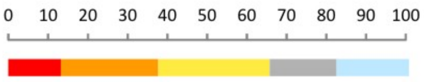
Complex 2: Howe Sound - Queen Charlotte Sound

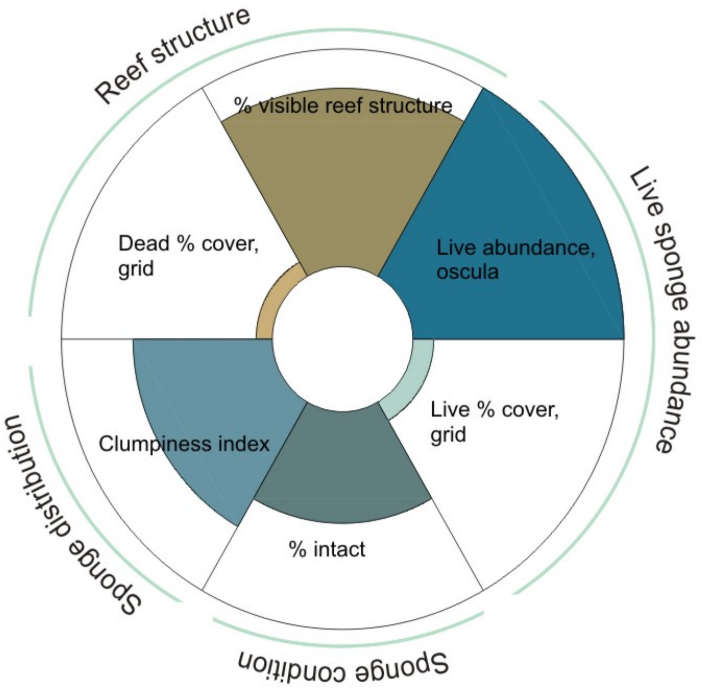
Temperature: 8.44 – 9.71°C

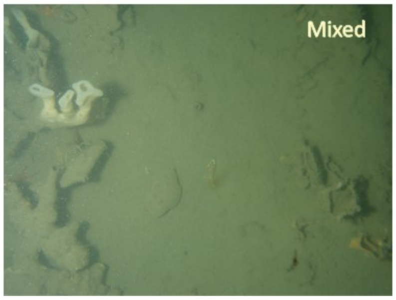
Salinity: 29.40 – 30.76 PSU

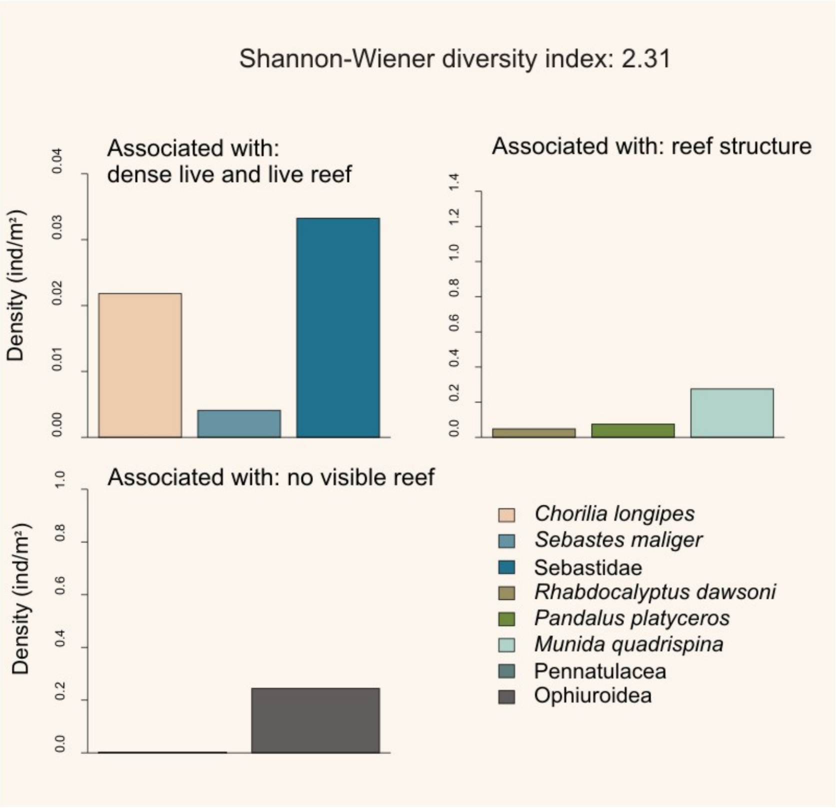
Depth surveyed: 30 – 128 m

Habitat categories:

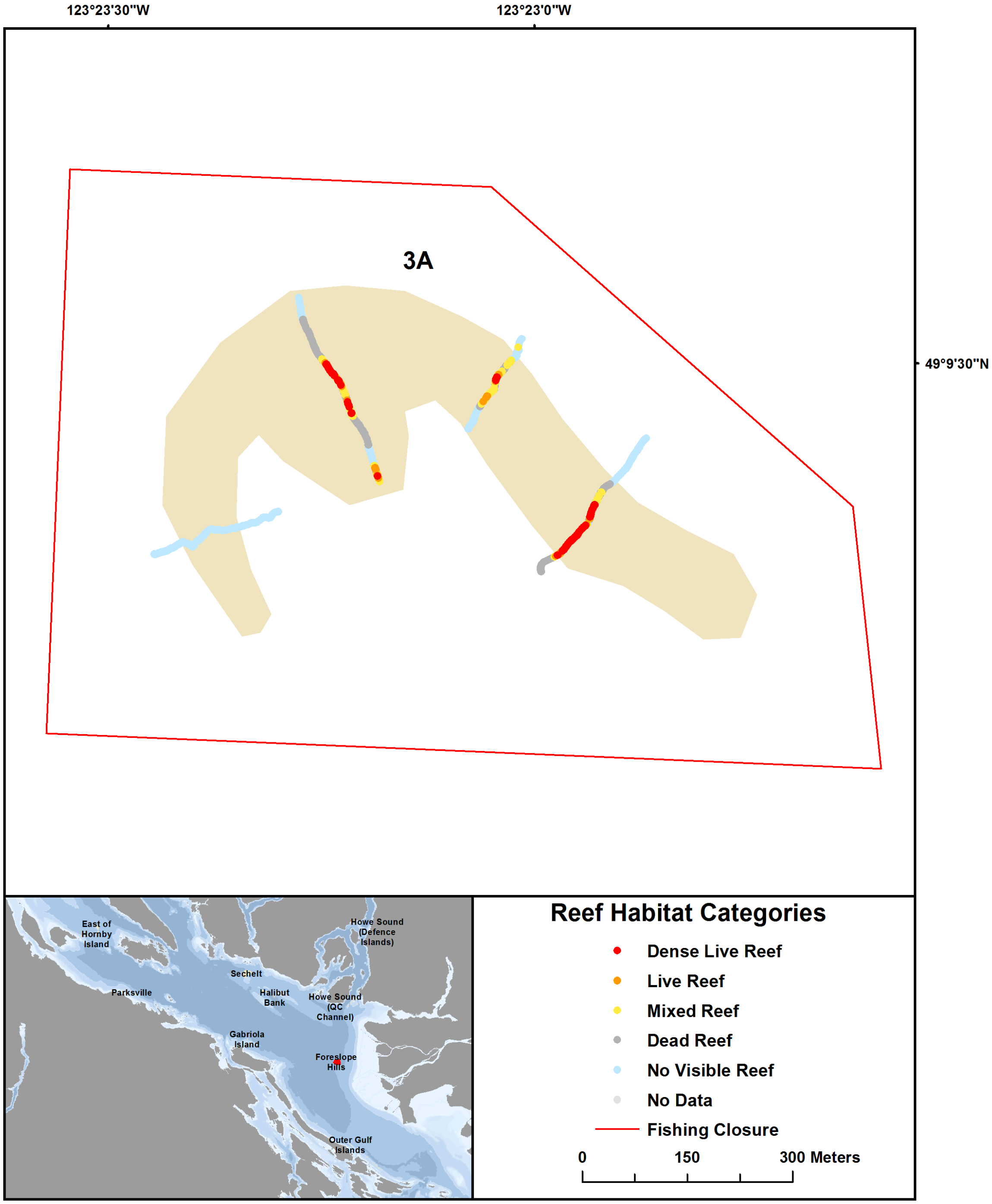








Foreslope Hills



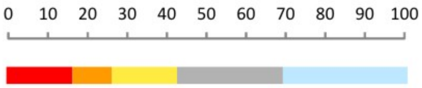
Complex 3: Foreslope Hills

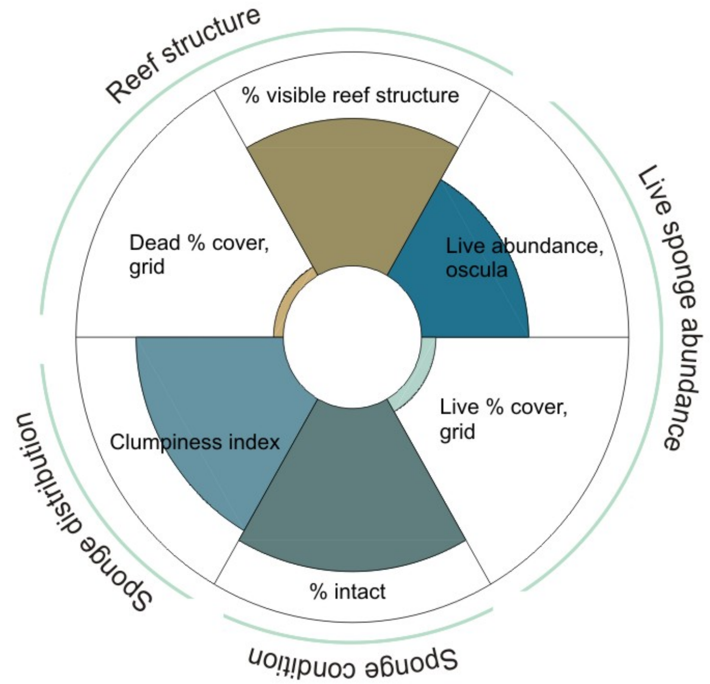
Temperature: 7.92 − 9.47°C

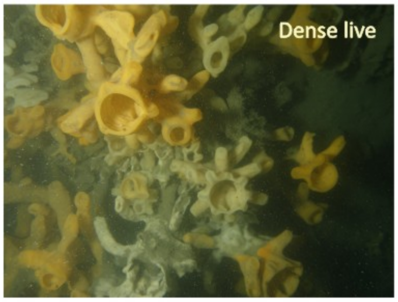
Salinity: 30.63 − 31.09 PSU

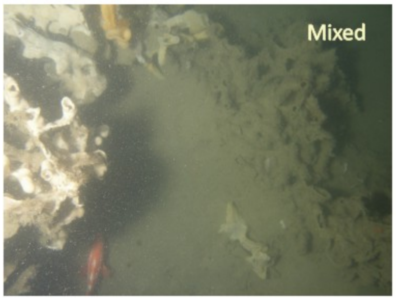
Depth surveyed: 148 − 191 m

Habitat categories:

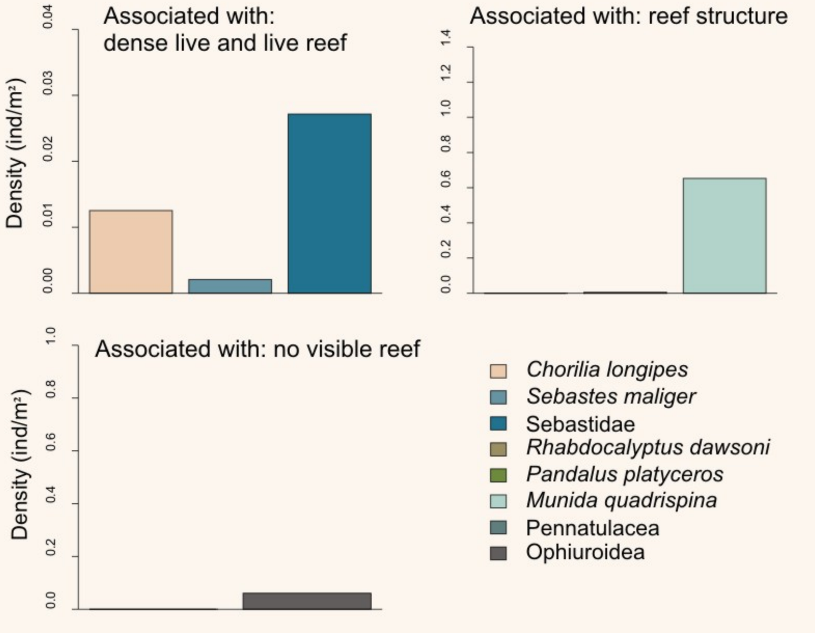




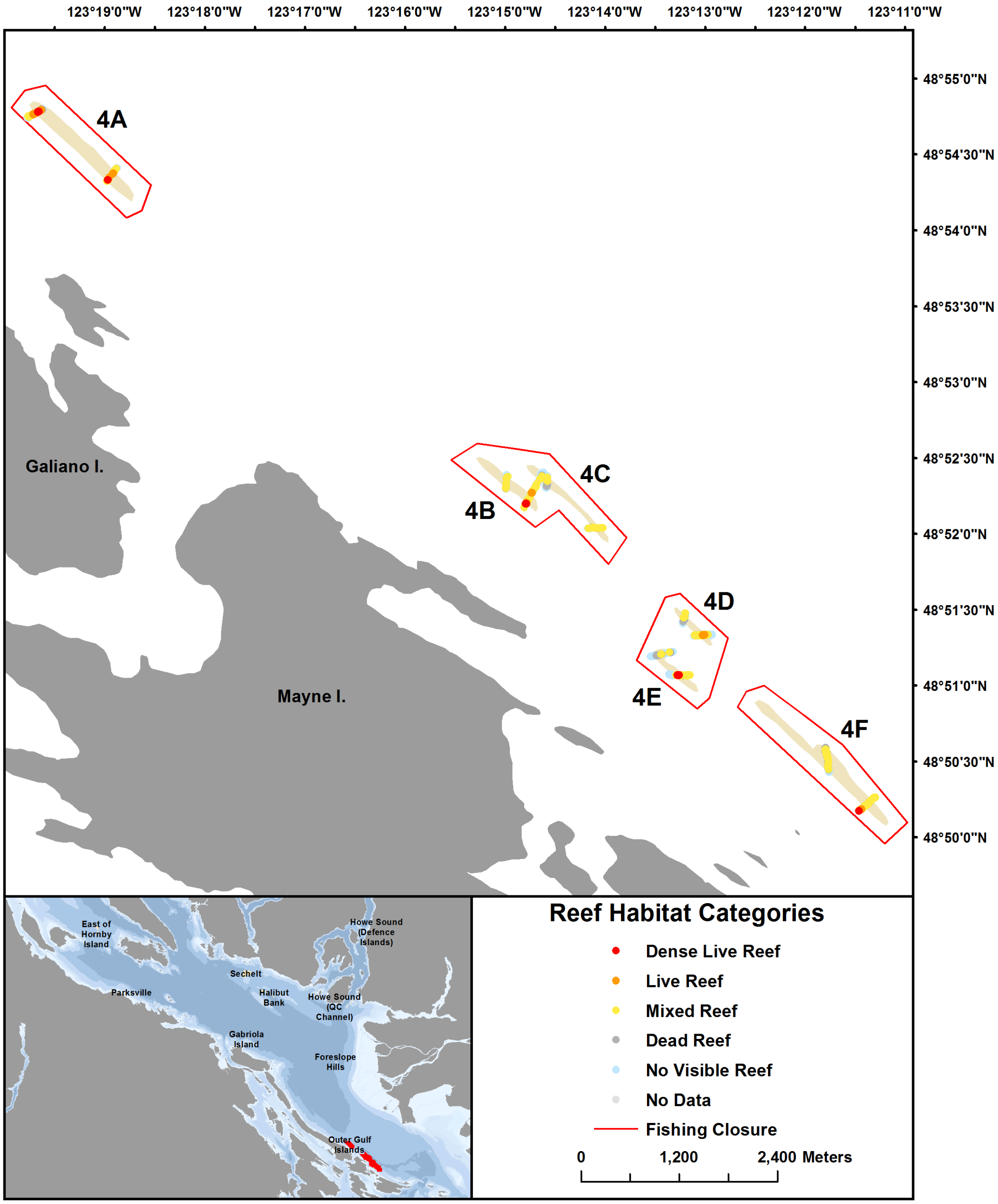




Shannon-Wiener diversity index: 2.90



Outer Gulf Islands



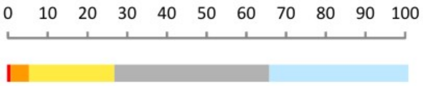
Complex 4: Outer Gulf Islands

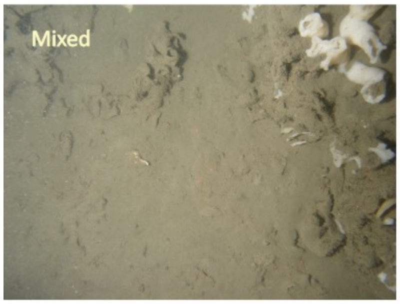
Temperature: 8.23 − 9.73°C

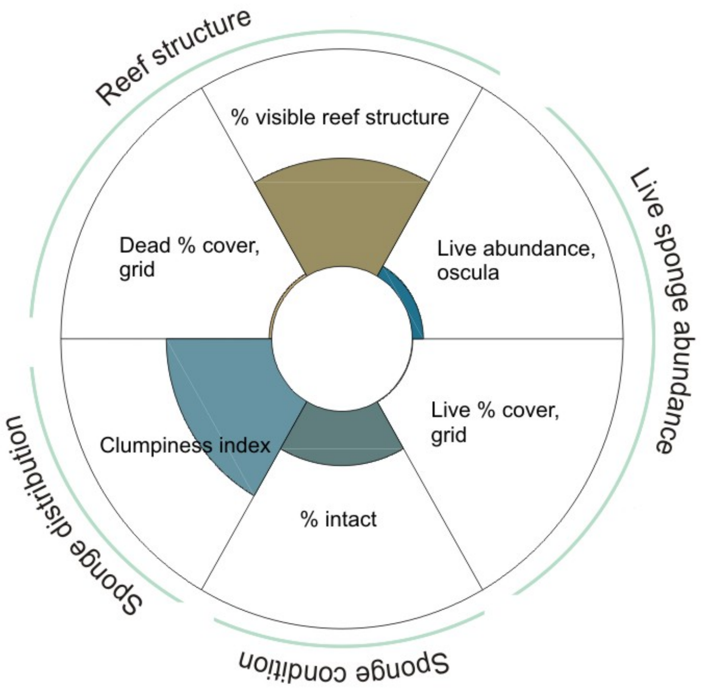
Salinity: 30.18 − 30.90 PSU

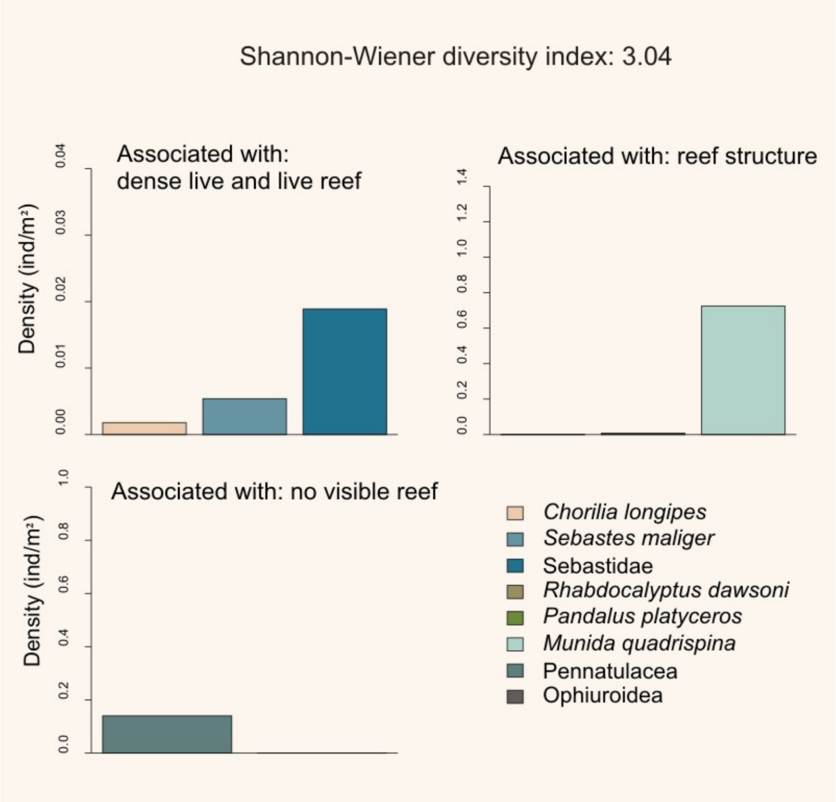
Depth surveyed: 72 − 158 m

Habitat categories:

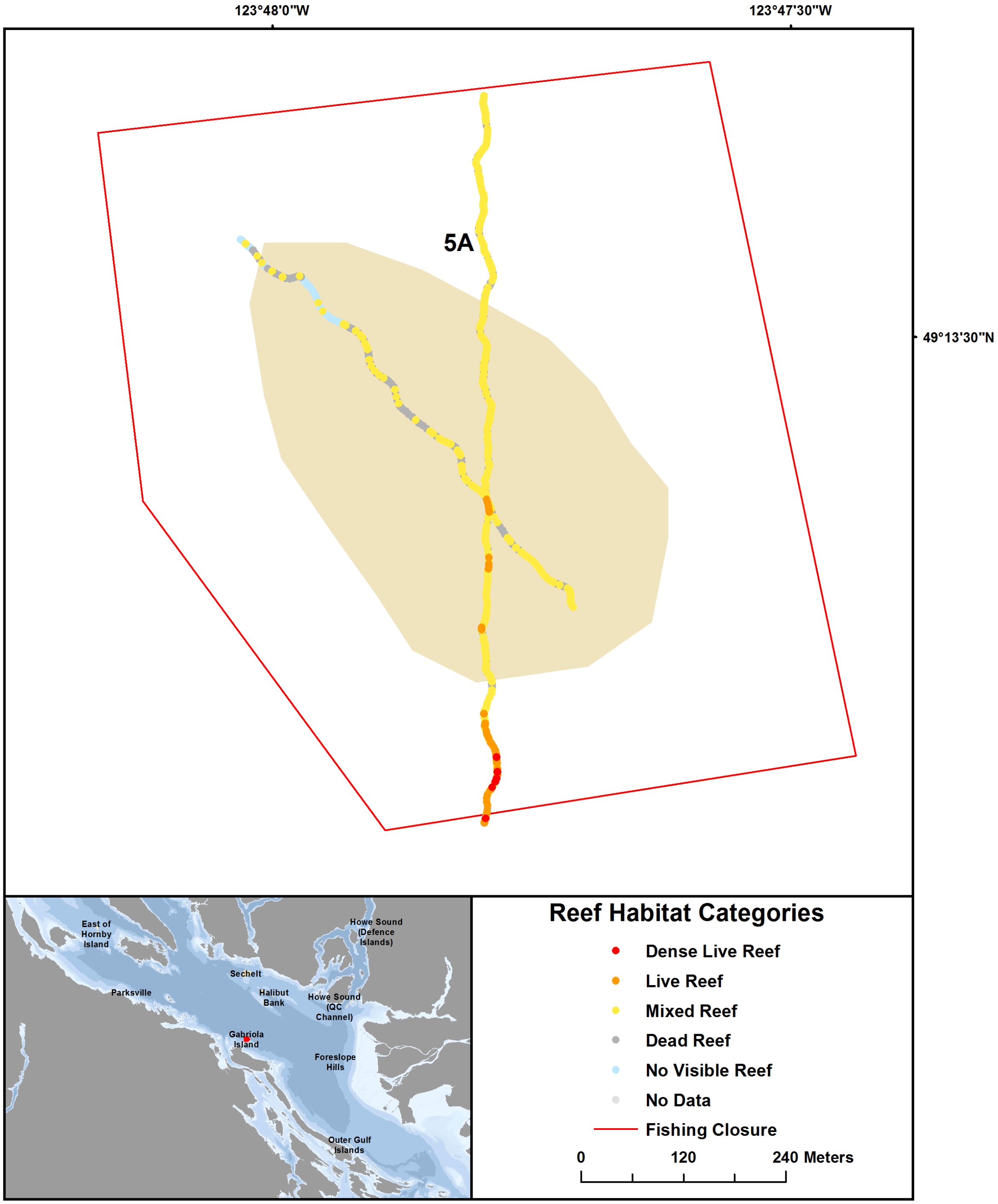








Gabriola Island



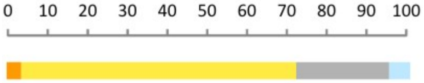
Complex 5: Gabriola Island

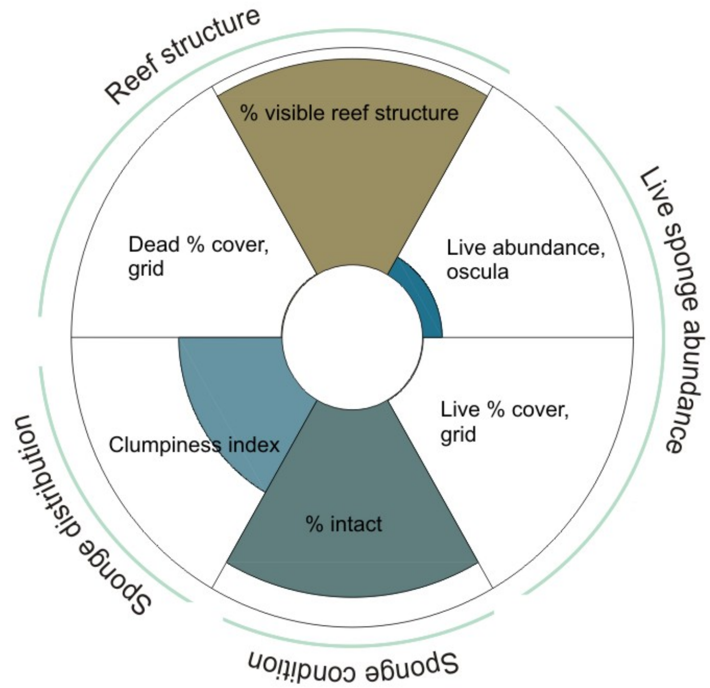
Temperature: 8.69 − 9.46°C

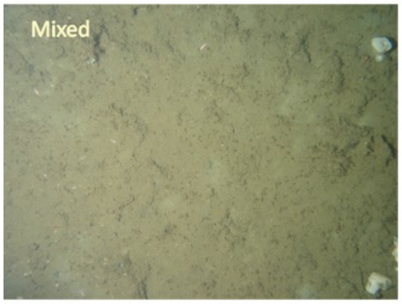
Salinity: 30.43 − 30.87 PSU

Depth surveyed: 108 − 151 m

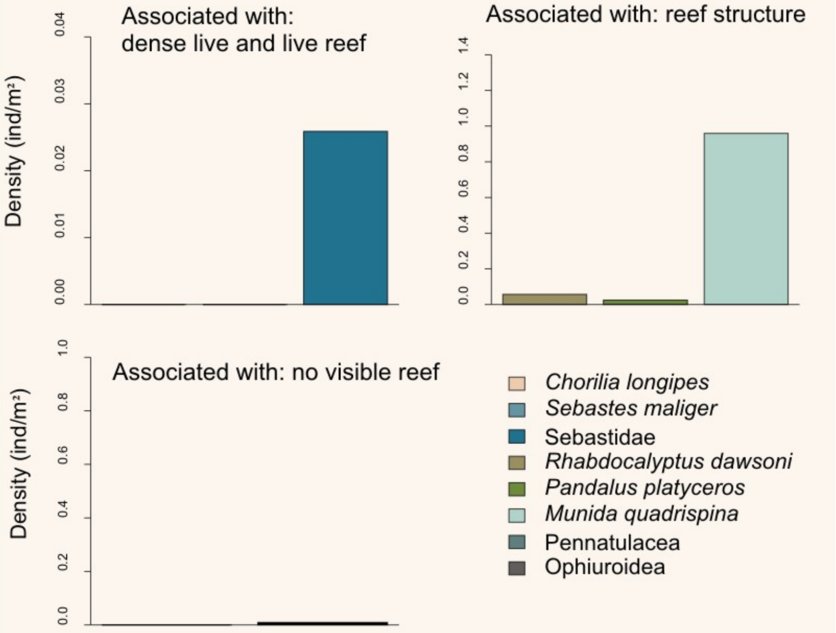
Habitat categories:



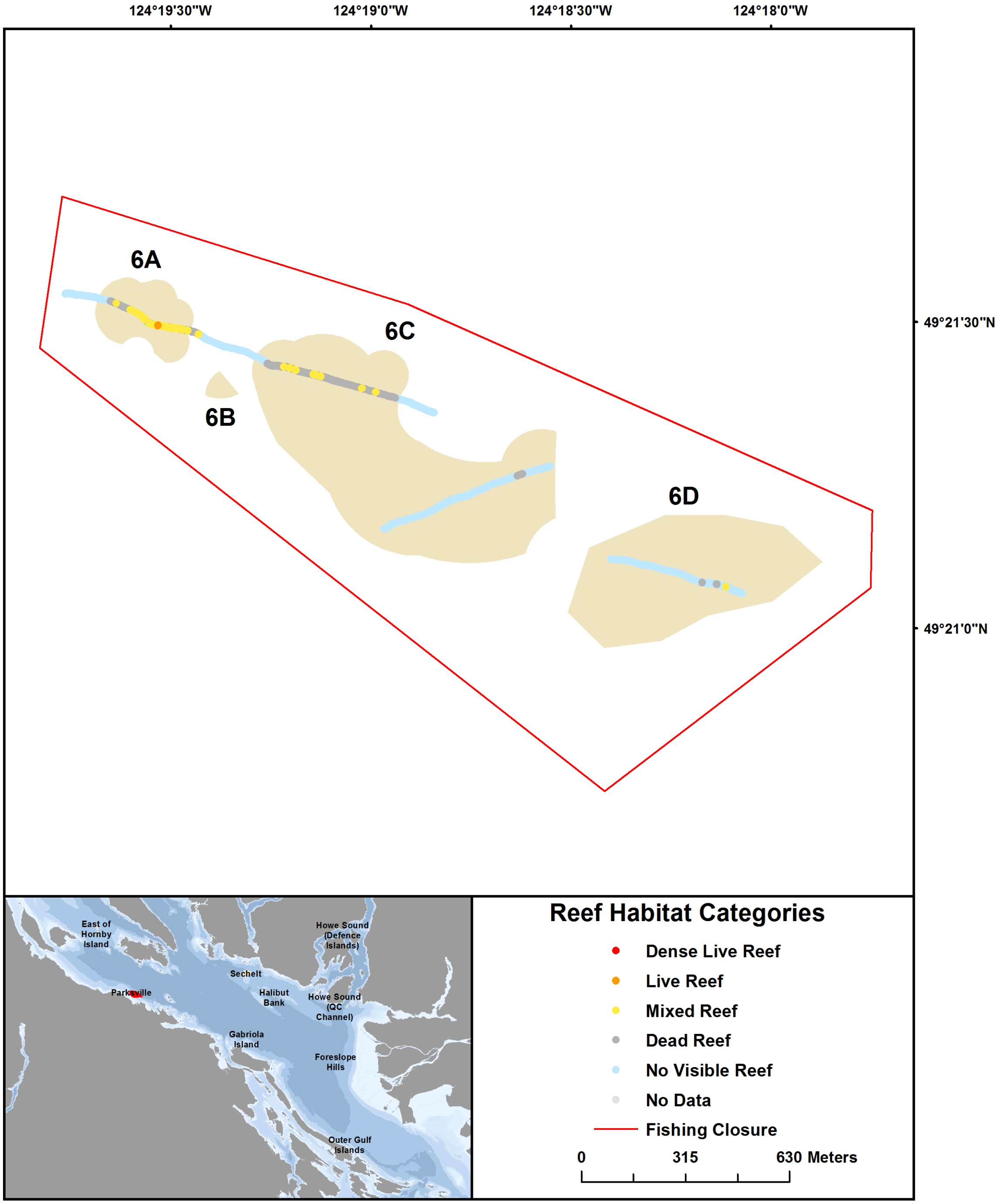




Shannon-Wiener diversity index: 1.97



Parksville


124°19'30"W
124°19'0"W
124°18'30"W
124°18'0"W
49°21'30"N
49°21'0"N
6A
6B
6C
6D
East of Hornby Island
Howe Sound (Defence Islands)
Sechelt
Parksville
Halibut Bank
Howe Sound (QC Channel)
Gabriola Island
Foreslope Hills
Outer Gulf Islands
Reef Habitat Categories
● Dense Live Reef
● Live Reef
● Mixed Reef
● Dead Reef
● No Visible Reef
● No Data
Fishing Closure
0
315
630 Meters

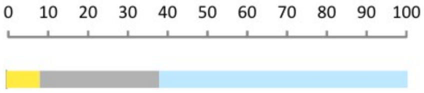
Complex 6: Parksville

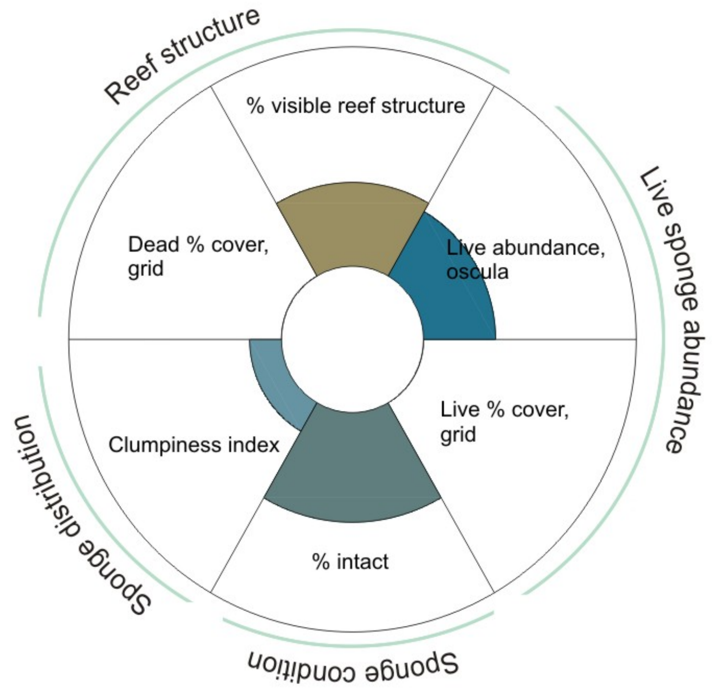
Temperature: 8.78 − 9.40°C

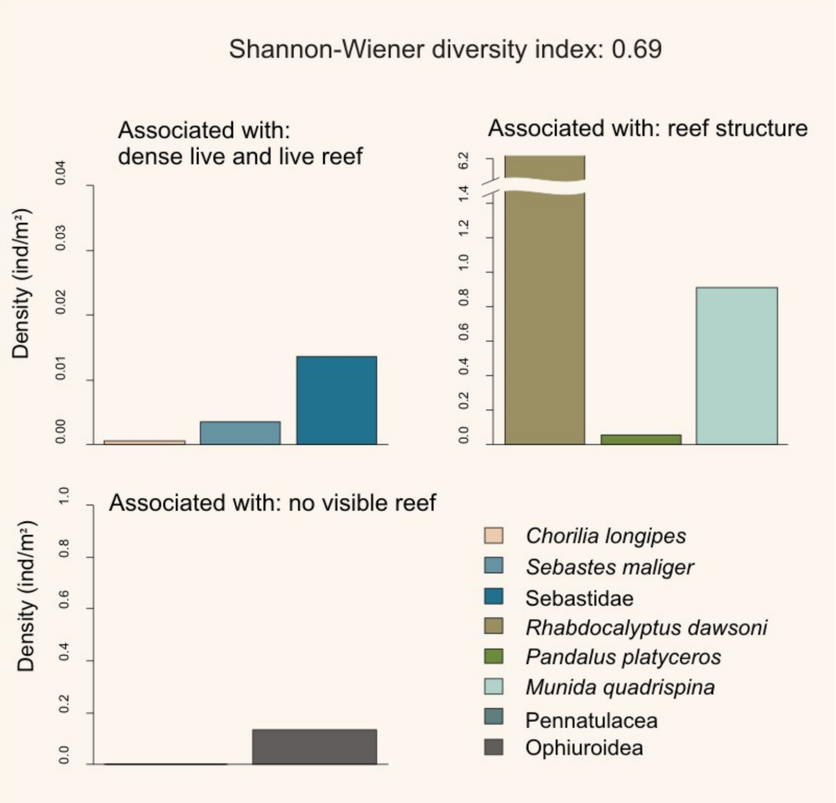
Salinity: 29.66 − 30.31 PSU

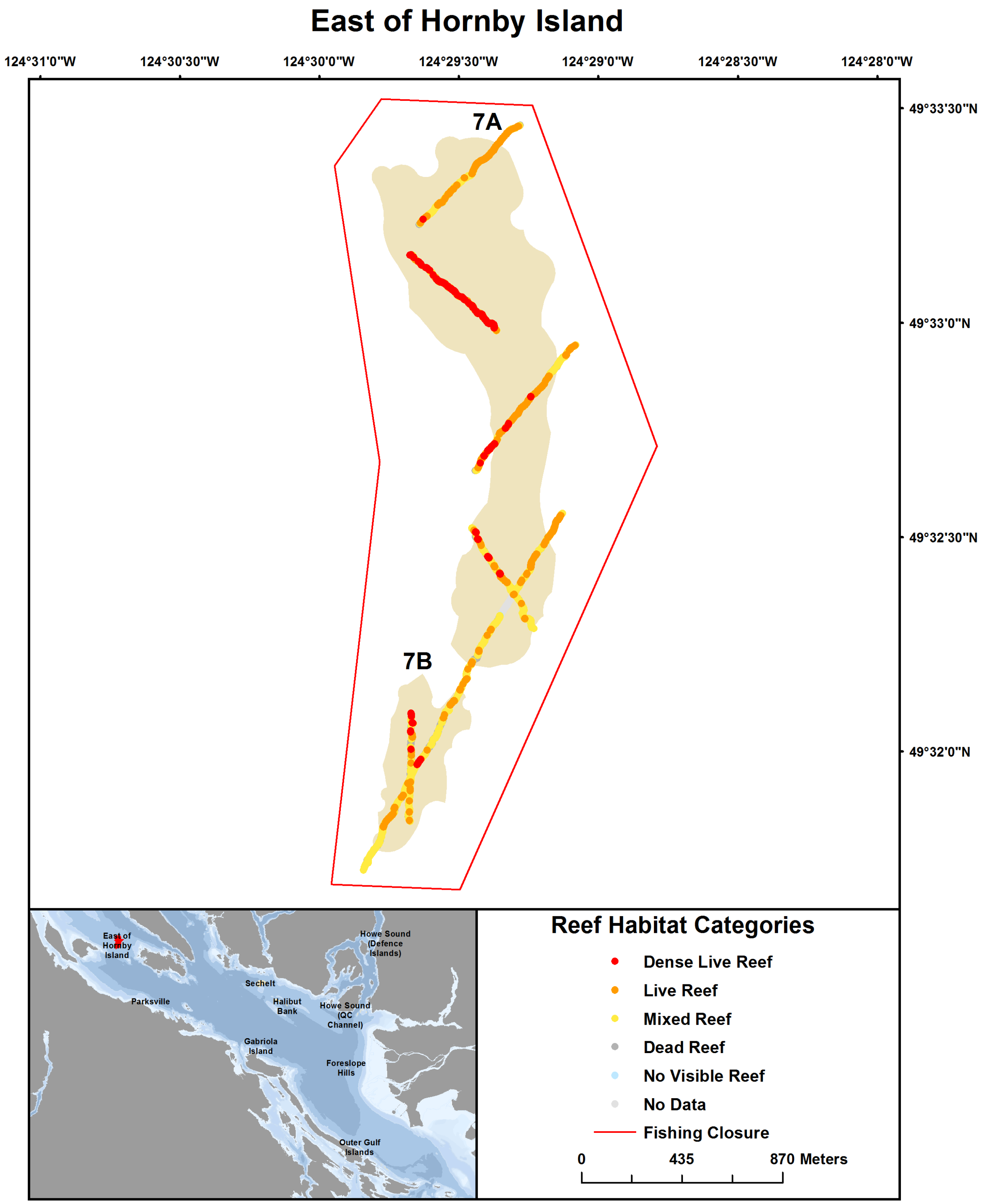
Depth surveyed: 55 − 80 m

Habitat categories:







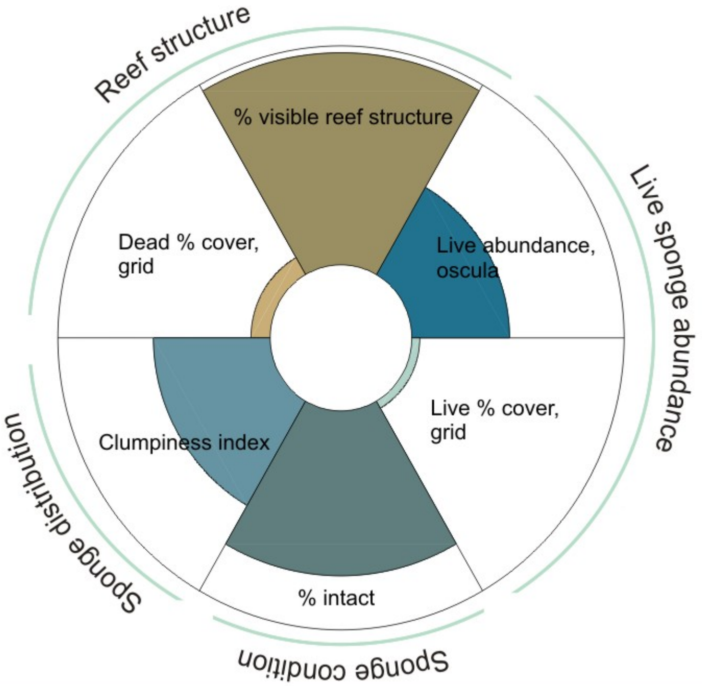

East of Hornby Island
124°31'0"W
124°30'30"W
124°30'0"W
124°29'30"W
124°29'0"W
124°28'30"W
124°28'0"W
49°33'30"N
49°33'0"N
49°32'30"N
49°32'0"N
7A
7B
East of Hornby Island
Howe Sound (Defence Islands)
Sechelt
Parksville
Halibut Bank
Howe Sound (QC Channel)
Gabriola Island
Foreslope Hills
Outer Gulf Islands
Reef Habitat Categories
Dense Live Reef
Live Reef
Mixed Reef
Dead Reef
No Visible Reef
No Data
Fishing Closure
0
435
870 Meters

Complex 7: East of Hornby Island

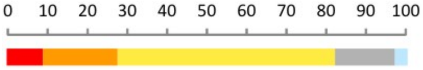
Temperature: 8.57 − 9.44°C

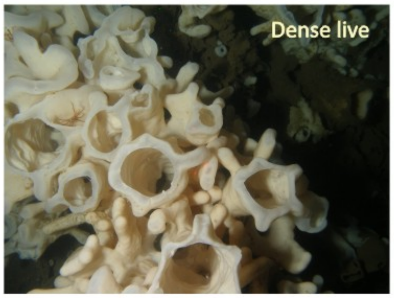
Salinity: 29.53 − 30.91 PSU

Depth surveyed: 58 − 146 m

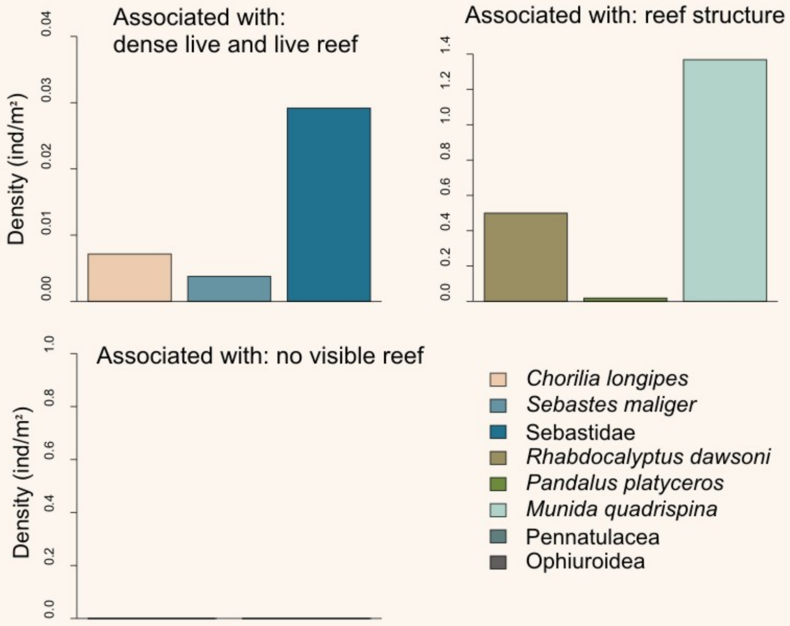


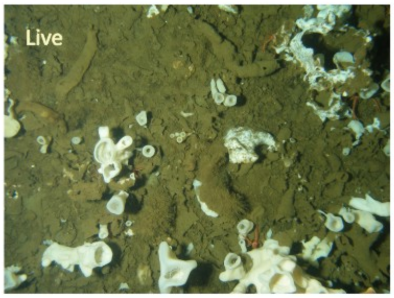
Habitat categories:

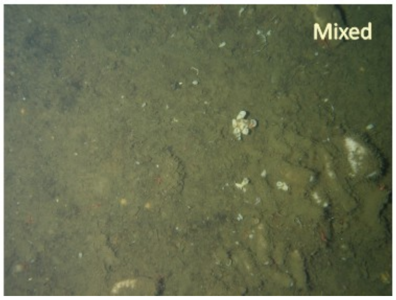




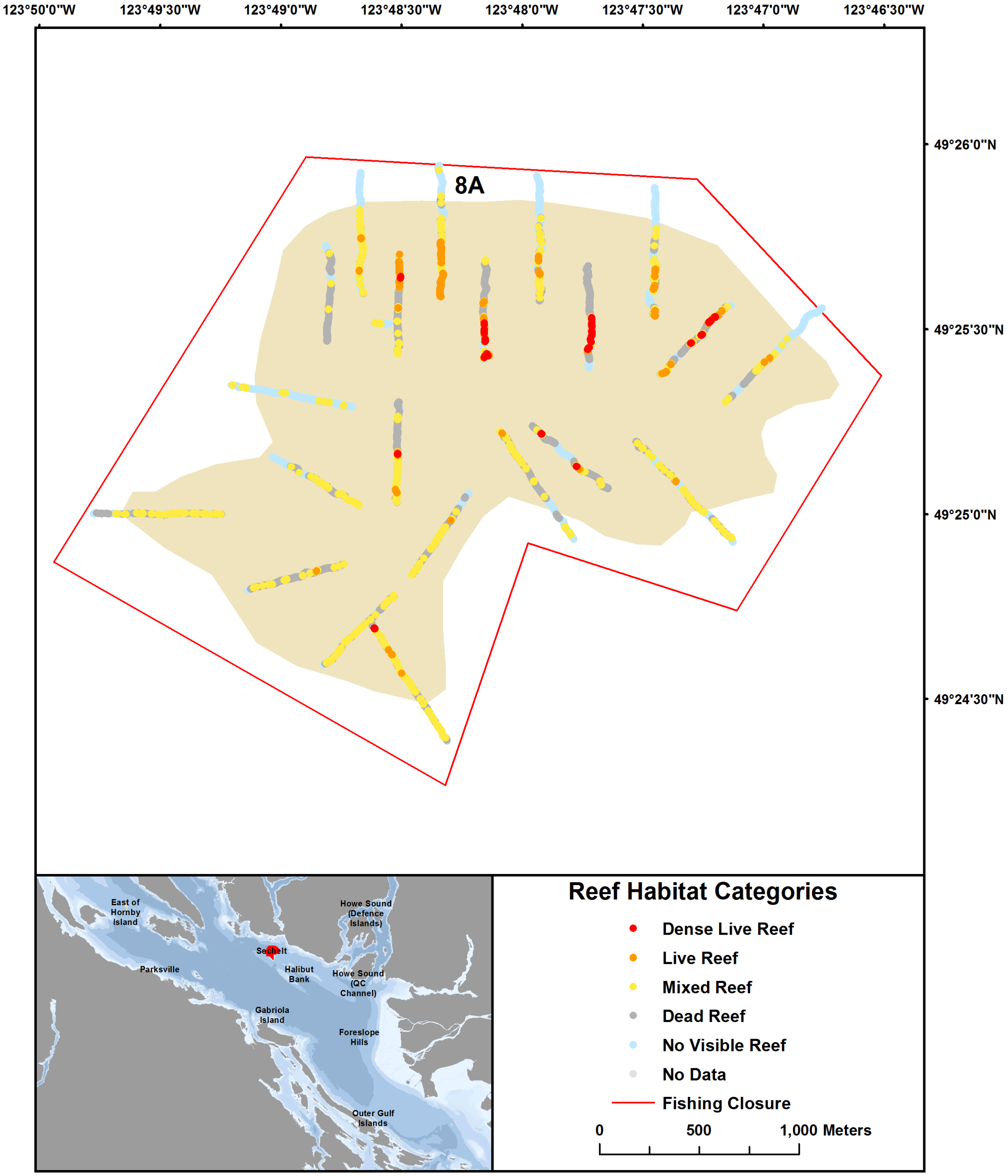
Shannon-Wiener diversity index: 1.78







Sechelt

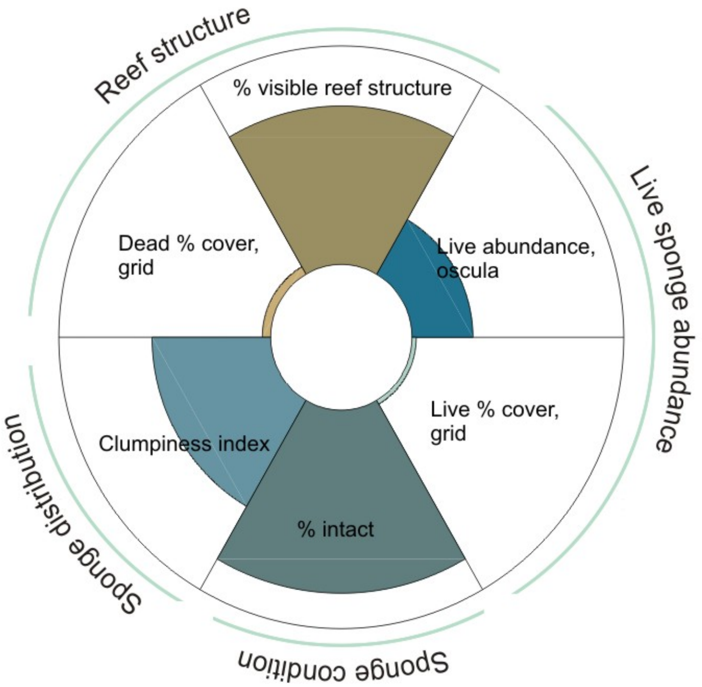

123°50'0"W
123°49'30"W
123°49'0"W
123°48'30"W
123°48'0"W
123°47'30"W
123°47'0"W
123°46'30"W
49°26'0"N
49°25'30"N
49°25'0"N
49°24'30"N
8A
East of Hornby Island
Howe Sound (Defence Islands)
Sechelt
Parksville
Halibut Bank
Howe Sound (QC Channel)
Gabriola Island
Foreslope Hills
Outer Gulf Islands
Reef Habitat Categories
● Dense Live Reef
● Live Reef
● Mixed Reef
● Dead Reef
● No Visible Reef
● No Data
Fishing Closure
0
500
1,000 Meters

Complex 8: Sechelt

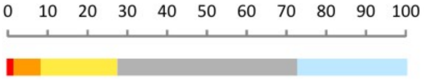
Temperature: 8.76 − 9.44°C

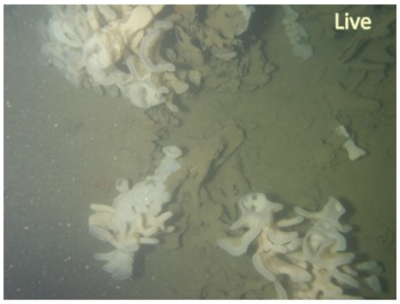
Salinity: 30.09 − 31.01 PSU

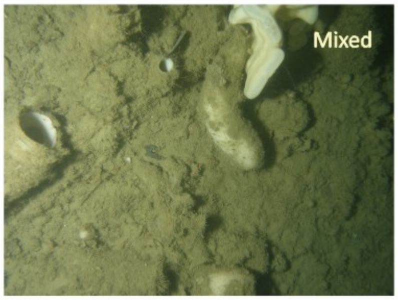
Depth surveyed: 71 − 198 m

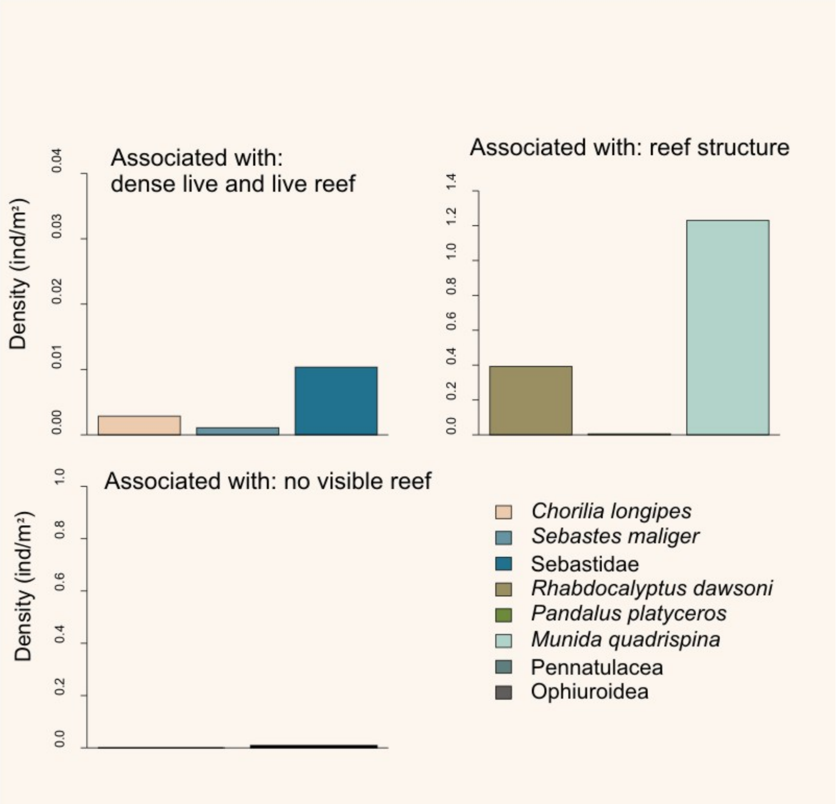


Habitat categories:

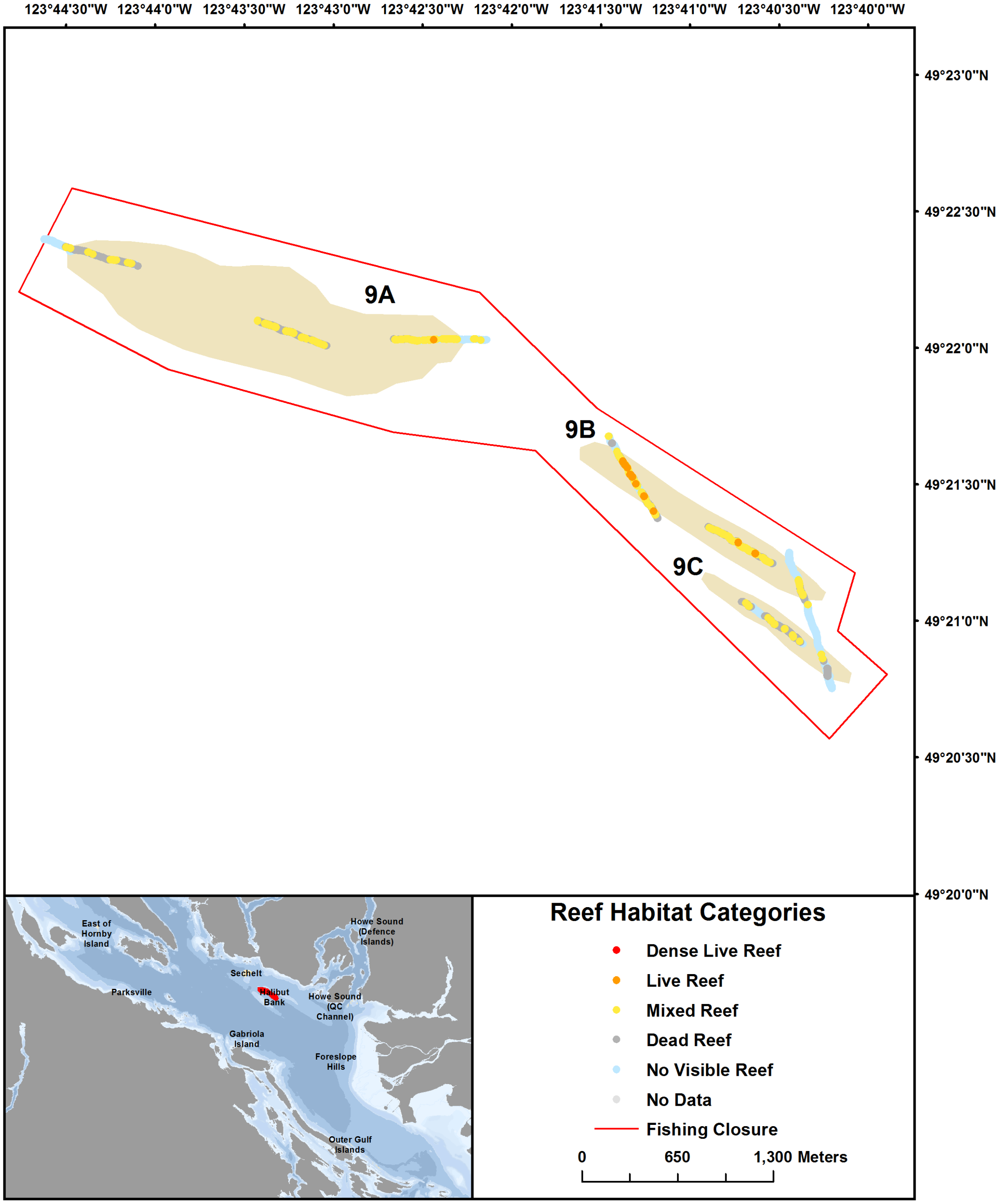








Halibut Bank



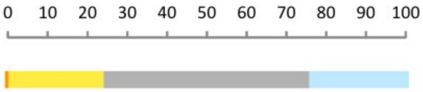
Complex 9: Halibut Bank

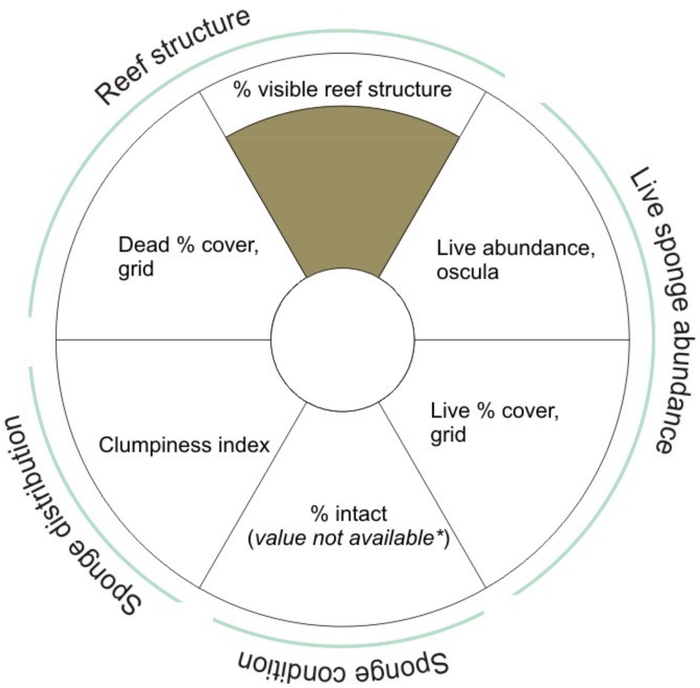
Temperature: 8.99 − 9.08°C

Salinity: 30.84 − 31.13 PSU

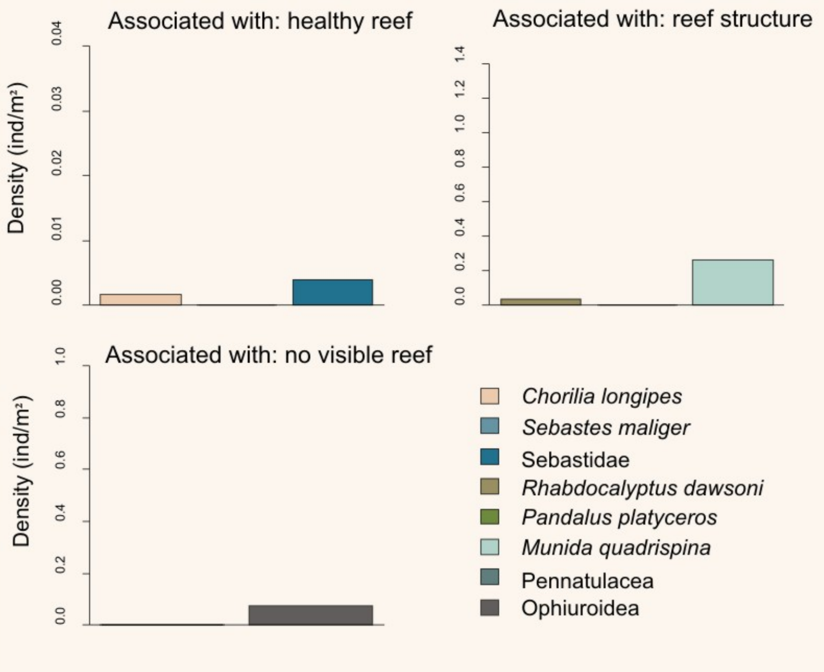
Depth surveyed: 166 − 230 m

Habitat categories:





Shannon-Wiener diversity index: 2.39



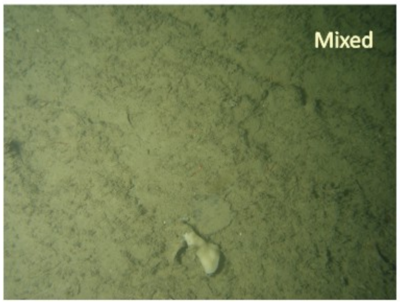


Table 28. Summary of indices calculated for characterizing nine glass sponge reef complexes in the Strait of Georgia and Howe Sound currently protected by bottom-contact fishing closures.

Suites of indices		**Index**	**Glass sponge reef complex**								
			Howe Sound - Defence Islands (1)	**Howe Sound - QCC (2)**	**Foreslope Hills(3)**	**Outer Gulf Islands (4)**	**Gabriola Island (5)**	**Parksville (6)**	**East of Hornby Island (7)**	**Sechelt (8)**	**Halibut Bank(9)**
Reef-building sponge-based	Live sponge abundance	Live abundance, oscula method (count/m^2)	2.22	7.43	3.84	0.39	0.69	2.52	3.42	2.14	0
		Live % cover, grid method	0.65	9.84	6.93	0.22	0.30	0.09	3.76	2.03	0.01
		Live sponge habitat categories combined (%)	54.3	65.3	42.5	26.8	71.8	8.4	81.9	27.6	24.4
	Live sponge distribution	Clumpiness index[1]	0.34	0.66	0.71	0.50	0.49	0.15	0.55	0.56	n/a[2]
	Live sponge condition	% images with intact sponges	81.2	51.2	76.2	25.0	86.2	50.0	75.3	83.8	n/a[2]
		% images with broken sponges	18.8	48.8	23.8	75.0	13.8	50.0	24.7	16.2	n/a[2]
	Recovery potential	Dead % cover, grid method	1.53	7.65	4.68	1.21	0.36	0.05	9.05	3.94	0.05
		Visible reef structure habitat categories combined (%)	89.2	81.9	68.9	65.4	94.8	38.2	96.9	72.5	75.4
Community-based	Community structure	Shannon-Wiener diversity index	2.28	2.31	2.9	3.04	1.97	0.69	1.78	1.47	2.39
	Indicator taxa of dense live and live reef (ind/m^2)	*Chorilia longipes*	0.003	0.022	0.013	0.002	0	0	0.007	0.003	0.001
		Sebastes maliger	0	0.004	0.002	0.005	0	0.004	0.004	0.001	0
		Family Sebastidae	0.001	0.033	0.027	0.019	0.026	0.014	0.029	0.010	0.004

Suites of indices		Index	Glass sponge reef complex								
			Howe Sound - Defence Islands (1)	**Howe Sound - QCC (2)**	**Foreslope Hills(3)**	**Outer Gulf Islands (4)**	**Gabriola Island (5)**	**Parksville (6)**	**East of Hornby Island (7)**	**Sechelt (8)**	**Halibut Bank(9)**
	Indicator taxa of visible reef structure (ind/m^2)	*Rhabdocalyptus dawsoni*	0.012	0.048	0	0.001	0.057	6.252	0.5	0.393	0.035
		Pandalus platyceros	0.038	0.075	0.005	0.008	0.025	0.040	0.018	0.006	0
		Munida quadrispina	0.100	0.275	0.653	0.725	0.959	0.914	1.369	1.230	0.261
	Indicator taxa of no visible reef (ind/m^2)	Order Pennatulacea	0.005	0.002	0	0.140	0	0.001	0	0	0
		Class Ophiuroidea	0.206	0.245	0.061	0	0.01	0.117	0	0.009	0.072

[1] The “clumpiness index” assesses the level of sponge aggregation, and is unique from other measures of “patchiness” or “density”. See the help files at McGarigal et al. (2002) for additional information. Maximum value recorded per transect is presented.

[2] Value not available due to insufficient number of live sponges in the image dataset used for index calculation.

3.5 MONITORING CONSIDERATIONS

Monitoring is required to provide the information necessary for effective management responsive to the state of the protected ecosystem. Monitoring strategies and protocols have been developed for a number of marine ecosystems where the majority of habitat complexity is provided by foundation species (biogenic habitats). For example, ecological monitoring of coral reefs, defined as repeated surveys collecting data on ecological attributes such as abundance of fish and coral, has been conducted since coral reef survey techniques were first described in the 1970s (Flower et al. 2017). Challenges in coral reef monitoring include the ecosystem's complexity, differences in sampling methods between surveys, and lack of consistent, long-term datasets (Jameson et al. 1998). Glass sponge reef ecosystems present an additional challenge of largely occurring outside of safe SCUBA diving limits, restricting visual survey methods to ROVs, AUVs, and drop cameras. These survey platforms can be expensive, time consuming, and logistically challenging. This underscores the importance of a carefully developed monitoring strategy that uses relevant metrics of reef health at appropriate spatial and temporal scales and provides well-resolved time series. Integrated, comprehensive monitoring should provide:

- Data to evaluate reef health status and determine trends that can be used to track recovery or decline;
- Indication of chronic and acute stressors of environmental or human origin that may be affecting the reefs; and
- Support for adaptive management decisions to guide management actions.

A final monitoring plan will have to be a detailed document and will include precise protocols that can be used for on-going data collection. Here, we provide science-based recommendations outlining how future monitoring could be structured. These recommendations are not intended to be prescriptive or exhaustive: they will not specify exactly how data should be collected, analyzed, or interpreted, but rather offer potential options and suggest the tools and approaches to achieve them.

3.5.1 Monitoring methods

A glass sponge reef monitoring survey would include choosing the appropriate survey design and methods, pre-survey planning, logistics and equipment set-up, conducting the survey, post-survey processing of imagery files, data analysis, and interpretation of results.

3.5.1.1 Survey design and methods

The nine sponge reefs are dispersed throughout the Strait of Georgia and Howe Sound with each reef exhibiting unique characteristics of sponge distribution, community composition, and overall character. Some of these reefs may be more prone to acute stressors (*e.g*. illegal fishing, human error leading to fishing within boundaries, impacts from activities allowed within closures) than others. In addition, genetic mixing has been suggested to occur across sponge reefs in the Strait of Georgia through larval dispersal (Brown et al. 2017) and thus recovery of a particular reef may be influenced by the status of other reefs. Therefore, the characterization of one reef cannot be extrapolated to another, even within close proximity; each reef complex requires separate monitoring. As described in section 3.2.2.2, it may be beneficial to treat the Northern (Galiano Ridge) and Southern reefs of the Outer Gulf reef complex as separate sub-complexes during monitoring surveys. Surveying all (or most) reefs would be required to be able to attribute observed changes in reef status to chronic or acute stressors.

Assessments should take place at appropriate time intervals to detect trends with sufficient early warning to allow for management actions. Glass sponges are slow growing organisms. Previous research on sponge growth rates in the Strait of Georgia resulted in estimates of 1 – 3 cm/year (Dunham et al. 2015) and 1 – 9 cm/year (Kahn et al. 2016) for *H. calyx* and *A. vastus* and 1.98 cm/year for *Rhabdocalyptus dawsoni* (Leys and Lauzon 1998). Monitoring the reefs annually would likely not allow for the detection of a measurable change in sponge growth. However, deterioration in sponge condition can be detected rapidly, with changes becoming readily apparent over a period of weeks or even days when observed in laboratory conditions, as sponge colouration changes from yellow/tan to brown as the sponge dies (A. Dunham, pers. obs.). Thus, acute stressors such as increased sedimentation or physical damage can have a measurable impact on sponges *in situ* in a short time period. Taking into consideration the relatively slow growth and recovery rates of glass sponges and their rapid deterioration, spacing of monitoring surveys must be adaptive to incorporate known status of the reefs and suspected stressors involved.

Due to economic and logistical factors, the trade-off between the frequency of monitoring and the sampling effort must be considered. Frequent sampling at a limited number of sites may not provide a true picture of the overall reef status and can still be time- and resource-intensive. The use of routine broad-scale surveys and a less frequent intensive, full assessment surveys (similar to the Great Barrier Reef Long-Term Monitoring Program described in Sweatman 2008) will provide the opportunities for adaptive management of the sponge reefs. It must be noted that this paper did not specifically address the survey frequency question. A general time frame of 3 to 10 years is suggested; it can be refined as the monitoring progresses and data on various trends is collected.

For **routine broad-scale surveys,** fixed transects are recommended. The use of fixed transects would allow for observed trends to be attributed to changes in reef status rather than changes in survey location. Small differences in fixed transect placement, centimeter- to meter-range “placement errors”, have been shown to contribute to considerable variation in coral cover estimates (Davidson 1997). Non-invasive markers along the transect routes (for example, thin poles) will facilitate consistency in area captured. The number and length of transects should be related to the total area of each reef complex in order to distribute survey effort evenly. Considerable variability in sponge distribution and condition was observed in our study within and between transects of the same reef complexes (Appendix 7), which demonstrates reef patchiness. Sampling units equal to or smaller than the scale of a typical patch will often produce highly variable abundance estimates as entire patches may be included or excluded from samples (Andrew and Mapstone 1987). In other words, the patchier the reef, the higher the likelihood that random transects will miss certain patch types (*e.g.* live sponge). High patchiness might necessitate a larger initial effort to quantify sponge distribution before transect locations are chosen. A resource-intensive, but well-designed and comprehensive example of detailed mapping of three reef areas using a grid survey pattern is described in Chu and Leys (2010*a*). It would also be beneficial to consider the depth range and bottom topography where individual reefs are situated, as dense sponge aggregations are often observed on tops of ridges and pinnacles.

Broad-scale survey transects could also include marked index sites where overlapping still images are taken to address knowledge gaps in sponge recruitment and recovery. Using fixed rather than randomly placed index sites has been suggested for improving power to detect coral recovery (Molloy et al. 2013); similar results are expected for glass sponges. A more detailed assessment of one reef complex during each routine monitoring cycle could also help address recruitment and recovery knowledge gaps.

During less frequent **intensive surveys,** stratified random transect placement where the number and length of transects are related to the total area of the reef complex and stratified by depth within each reef complex is recommended. A larger number of transects than that completed during the routine surveys would be advisable. Including transects that cross reef footprint and closure boundaries to assess the reef's spatial extent (*i.e*. whether reefs are shrinking or expanding) would be beneficial. If dense live sponge areas are detected outside of reef footprints or closure boundaries, a multibeam sonar survey can be conducted to update and refine reef footprint delineation if needed. An expansion of the reef protection zone may also be considered even in the absence of multibeam sonar data to include glass sponge gardens or aggregations as sponge gardens have been shown to support diverse and productive communities (Marliave et al. 2009, Maldonado et al. 2016).

The choice of sampling platform and methods depends on the types of data to be collected. Input data described in this paper can be broadly divided into two categories that relate to the sampling unit: still images and video. Table 29 summarizes suggested monitoring indices and corresponding data collection techniques.

Table 29. Suggested suites of monitoring indices and corresponding data collection techniques. Indices recommended for routine broad-scale assessment are bolded. Grey "x" denotes data collection techniques that are possible for the index in question, but were not employed in the present study.

Group of indices		**Type of index**	**Data collection technique**	
			Video	**Still imagery**
Reef-building sponge-based	Live sponge abundance	Live abundance, oscula method	-	x
		Live % cover, grid method	-	x
		Live sponge habitat categories combined (%)	x	x
	Live sponge distribution	Clumpiness index	-	x
	Live sponge condition	**% intact**	x	x
		% broken	x	x
	Recovery potential	**Dead % cover, grid method**	-	x
		Visible reef structure habitat categories combined (%)	x	x
Community-based	Community structure	Shannon-Wiener diversity index	x	x
	Indicator taxa of dense live and live reef (ind/m^2)	**Quillback Rockfish**[1]	x	x
		Longhorn Decorator Crab	x	x
	Indicator taxa of visible reef structure (ind/m^2)	Boot Sponge	x	x
		Spot Prawn	x	x
		Squat Lobster	x	x

Group of indices		Type of index	Data collection technique	
			Video	Still imagery
	Indicator taxa of no visible reef (ind/m^2)	**Sea Pen**	x	x
		Brittle Star	x	x

[1] If sampling method and/or data processing does not allow reliably distinguishing among rockfish species, abundance of Family *Sebastidae* can be used as proxy indicator for dense live and live reef. However, whenever possible, *Sebastes maliger* should be counted, as this species appears to be driving the association (see Tables 23 and 24).

These indices address the structural habitat, biodiversity, and ecosystem function – the three components of the conservation objective for the Hecate Strait Glass Sponge Reef Marine Protected Area. Suggested sponge-based indices may be viewed as state indicators used to collect information on long-term trends in response to environmental factors (Kenchington et al. 2012).

Intensive surveys would ideally collect input data for calculating all of the indices listed in Table 27, as well as any other relevant indices or metrics identified in the future. Routine broad-scale surveys could focus on addressing the following five indices: live % cover, dead % cover, % broken and intact sponge, and densities of Quillback Rockfish and Sea Pens. Rockfish and Sea Pens are relatively easy to quantify regardless of imagery resolution or definition, as both of these taxa consist of large, easily identified organisms.

Transect length and number of images to collect should be related to the reef complex size and type of analysis planned. For example, a species accumulation curve can be used to determine sampling effort required to accurately assess community structure (Ugland et al. 2003). In this study, most species accumulation curves for individual reef complexes only just began to reach the asymptote at an equivalent to approximately 167 minutes of video and over 140 still images (see Fig. 20). However, if the monitoring questions are directed toward indicator taxa, reaching the asymptote may not be necessary. Overall, the more imagery collected during a survey, the more imagery available for processing, resulting in higher statistical power to test the hypotheses of interest.

3.5.1.2 Logistical aspects

Considerations for the time of year to conduct monitoring surveys should include planning for optimal tides such as neap tides, where tidal currents have a decreased speed, whenever possible. Avoiding surveys in the spring when water clarity can be poor due to spawning events and algal blooms would allow for better image and video quality. For efficiency, glass sponge reef monitoring should be coordinated with the Department's other monitoring programs in the area, whenever practical.

Detectability of certain taxa may differ inherently between sampling platforms (Althaus et al. 2015): for example, high-resolution still images allow for detection of many more small organisms than standard definition video. It is crucial for the monitoring program to use standardized, compatible sampling platforms (*e.g.* camera resolution) and data processing hardware (*e.g.* monitor settings) to allow for comparisons across space and time. If a change to the sampling platform is necessary, surveys should be conducted using both the old and new platforms to allow for statistical comparison of indices calculated from imagery collected in the same location at the same time. This will allow data collected using the new platform to be compared to existing data in a meaningful way.

3.5.1.3 Additional elements of the monitoring program

Recording anthropogenic objects within the reef footprints is recommended during monitoring surveys. The numbers and types of these objects could provide clues to the types and intensities of anthropogenic pressures the reefs are experiencing. Anthropogenic objects observed during this study are expected to persist in the environment for long periods of time before decomposing.

In addition, we recommend incorporating an aquatic non-indigenous species component into the monitoring plan, listing non-indigenous species with documented presence in the Strait of Georgia and Howe Sound and those that can potentially spread from nearby areas (*e.g.* European green crab, ascidians, and bryozoans).

3.5.2 Data analysis

Annotated data are counts or judgements based on integration and reduction of a large amount of visual information which makes the data prone to annotator bias and procedural errors. Although annotator continuity would be ideal, monitoring programs, and especially long-term ones, are bound to have staff changes. Development of materials and procedures to ensure annotation consistency are critical for achieving consistent and reliable results. These would include, but are not limited to, quality assurance/quality control between annotators on both taxonomic identification and percent cover estimates. Building the capacity for imagery annotation within the Pacific Region is essential to ensure consistent results in future surveys and can be accomplished through training involving both field sampling and imagery review. The production of a Strait of Georgia glass sponge reef species inventory technical report following the model used by Du Preez et al. (2015) will help to expand species identification knowledge for sponge reef communities and facilitate knowledge transfer. This report should include a set of decision rules listing the characteristics which clearly and unambiguously define problematic taxa (Mundy 1991, Carleton and Done 1995) to help minimize annotator inconsistencies.

In this study, select data processing methods were found to be problematic for the quantitative assessment of sponge reef status. The ACFOR scale of relative abundance created an uncertainty related to how frequently annotators defaulted to relative abundance versus direct counting of organisms. The threshold or circumstances to guide the annotator to a relative abundance score over determining a total count should be rigorously defined in the methodology prior to being used a metric in the future. Similarly, the 10 second video bin approach to video processing resulted in in annotation inconsistency; occasionally elapsed time for video bins was greater or less than 10 seconds resulting in difficulties for quality control checks of video annotation during expert review.

Finally, it is important to carefully document and report sampling effort and resulting variance associated with calculations of various indices from each monitoring survey's dataset. This information will be crucial for enabling detection of trends in particular indices and in overall sponge reef status.

3.5.3 Interpretation of Results

For monitoring data to provide feedback to management, managers need a framework not only for collecting data but also for interpreting it (Renken and Mumby 2009, Houk and Van Woesik 2013). Due to patchiness of the reefs and the resulting high variance in quantitative assessment metrics – and given that functional relationships within the reef ecosystem are not yet fully understood – it is difficult to establish clear thresholds for any of the suggested monitoring indices. At present, we believe it would be most appropriate for management decisions to be based on **trend analysis** and to **consider suites of indices in combination**, rather than a one-off increase or decrease in a certain index. It would also be beneficial to determine whether observed changes affect all reef complexes equally or are specific to certain reefs or areas.

A diagnostic approach has been suggested by Downs et al. (2005) for improving the use of coral monitoring data in management decisions. The paradigm is similar to that used in the field of medicine: a clinical examination of the subject (reef), which includes a review of the subject's history and an examination of the current state of health to identify the cause of the illness (Downs et al. 2005). A diagnostic approach that focuses on making the best use of commonly collected, or easy to collect, data has been suggested for better integration of monitoring data with management actions in coral reefs (Flower et al. 2017). Figure 23 provides a diagnostic tree for glass sponge reefs in the Strait of Georgia and Howe Sound based on the results of this paper. The questions included in the "branches" of the diagnostic tree can be answered using the outputs of the routine broad-scale surveys; the resulting pathways lead to the stressor(s) that may be affecting the reef(s) and suggestions on monitoring-related management actions.

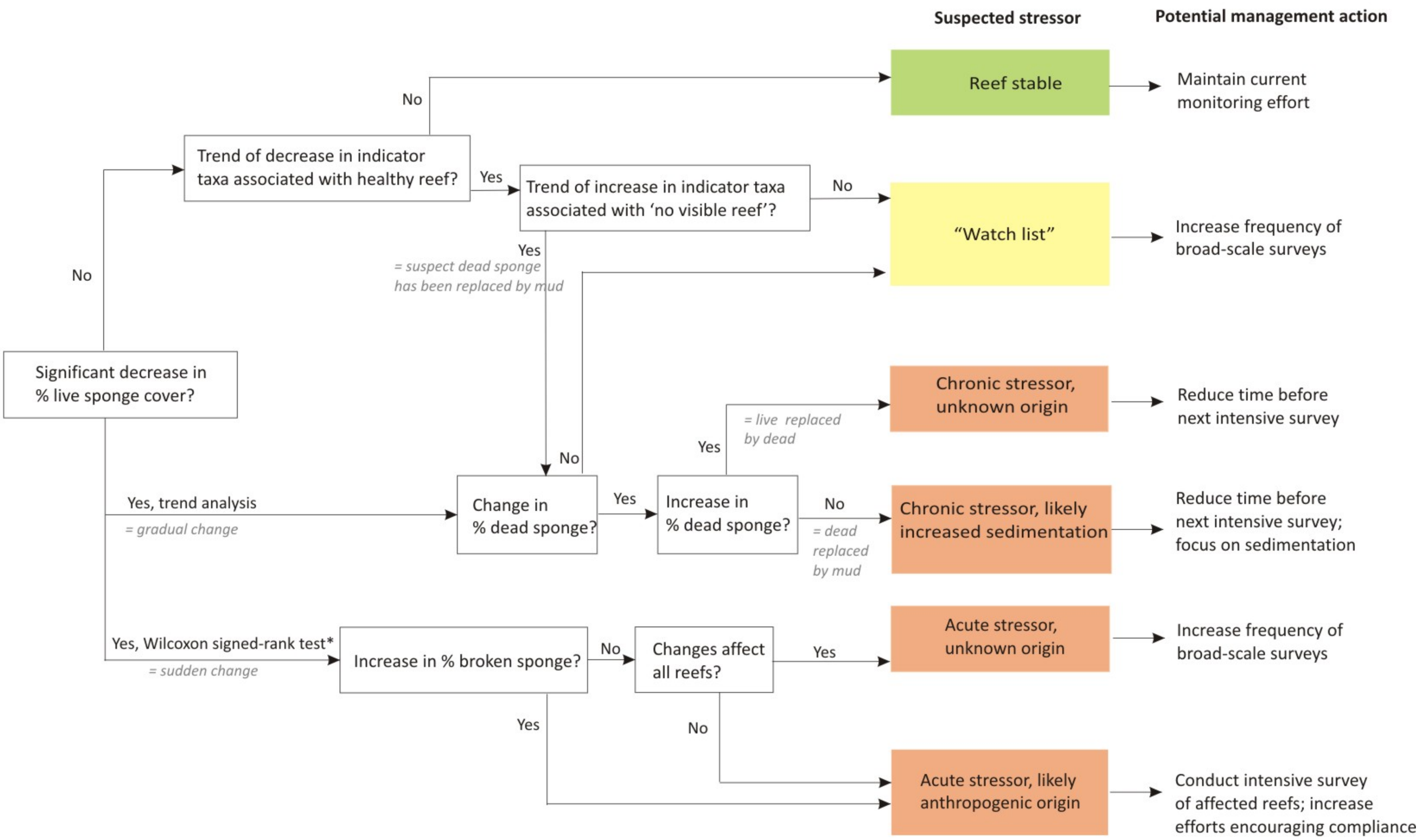


*Figure 23. Diagnostic decision tree for monitoring glass sponge reefs in the Strait of Georgia and Howe Sound. Note that the "Yes, trend analysis" and "Yes, Wilcoxon signed-rank test" pathways are not mutually exclusive. *Difference is statistically significant when compared against immediately preceding sampling period.*

Consider a hypothetical scenario in which a decrease in percent live sponge cover is observed in a reef. Trend analysis does not reveal a significant downward trend, but Wilcoxon signed-rank test shows a statistically significant difference when compared against the immediately preceding survey. This result suggests a somewhat sudden decrease in live sponge cover. Percent of images capturing broken sponges is not significantly higher than during previous assessment; live sponge cover has not decreased significantly in any other reefs. This points to “acute stressor, likely anthropogenic origin” such as a localized pollution event or fishing due to human error or lack of compliance. In this case, it would be beneficial to conduct an intensive survey of the affected reef complex and to consider increasing efforts to boost knowledge and compliance.

This example illustrates the need for monitoring to be **adaptive**: if effects of acute stressors are detected or suspected, more frequent and/or intensive monitoring can be initiated to track recovery or decline and to determine the likely causes of the observed changes. A well-designed long-term monitoring program will provide an opportunity to refine and further develop the diagnostic tree.

4. CONCLUSIONS

4.1 REEF STATUS ASSESSMENT

Through the maps and descriptions provided, this work demonstrated that the nine reef complexes assessed have unique characteristics and reef-specific community structure. Grid and oscula methods (which were found to be most effective in estimating percent live sponge cover and filtration capacity, respectively) resulted in similar relative ranking of the reef complexes. Live sponge cover ranged from 0.01 to 9.84%; oscula densities ranged from 0 to 7.43 oscula/m^2. Highest live sponge cover and oscula density values were observed at Howe Sound – Queen Charlotte Sound, Foreslope Hills, and East of Hornby Island complexes. Visible dead sponge cover ranged from 0.05 to 9.05%. The frequency of occurrence of the five habitat categories – dense live reef, live reef, mixed reef, dead reef, and no visible reef - varied between reef complexes. All complexes had at least some areas designated as ‘live reef’: from 0.2% at Parksville to 24.3% at Howe Sound – Queen Charlotte Channel. Areas with any visible reef structure designation (*i.e.* all except ‘no visible reef’ habitat category) accounted for 38.2% (Parksville) to 96.9% (East of Hornby Island) of the surveyed area.

Live reef-building sponges were found outside of all reef footprints delineated by multibeam bathymetry. Gabriola Island and East of Hornby Island had the highest frequency of occurrence of sponge habitat categories outside of reef footprints. Dense live and live reef areas were also noted outside of the fishing closure boundary of the Gabriola Island complex.

Diverse megafaunal communities, including 9 phyla and 101 unique taxonomic groups, were observed in association with the glass sponge reefs. The taxonomic list generally agrees with and expands the lists developed in earlier studies. The identity of the dominant taxonomic groups differed between complexes. Species richness, diversity and evenness indices, as well as total megafaunal density differed significantly between reef complexes. Community structure was also significantly different across reef complexes, with Howe Sound – Queen Charlotte Channel and Parksville complexes exhibiting the most distinct community structure. A number of significant associations of taxa with habitat categories within reef complexes were identified. Combining the results of video- and still image-based indicator species analyses, we suggested seven indicator taxa for monitoring glass sponge reefs in the Strait of Georgia and Howe Sound.

The information presented in the reef status summaries section and throughout this paper to characterize the nine glass sponge reef complexes in the Strait of Georgia and Howe Sound can be viewed as the best available reference of reef status prior to the implementation of the bottom-contact fishing closures (recognizing, however, that these before-closure reference points apply only to select indices and metrics covered in this paper).

4.2 METHODS AND SAMPLING EFFORT CONSIDERATIONS

In this study, the 2012 and 2013 datasets were processed using the same protocol, but by two different annotators. Rigorous quality control protocols were implemented to increase consistency in video interpretation and to assess annotator variability. Overall annotation success (36%) fell in the range of what is commonly considered ‘fair agreement’. The uncertainty introduced by the lower annotation success in our dataset is partially offset by the fact that both annotators reviewed transects from each reef complex, thus balancing data processing design. Although annotator continuity may be considered ideal, real life monitoring programs, and especially long-term ones, are bound to have staff changes. Development of materials and procedures to ensure annotation consistency are critical for achieving reliable results. Quality assurance/quality control between annotators on both taxonomic identification and percent cover estimates, rigorous training involving both field sampling and imagery review, and producing a Strait of Georgia glass sponge reef species inventory technical report will help expand knowledge, facilitate knowledge transfer, and ensure annotation consistency in the future.

All methods for estimating sponge abundance that were tested in this paper had positive and negative aspects and differed in terms of effort and resources required. Still image-based grid method was found to be accurate and cost-effective for assessing sponge cover. Oscula count method offers a way to efficiently assess filtration capacity, but cannot be used as a proxy for live sponge cover, because of the wide variation in size and number of oscula per area of live reef building sponge cover.

The results of the sampling effort analyses suggest that for characterizing sponge cover using field techniques and grid method of still image analysis described in this paper, a minimum of 3 transects of approximately 500 m length per reef complex should be surveyed and a minimum of 38 images per transect should be analyzed. This would cover 1,500 linear m, or 1,950 m^2 of the reef complex area and result in approximately 100 minutes of video (33 minutes per transect) and 114 images. In our study, the still image cut-off was met for all 9 reef complexes. For characterizing associated megafaunal community composition using field techniques and analytical methods described in this paper, a minimum of 5 transects of approximately 500 m length should be surveyed, with a minimum of 167 minutes of video and 140 images analyzed per reef complex. This would cover 2,500 linear m, or 3,250 m^2 of the reef complex. Over 2,500 m, or 3,250 m^2 of the reef area were surveyed in 4 out of 9 reef complexes in this study.

4.3 MONITORING IMPLICATIONS

The nine sponge reefs are dispersed throughout the Strait of Georgia and Howe Sound with each reef exhibiting unique characteristics of sponge distribution, community composition, and overall character. Some of these reefs may be more prone to acute stressors (*e.g*. illegal fishing, unintentional non-compliance, or impacts from activities allowed within closures) than others. The characterization of one reef cannot be extrapolated to another, even within close proximity; each reef complex requires separate monitoring.

Considerable variability in sponge distribution and condition was observed in our study within and between transects of the same reef complexes. Fixed transects and marked index sites are recommended for routine broad scale monitoring surveys, to allow for observed trends to be attributed to changes in reef status rather than changes in survey location. In addition to frequently surveyed fixed transects, we recommend less frequent intensive surveys. The intensive surveys should have stratified random transect placement where the number and length of transects are related to the total area of the reef complex and stratified by depth within each reef complex. These surveys should include transects that cross reef footprint and closure boundaries to assess the reef's spatial extent. When dense live sponge areas are detected outside of reef footprints or closure boundaries, a multibeam sonar survey can be conducted to update and refine reef footprint delineation.

Based on the results of this paper and best available knowledge to date, we suggested 16 sponge-based and community-based monitoring indices and corresponding data collection techniques (Table 29). These indices assess live sponge abundance, distribution, and condition, reef recovery potential, community structure, and indicator taxa densities. It is important to note, however, these indices are only a subset of the potential metrics that can be used to characterize and monitor glass sponge reefs. Future assessments may need to incorporate different metrics, based on new knowledge and improved understanding of reef biology and ecology, as it becomes available. Evaluation of potential indices as indicators to support ecosystem-based management of human activities affecting glass sponge reefs (*e.g.*, theoretical basis, sensitivity, specificity, cost-effectiveness) cannot be accomplished without explicit conservation objectives for the sponge reef complexes and further knowledge of glass sponge reef ecology and their response to stressors.

Finally, we recommended that management decisions be based on trend analysis and consider multiple indices in combination. We provided a diagnostic decision tree that could be used to incorporate information from a number of indices to guide adaptive management decisions. A well-designed long-term monitoring program will provide an opportunity to refine and further develop the diagnostic tree approach.

5. UNCERTAINTIES, GAPS, AND FUTURE DIRECTIONS

5.1 UNCERTAINTIES AND LIMITATIONS

The following limitations and uncertainties were noted during the completion of this study:

- Despite incorporating over 39 hours of high-quality video and accompanying still imagery, the visual survey datasets underlying our analyses covered only a small percentage of each reef complex (0.24-0.78%).
- In several cases we observed discrepancies between the biological reef footprints and the geological (multibeam-based) reef delineation used for fishing closure placement. The degree to which the geological features overlap the biological features intended to be protected is presently uncertain. We suggest an update or review of the multibeam bathymetry data in the future to assess the ongoing accuracy of the reef footprint definitions.
- All sponge cover estimates were based on two-dimensional (top view) measurements. Due to sponge habitat complexity, true area available for new recruits is likely greater than our estimates.
- Consistent and reliable visual species identification in the absence of physical samples is a key source of uncertainty.
- Involvement of multiple annotators introduced challenges for data analyses. The uncertainty introduced by the 'fair' level of annotation success was partially offset by the fact that each annotator reviewed transects from all reef complexes, thus balancing data processing design.

- All visual survey methods in general are likely to underestimate the abundance and richness of megafaunal organisms in dense sponge areas, as they are harder to see due to habitat complexity. In addition, some mobile taxa are quick to move out of the field of view and may thus be underestimated and/or underrepresented in species counts. This caveat, however, does not diminish the significance of any of the findings incorporated into monitoring advice: they are based on datasets collected solely through visual survey, and thus this caveat would apply to them in a consistent manner, enabling quantitative comparisons over time.
- There is insufficient understanding of glass reef ecology and ecosystem function to define and assess reef “health” at this time. Instead, we developed suites of potential quantitative indices characterizing reef-building glass sponges and associated megafaunal communities were developed and evaluated based on consistency, ability to distinguish between reefs of qualitatively different status, and data processing effort involved.
- Reef complex status summaries presented in this paper include indices that showed most promise in characterizing the reef complexes in this study, based on best available knowledge to date. These summaries can be viewed as a reference for future monitoring, recognizing, however, that they represent before-closure reference points only for select indices covered in this paper. In other words, status summaries should not be viewed as comprehensive baselines, but rather as best available reference of reef status prior to implementation of the bottom-contact fishing closures.
- The monitoring recommendations provided in this paper are intended to guide the development of an effective monitoring program, but are not intended to be prescriptive or exhaustive.
- Considerable variability currently limits the ability to detect real change in reef character over time. As such, we are limited in our ability to attribute any differences observed during the next visual survey time period to measurement error, seasonal variability, and/or inherent ecosystem variability, rather than change in reef character due to ecosystem recovery/decline. Incorporating repeat transects into future survey designs will allow for estimation of measurement error associated with the visual survey method. Gathering long-term datasets from fixed transects or index sites will shed light on the natural variability of sponge reef ecosystems.
- The findings and recommendations of this study are specific to the nine glass sponge reefs in the Strait of Georgia and Howe Sound. The methods and indices developed will likely be applicable to other reefs found in Howe Sound by G. Dennison and collaborators (Glen Dennison, Marine Life Sanctuaries Society of British Columbia, North Vancouver, BC, pers. comm.). However, the indices may first need to be adapted, or additional suites of indices may need to be developed, for glass sponge reefs found in other areas. In particular, Hecate Strait reefs include a third reef-building glass sponge species, *Farrea occa* (Conway 1999), and may thus require modified suites of indices.

5.2 KNOWLEDGE GAPS AND FUTURE DIRECTIONS

A number of additional indices may be incorporated into the assessment of reef character in the future as knowledge of glass sponge reefs and associated communities, as well as understanding of function and diversity in biogenic habitats in general, expands. For example, a community biodiversity component may be expanded once a better understanding of the functional linkages between various trophic levels in glass sponge reef ecosystem is achieved. Seascape ecology metrics such as contagion of habitat and clumpiness of live sponge patches can be incorporated as well, after autocorrelation of sponge distribution and associations between habitat distribution metrics and indicator taxa are explored further. Finally, recovery potential metrics can be refined and strengthened through a better understanding of sponge larval ecology and recruitment, as well as resilience and recovery of individual sponges and sponge reefs as a whole. Once these and other key research questions are addressed that are needed to better understand sponge reef biology, ecology, and ecosystem function, as well as key attributes of overall reef "health", a composite index of reef health may be developed and applied.

Once explicit conservation objectives and management goals are in place for the reef complexes, it will be important to carry out a comprehensive evaluation of potential monitoring indices to determine their relative utility based on theoretical basis, sensitivity/responsiveness, specificity, cost-effectiveness, and other screening criteria (*e.g.*, Rice and Rochet 2005, Kershner et al. 2011).

Promising new directions include testing passive acoustics methods for monitoring communities associated with glass sponge reefs, including acoustic complexity and richness indices (Archer et al., in prep.). The application of three-dimensional imaging systems (*e.g.* stereo cameras) may help further advance the quantitative assessment of live sponge cover and the application of seascape ecology metrics to characterizing sponge reef structure, function, and status. To further assess reef function and understand growth and recruitment processes within these biogenic habitats, it would be beneficial to describe small-scale surface structure (microtopography) which can be quantified by measuring surface roughness, or rugosity (Du Preez and Tunnicliffe 2012).

Future glass sponge reef research and monitoring efforts should include transects that cross reef footprint and closure boundaries to assess accuracy of, and future changes to, the reef's spatial extent. When dense live sponge areas are detected outside of reef footprints or closure boundaries, a multibeam sonar survey may need to be conducted to update and refine reef footprint delineation.

In terms of practical considerations, once managers determine monitoring needs, and resources are allocated to conduct the monitoring, discussions can be facilitated between DFO Science and Management regarding the level of detail and expected outcomes of the monitoring plan. Specific survey protocols and accompanying data processing and analysis guides can subsequently be developed for required monitoring levels.

6. RECOMMENDATIONS

- The information presented in this paper to characterize the nine glass sponge reef complexes in the Strait of Georgia and Howe Sound can be utilized as best available reference of reef status prior to the bottom-contact fishing closure implementation. Reef status was characterized using quantitative indices based on empirical data and best available knowledge to date. Future assessments may need to incorporate other indices, based on new knowledge and improved understanding of reef biology and ecology, as it becomes available.
- For future assessments, we recommend still image-based grid and oscula methods for estimating live sponge cover and assessing filtration capacity, respectively. Suites of sponge-based and community-based indices should be considered in combination rather than in isolation.
- There is a need for a comprehensive evaluation of potential indices to determine their relative utility as indicators to support ecosystem-based management of human activities affecting glass sponge reefs. Indices can be screened based on theoretical rationale,

sensitivity, specificity, cost effectiveness, and other attributes once explicit conservation objectives for the reef complexes are developed.

- Given the dynamic nature of sponge reef ecosystems, incorporating transects that cross both reef footprint and fishing closure boundaries into the survey design will assist in the assessment of the necessity for adjustments to the protection zones around the reef complexes. When dense live sponge areas are detected outside of reef footprints or closure boundaries, a multibeam sonar survey may be conducted to update and refine reef footprint delineation.
- The nine sponge reefs are dispersed throughout the Strait of Georgia and Howe Sound with each reef exhibiting unique characteristics of sponge distribution, community composition, and overall character. The characterization of one reef cannot be extrapolated to another, even within close proximity; each reef complex requires separate monitoring.
- Due to distinct differences in reef characteristics, we recommend treating the northern and southern reefs of the Outer Gulf Islands reef complex as separate sub-complexes for monitoring purposes.
- Regular monitoring is recommended using a combination of broad-scale and intensive surveys of all reef complexes every 3 to 10 years.
- It is crucial for the monitoring program to use standardized, compatible sampling platforms and data processing hardware and protocols to allow for valid comparisons across space and time. If a change to the sampling platform is necessary, surveys should be conducted using both the old and new platforms to allow for statistical comparison of indices calculated from imagery collected in the same location at the same time. This will allow data collected using the new platform to be compared to previously collected datasets in a meaningful way.
- Development of materials and procedures to ensure annotation consistency are critical for achieving reliable results. Quality assurance/quality control between annotators on both taxonomic identification and percent cover estimates, rigorous training involving both field sampling and imagery review, and producing a Strait of Georgia glass sponge reef species inventory technical report are recommended for facilitating knowledge transfer and ensuring annotation consistency in the future.
- For characterizing sponge cover using field techniques and grid method of still image analysis described in this paper, we recommend a minimum of 3 transects of approximately 500 m length per reef complex and a minimum of 38 images per transect analyzed. This would cover 1,500 linear m, or 1,950 m^2 of the reef complex area and result in approximately 100 minutes of video (33 minutes per transect) and 114 images. For characterizing associated megafaunal community composition using field techniques and analytical methods described in this paper, a minimum of 5 transects of approximately 500 m length are recommended, with a minimum of 167 minutes of video and 140 images analyzed per reef complex. This would cover 2,500 linear m, or 3,250 m^2 of the reef complex.
- At present, no thresholds for any of the proposed indices are recommended. We recommend that management decisions be largely based on trend analysis and consider suites of indices in combination, rather than a one-off increase or decrease in a certain index. The one exception is a dramatic, statistically significant decrease in live sponge cover, which should be viewed as evidence of an acute stressor.

- Monitoring should be adaptive: if effects of stressors are detected or suspected, more frequent and/or intensive monitoring can be initiated to track recovery or decline and to determine the likely causes of the changes observed.
- Scientific research should be continued to fill knowledge gaps, to iteratively improve existing monitoring methods, and to explore novel monitoring approaches and techniques. As more data becomes available, proposed indices could be refined and new ones incorporated, while consistent, comprehensive, and well-resolved time series datasets are maintained.

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APPENDICES

APPENDIX 1. BOTTOM-CONTACT FISHING CLOSURES, REEF COMPLEX, AND INDIVIDUAL REEF LOCATIONS AND FOOTPRINT AREAS.

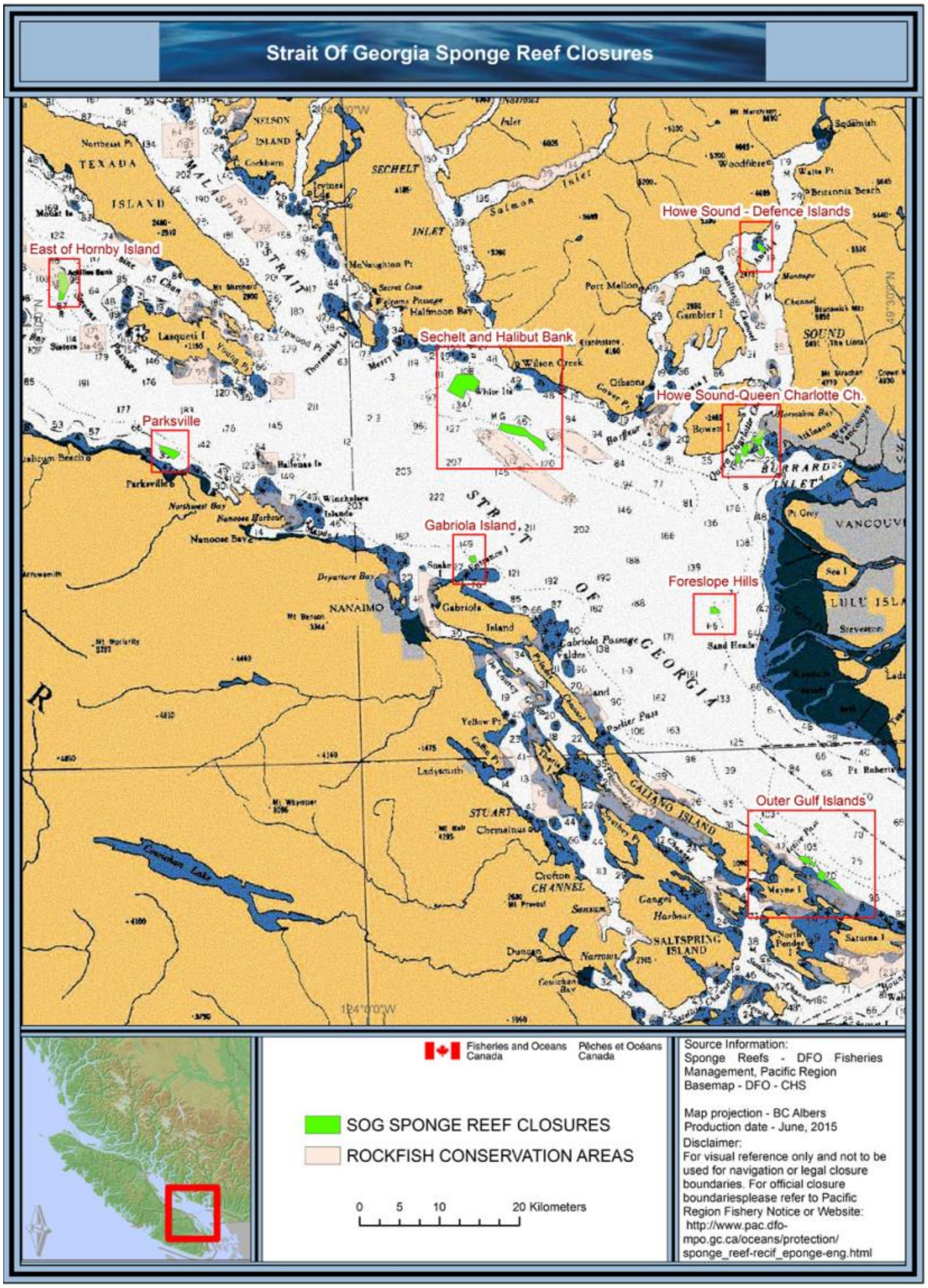


Figure A1-1: Bottom-contact fishing closures implemented in 2015 to protect 9 glass sponge reefs in the Strait of Georgia and Howe Sound.

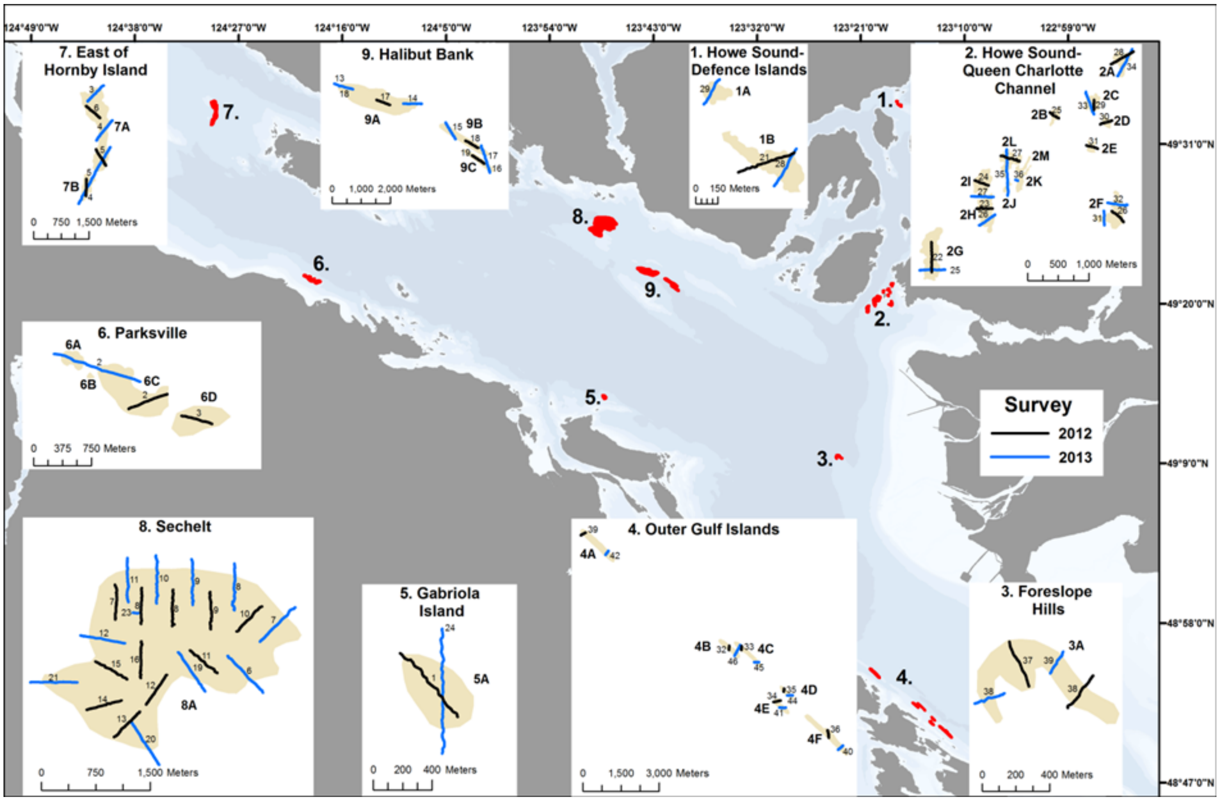


Figure A1-2. Maps of nine reef complexes showing individual reefs (identified by letters) and transect placements (identified by transect number) during 2012 and 2013 ROV surveys.

Table A1-1. Identification, location of footprint centroid, and total area (m²) of individual sponge reefs within bottom-contact fishing closures.

Reef complex	Reef ID*	Latitude	Longitude	Reef area (m²)
Howe Sound - Defence Islands (1)	1A	49.565548° N	-123.285694° W	20,919
	1B	49.561247° N	-123.280469° W	78,875
Howe Sound – Queen Charlotte Channel (2)	2A	49.355376° N	-123.292972° W	73,342
	2B	49.347395° N	-123.307992° W	30,931
	2C	49.349270° N	-123.299471° W	55,564
	2D	49.346469° N	-123.296322° W	22,425
	2E	49.342763° N	-123.299781° W	20,639
	2F	49.333025° N	-123.294624° W	125,829
	2G	49.326958° N	-123.335577° W	198,790
	2H	49.333129° N	-123.323286° W	118,774
	2I	49.337447° N	-123.324171° W	98,687

Reef complex	Reef ID*	Latitude	Longitude	Reef area (m²)
	2J.	49.336890° N	-123.318645° W	13,642
	2K	49.337812° N	-123.315437° W	34,604
	2L	49.340580° N	-123.318184° W	81,599
	2M	49.339913° N	-123.315076° W	19,960
Foreslope Hills (3)	3A	49.157221° N	-123.385653° W	176,761
Outer Gulf Islands (4)	4A	48.908681° N	-123.320335° W	261,196
	4B	48.872124° N	-123.249151° W	99,977
	4C	48.870419° N	-123.239540° W	101,063
	4D	48.856374° N	-123.218352° W	45,333
	4E	48.851804° N	-123.221609° W	70,077
	4F	48.841469° N	-123.196130° W	281,401
Gabriola Island (5)	5A	49.223622° N	-123.796950° W	168,114
Parksville (6)	6A	49.358128° N	-124.325802° W	52,774
	6B	49.356218° N	-124.322657° W	5,128
	6C	49.354415° N	-124.315174° W	353,535
	6D	49.351329° N	-124.303234° W	202,803
East of Hornby Island (7)	7A	49.547832° N	-124.489645° W	925,460
	7B	49.532620° N	-124.493335° W	172,235
Sechelt (8)	8A	49.420935° N	-123.802971° W	4,999,438
Halibut Bank (9)	9A	49.368120° N	-123.723118° W	1,462,331
	9B	49.355912° N	-123.681852° W	379,300
	9C	49.349468° N	-123.674446° W	163,335

APPENDIX 2. SUMMARY OF LITERATURE DATA ON NINE GLASS SPONGE REEF COMPLEXES IN THE STRAIT OF GEORGIA (SOG) AND HOWE SOUND.

Howe Sound - Defence Islands (closure #1, 2 reefs)

The Howe Sound - Defence Islands reef complex is located approximately 1.5 km south of the Defence Islands in northern Howe Sound. Tidal currents range from 1 to 5 cm/s in Howe Sound with the highest velocities occurring near the surface (Leys et al. 2004). Sedimentation in this complex results from glacial meltwater delivered by the Squamish River which flows into the head of Howe Sound with a mean annual discharge of 300 m^3 per second (Leys et al. 2004).

Leys et al. (2004) analysed photographs and transcripts of manned submersible dives from 1981 to 1984 in Howe Sound; dictyonine sponges were frequently observed at depths less than 100 m and were noted as shallow as 18 m. Small live dictyonine sponges were recorded as abundant between depths of 20 to 200 m throughout Howe Sound sites, with a maximum density of 60 per 10 m^2. High numbers of dead dictyonine sponges (>60 individuals) were observed in the vicinity of Defence Islands at depths between 160 and 220 m. In contrast, high glass sponge density was observed at a depth of 248 m. Dead dictyonine sponges were observed at the base of the sill where sediment accumulates from the Squamish River, indicating that sediment loading may be a mitigating factor as well as oxygen deficiency and contamination from industrial sites.

Marliave et al. (2009) described the associated community from video collected by divers in 2004 to 2007 and ROV dives in 2008 to 2009 at two locations between 28 to 35 m depth (1- east of Defence Island at 49.34.67 N, 123.16.26 W and 2- adjacent shoreline of Defence Island at 49.34.67 N, 123.16. 42 W); these sites were referred to as the Defence Islands Bioherm and are located north of reefs 1A and 1B. *Aphrocallistes vastus* was the sole reef-building glass sponge species present in this survey with no observations of *H. calyx*. Community composition was characterized by high abundances of squat lobster *M. quadrispina* and decapod *Eualus* sp. (Marliave et al. 2009).

Howe Sound - Queen Charlotte Channel (closure #2, 13 reefs)

The Howe Sound - Queen Charlotte Channel complex is located on sloped bathymetry at the mouth of Howe Sound at a depth of 50 to 160 m, a sedimentation rate of less than 0.75 g/cm²/yr and organic flux of less than 10 mg C/cm²/yr (Cook et al. 2008).

The reefs represent substantial silicon sinks; reef 2F has an estimated total biogenic silica (bSi) reservoir of 141 tons (assuming an average reef height of 0.6 m) and a mass of 7.3 kg of bSi per m² of reef (Chu et al. 2011). Kahn et al. (2015) estimated component flux rates mediated by sponges for the Howe Sound - Queen Charlotte Channel reef complex (individual reefs not specified) based on in situ measurements of previous studies (Chu and Leys 2010*a*, Chu and Leys 2010*b*, Leys et al. 2011); benthic grazing and volumetric pumping rates were estimated at 85±15 and 108 ±19 $m^3/m^2/d$, respectively (mean±SE). Each square meter of reef was estimated to consume 1.8±0.7 g $C/m^2/d$ and 0.34±0.16 bacterial $N/m^2/d$ (mean±SE). This complex has a bacterial, oxygen and ammonia consumption rate of $5.8 \times 10^{13} \pm 1.3 \times 10^{13}$ cells/m^2/d, 16.8±6.99 *u*mol/m^2/d and 20±9 mmol/m^2/d, respectively. Kahn et al (2015) estimated that this complex has a bacterial carbon consumption of $1.83 \times 10^5 \pm 7.46 \times 10^4$ g C/d from 104,231 m² of live reef.

Brown et al. (2017) assessed the genetic structure of *A. vastus* between individuals within reefs, between reefs, and between populations in and outside the SoG using single nucleotide polymorphisms from tissue samples collected during ROV dives on 4 glass sponge reefs from 2007 to 2011. Genetic distance between individuals within reefs sampled (Reef 2F in the Howe Sound- Queen Charlotte Channel Complex, Reef 4A in the Outer Gulf Reef Complex, Sechelt, and Foreslope Hills) and across the SoG basin did not vary with geographic distance (r=-0.005-

0.014) suggesting extensive larval dispersion within one main population in this area; population(s) within the SoG basin were genetically distinct from nearby populations in Barkley Sound. Genetic distinctness between adjacent sponges within reefs with no indication of identical multilocus genotypes paired with genetic distinctness between reefs suggest that sponges are a result of sexual reproduction on both a fine (1 to 5 m diameter) and broad spatial scale in the SoG (Brown et al. 2017).

Chu and Leys (2010*a*) surveyed reef 2F (referred to as Howe reef) in a grid pattern in 2007-2009 using ROVs which revealed patchy live dictyonine sponge cover of 11.6% (total survey area: 166500 m², live sponge area: 10242 m², dead sponge area: 9083 m²). Live sponge cover in Reef 2F occurred in sparse patches with less than 20% cover in the majority of areas and few areas over 50% cover; dominant substrate between sponge patches consisted of fine-silt clay. Reef 2F contained glass sponges with an average oscula size of 12.8 cm² at a density of 5.5 oscula per m²; a density of 30.9 oscula per m² was found within continuous patches of live sponge (Chu and Leys 2010*a*). Fine scale sponge distribution map and estimates of associated faunal density for reef 2F can be found in Chu and Leys (2010*a*).

Marliave et al. (2009) described the associated community from ROV videotapes of two transects running through reefs 2F and 2I in 2007 (referred to as Passage Island bioherm). *Aphrocallistes vastus* sponges were the only glass sponge species observed in reefs 2F and 2I with no occurrence of *H. calyx*. The main species identified were flatfishes, Dungeness crab, pink shrimp, eelpouts, and rockfish species *S. sebastes, S. elongates* and *S. proriger* with comparable biodiversity estimates to the shallower area surveyed near the Howe Sound - Defence Islands reef complex. No rockfish under 6 cm were observed in Reefs 2F or 2I; communities were dominated by fishes and decapod crustaceans (Marliave et al. 2009). Chu and Leys (2010*a*) described community composition of reef 2F noting high abundance of the sea whip *H. willemoesi*, with significantly higher abundances of fish and crustacean species, and lower abundances of other sponge species and molluscs, in the presence of glass sponges.

Foreslope Hills (closure #3, 1 reef)

The Foreslope Hills reef is located on the Fraser Ridge—an elevated mound capped by glacial sediments with a depth range of 150 to 180 metres and organic flux greater than 30 mg C/cm²/yr (Conway et al. 2004; Cook et al. 2008; Kahn et al. 2015). Average reef structure height is 8 m and maximum reef height is 14 m (Conway et al. 2005). This reef has a sedimentation rate greater than 4 g/cm²/yr as it lies in the path of the Fraser River outflow which is the source of 73% of freshwater and 64% of particles entering the SoG (Cook et al. 2008; Kahn et al. 2015). Sediment accumulation from 2007 to 2009 was 137 mm per year with no net change due to continual sediment erosion (Kahn et al. 2015). The reef represents a substantial silicon sink with an estimated total biogenic silica (bSi) reservoir of 180 tons (assuming an average live reef height of 0.6 m) and a mass of 8.7 kg of bSi per m² of reef (Chu et al. 2011).

Kahn et al. (2015) collected water samples to assess the *in situ* flux of carbon and nitrogen through *A. vastus* during nine dives on this reef in July 2005 using the ROPOS ROV and estimated flux rates. Nutrient concentrations of nitrate and nitrite combined, phosphate, and dissolved silica at Foreslope reef peaked at 5 m above the reef surface (mean±SE: NO_x= 26.0±0.6 *u*mol/L, dSi =49±2 *u*mol/L and PO_4 =2.31±0.09 *u*mol/L) with, on average, lower ammonium concentration among sponges (mean±SE: 571±110 nmol/L) than at 5 m intervals above reef surface up to 20 m. Bacteria concentrations were lowest among sponges at 0 m height (mean±SE: $6.7 \times 10^5 \pm 3.5 \times 10^4$ cells/ml) while total organic carbon (TOC) was highest (mean±SE: 65±3.3 *u*mol/L). Ambient water among sponges was moderately low in oxygen and nutrient enriched; dissolved inorganic nitrogen formed approximately 89% of total nitrogen and dissolved organic carbon formed approximately 85% of TOC in water (Kahn et al. 2015).

Particulate organic matter was low (mean±SE: 10.5±4.2 *u*mol/L), nitrogen poor and consisted partly of picoplankton in which bacteria was dominant over picoeukaryotes. *Aphrocallistes* removed bacteria at up to 90% efficiency; the number of bacteria removed by individual sponges increased linearly with ambient bacteria concentrations. Benthic grazing and volumetric pumping rates for this complex were 165±29 and 210 ±35 m^3/m^2/d, respectively (mean±SE). Each square meter of reef was estimated to consume 3.4±1.4 g C/m^2/d and 0.66±0.31 bacterial N/m^2/d; excretion rates of nitrogenous waste were comparable to the rate of uptake of bacterial nitrogen (0.04±0.02 mol ammonium/m^2/d or 0.55±0.23 g N/m^2/d). Bacterial carbon consumption of the entire Foreslope reef was estimated at 1.0 x 10^5± 0.4 x 10^5 g C /d (Kahn et al. 2015).

Brown et al. (2017) assessed the genetic structure of *A. vastus* between individuals within this reef, between reefs, and between populations in and outside the SoG (for details see description of Howe Sound – Queen Charlotte Channel above).

This reef was assessed as ‘undamaged’ from video transects collected by the Pacific Geoscience Centre in 2002; an undamaged reef was defined as consisting mainly of areas of live reef-building sponges (*A. vastus* and *H. calyx*) and standing dead sponge growing on a mound of dead sponge skeletons and skeletal fragments in a sediment matrix (Cook et al. 2008). Similarly, Conway et al. (2007) qualitatively described this reef as ‘healthy’ based on ROV video transects by ROPOS in November 2004 and October 2005 and previous reports published in Conway et al. (2004) and Conway et al. (2005); however, ‘healthy’ was not defined. Glass sponges on this reef have been described as small and densely clustered with particularly bright coloration (Conway et al. 2005). Chu and Leys (2010*a*) surveyed this reef (referred to as the Fraser reef) in a grid pattern in 2007-2009 using ROVs and reported live dictyonine sponge cover of 14.5% (total survey area: 142775 m², live sponge area: 13774 m², dead sponge area: 6945 m²). Live sponge cover occurred in hotspots with four nodes of greater than 80% cover; glass sponges were distributed on the leeward side of the Fraser ridge. This reef contained glass sponges with an average oscula size of 38.2 cm² at a density of 9.4 oscula per m²; a density of 23 oscula per m² was found within continuous patches of live sponge (Chu and Leys 2010*a*). Leys et al. (2011) calculated an average excurrent flow speed of 2.8± 0.40 cm/s (mean±SE) for sponges on this complex.

Kahn et al. (2016) assessed recruitment, growth and recovery from small and large scale damage at this complex and reef 4A in 2005, 2007, 2009, 2011, 2013 and 2014 using still images from ROV dives; juvenile sponges (defined as sponges with an osculum width less than 10 cm and minimal branching) were significantly smaller at Foreslope with maximum osculum width of 1.6±0.8 cm (mean±SD) and exhibited a narrower size distribution. The number of juvenile sponges within one distinct size class of osculum diameter (1-3 cm) was interpreted as evidence of one or more reproductive events per year. Growth rates were estimated as 1 to 3 cm per year for juvenile sponges and rates of 7 to 9 cm per year for sponge projections. Natural sedimentation levels at Foreslope are higher than at other reefs, but sedimentation does not appear to impede recruitment at current levels (Kahn et al. 2016).

Despite this reef’s exceptionally high sedimentation rate, glass sponge colonization is possible as the ridge remains free of deposition due to strong tidal currents which result in sediment suspension. Reef building sponges in this complex exhibit tube-shaped morphology with narrow oscula in comparison to sponge reefs in the Queen Charlotte Basin, which has been suggested as an environmental adaptation to high sedimentation rates (Cook et al. 2008). This contrasts the significantly larger mean oscula size observed by Chu and Leys (2010*a*) at this reef in comparison to Reef 2F and 4A; Chu and Leys (2010*a*) suggest that narrow oscula may be a morphological adaptation to local hydrodynamic patterns. Further, hydrodynamic patterns may also impact the distribution of glass sponge along the leeward side of the Fraser Ridge where strong southward riverine outflow results in stratification that limits downwelling of surface waters and induces a predominant northern flow of bottom waters. Current velocity in this complex are high with speeds up to 92 cm/s during the flood tide, although currents vary due to a mixed semidiurnal tide schedule (Chu and Leys, 2010*a*; Kahn et al. 2015).

Chu and Leys (2010*a*) described community composition of this reef noting high abundance of the demosponge *Tetilla* sp. with significantly higher abundance of fish and crustacean species, and lower abundances of other sponge species and molluscs, in the presence of glass sponges. Corals, consisting mostly of large gorgonians, were associated with this reef although it had the lowest taxonomic diversity of all reefs surveyed by Cook et al. (2008). Fine scale sponge distribution map and estimates for associated faunal density for this reef can be found in Chu and Leys (2010*a*).

Outer Gulf Islands (closure #4; 6 reefs)

The Galiano Ridge is a submarine crest running in a northwest to southeast direction parallel to the eastern shoreline of Galiano Island. The Outer Gulf Islands complex discontinuously covers an approximately 5-6 km stretch of the ridge north and south of Active Pass (Conway et al. 2007; Chu and Leys 2010*a*). Reef 4A has been referred to in the literature as Active Pass North or Galiano Ridge while reefs 4B to 4F inclusively have been identified as Active Pass South (Chu and Leys 2010*a*, Dunham et al. 2015). This complex has a depth range of 90 to 140 metres, a sedimentation rate greater than 2.5 g/cm²/yr and organic flux of greater than 25 mg C/ cm²/yr (Cook et al. 2008). Kahn et al. (2016) measured sediment accumulation of 97 mm per year with no net change due to continual sediment erosion for Reef 4A from 2007 to 2009. Dissolved silica levels in the waters above and around reefs in SOG are approximately 50 µmol/L and the reefs represent substantial silicon sinks; Reef 4A has an estimated total biogenic silica (bSi) reservoir of 595 tons (assuming an average live reef height of 0.6 m) and a mass of 11.2 kg of bSi/ m² of reef (Chu et al. 2011). Dissolved oxygen levels in this area range from 140 to 210 µmol/l (Johannessen et al. 2014). Chu and Leys (2010*a*) estimated that Reef 4A would process water at 83000 litres/s and total organic carbon (TOC) removal and nitrogen excretion rates would be 0.96 g C/m²/day and 0.16 g N/m²/day, respectively.

Kahn et al. (2015) estimated component flux rates mediated by sponges for Reef 4A based on in situ measurements of previous studies (Chu and Leys 2010*a*; Chu and Leys 2010*b*; Leys et al. 2011); benthic grazing and volumetric pumping rates were estimated at 198±34 and 252±42 $m^3/m^2/d$, respectively (mean±SE). Each square meter of reef was estimated to consume 4.1±1.6 g $C/m^2/d$ and 0.79±0.37 bacterial $N/m^2/d$ (mean±SE). Reef 4A has a bacterial, oxygen and ammonia consumption rate of $1.4 \times 10^{14} \pm 0.29 \times 10^{14}$ cells/m^2/d, 39.3±15.6 *u*mol/m^2/d and 47±20 mmol/m^2/d, respectively. Kahn et al. (2015) estimated that Reef 4A has a bacterial carbon consumption of $9.20 \times 10^5 \pm 3.67 \times 10^4$ g C /d from 224,328 m² of live reef.

Dunham et al. (2015) analysed video and still images collected from ROV transects between 2008 and 2012 in Reef 4A to assess the effects of underwater cables. Water temperature ranged from 8.11 to 8.42°C and salinity ranged from 29.82 to 30.39 psu. Biogenic (sponge reef) habitat was dominant, with bedrock and mud with occasional boulders representing the most common secondary substrates.

Cook et al. (2008) described this complex from video transects collected by the Pacific Geoscience Centre in 2005; Reef 4A was assessed as ‘undamaged’ while Reef 4C was assessed as ‘damaged’. The remaining 4 reefs have not been assessed using visual survey techniques (Cook et al. 2008). Cook et al. (2008) defined an undamaged reef as consisting mainly of areas of live reef-building sponges (*A. vastus* and *H. calyx*) and standing dead sponge growing on a mound of dead sponge skeletons and skeletal fragments in a sediment matrix; damaged reef was defined as consisting mainly of areas of broken and fragmented dead sponge with some possible small areas of standing dead sponge and few isolated live reef-building sponges. Conway et al. (2007) qualitatively described reef 4A as ‘healthy’ and Reef 4C as ‘Largely or Completely Dead’ based on ROV video transects by ROPOS in November 2004 and October 2005 and previous reports published in Conway et al. (2004) and Conway et al. (2005); however, the term ‘healthy’ was not defined. Chu and Leys (2010*a*) surveyed reef 4A in a grid pattern in 2007 to 2009 using ROVs with an estimated patchy live dictyonine sponge cover of 26% (total survey area: 208250 m², live sponge area: 23432 m², dead sponge area: 29799 m²); live sponge cover formed multiple hotspots in concentric patterns along the crest with increasing percent live cover occurring as slope increased down the crest. Despite these findings, live sponge cover was not correlated with slope angle. Live glass sponges in reef 4A were found on both sides of the Galiano ridge where flow runs parallel to the ridge in a predominantly southeastern direction resulting in small-scale, localized upwelling; the resulting increased current velocities may be beneficial to suspension feeders by removing waste waters and renewing source waters (Chu and Leys 2010*a*).

Reef 4A contained glass sponges with an average oscula size of 23 cm² at a density of 17.4 oscula per m²; a density of 46.3 oscula per m² was found within continuous patches of live sponge (Chu and Leys 2010*a*). *Aphrocallistes vastus* growth rate in this reef was estimated at 1 to 3 cm/year (Dunham et al. 2015). Fine scale sponge distribution map and estimates for associated faunal density for reef 4A can be found in Chu and Leys (2010*a*).

Kahn et al. (2016) assessed recruitment, growth and recovery from small and large scale damage at the Foreslope reef complex and reef 4A in 2005, 2007, 2009, 2011, 2013 and 2014 using still images from ROV dives; juvenile sponges (defined as sponges with an osculum width less than 10 cm and minimal branching) were significantly larger in Reef 4A with maximum osculum width of 2.6±1.7 cm (mean±SD). The number of juvenile sponges within one distinct size class of osculum diameter (1-3 cm) was interpreted as evidence of one or more reproductive events per year. Growth rates were estimated at 1 to 3 cm per year for juvenile sponges and rates of 7 to 9 cm per year for sponge projections. Recovery from small scale damage of projections of individual sponges occurred within one year; however, there was no evidence of recovery from large scale damage of 1.5 x 2 m area of reef after a three year period (Kahn et al. 2016). Live glass sponges along the margin of the damaged site grew into the area but there was no evidence of recruitment within the site suggesting that damage to the underlying skeletal structure may significantly impede sponge recruitment. Undisturbed large scale damage control sites exhibited new growth of *A. vastus* and *H. calyx* as well as the demosponge *Desmacella austini* on sponge skeletons, and death of sponge patches occurred over the same time period (Kahn et al. 2016).

Brown et al. (2017) assessed the genetic structure of *A. vastus* between individuals within Reef 4A using single nucleotide polymorphisms from tissue samples collected during ROV dives from 2007 to 2011. While genetic distance between individuals within 1 to 5 m diameter clumps on Reef 4a did not vary, one of three adjacent oscula located within 5 m had a genetic difference of 16.9% indicating that densely associated oscula may be attributed to separate sponges rather than a single individual.

Chu and Leys (2010*a*) described community composition of reef 4A noting high abundance of squat lobster *M. quadrispina*, spot prawn *P. platyceros,* and rockfish *Sebastes* sp., with significantly higher abundance of fish and crustacean species and lower abundances of other sponge species and molluscs in the presence of glass sponges. Dunham et al. (2015) observed similar community composition in the area of reef 4A; Arthropoda was the most abundant taxon, with squat lobster *M. quadrispina,* and spot prawn *P. platyceros* observed at maximum densities of 0.48 and 0.13 individuals per m², respectively. Rockfish were observed at maximum densities of 0.26 individuals per m².

Cook et al. (2008) determined that reef 4A had the highest taxonomic diversity and the highest relative abundance of rockfish of reefs surveyed in the SOG while Reef 4C was the only reef associated with a high relative abundance of brittle stars and sea urchins. Corals, consisting mostly of large gorgonians, were also present at reef 4A (Cook et al. 2008). Spicules of *A. vastus and H. calyx* as well as the encrusting demosponge *D. austinii* were found in gut and fecal samples of Dorid nudibranchs, *Peltodoris lentiginosa* and *Archidoris odhneri*, collected at Reef 4A; this represents the first confirmed case of predation on a glass sponge in the SoG (Chu and Leys 2010*a*; Chu and Leys 2012). *Desmacella,* observed by Kahn et al (2016) in control sites as part of a large scale damage recovery experiment, was most common on dead glass sponge skeletons; however, colonization also occurred at the base of individual live sponges suggesting that *Desmacella* may be a source of competition for reef building glass sponges.

Gabriola Island (closure #5, 1 reef)

This complex has a depth range of 110 to 150 m, a sedimentation rate of less than 0.75 g/cm²/yr and organic flux of less than 15 mg C/ cm²/yr. Cook et al. (2008) assessed this complex, referred to as Nanaimo Reef, as 'damaged, possibly recovering' from video transects collected by the Pacific Geoscience Centre in 2004; a damaged, possibly recovering reef was defined as consisting mainly of areas of mostly broken and fragmented dead sponge with widespread areas of colonizing, young reef-building sponges on fragmented, dead sponge skeletons. Conway et al. (2007) qualitatively described this reef as 'Largely or Completely Dead' based on ROV video transects by ROPOS in November 2004 and October 2005 and previous reports published in Conway et al. (2004) and Conway et al. (2005). The presence of young reef-building sponges on this reef was suggested as evidence of recolonization. This reef and reef 9A in Halibut Bank had the highest relative abundance of demosponges and lyssacine sponges, as well as being the only reefs where shortspine thornyhead *Sebastolobus alacanus* was observed. This reef had the highest taxonomic diversity of the northern complexes surveyed in the SoG by Cook et al. (2008).

Parksville (closure #6, 4 reefs)

This reef complex has a depth range of 90 to 110 metres, a sedimentation rate of less than 0.75 g/cm²/yr and organic flux of less than 15 mg C/ cm²/yr (Cook et al. 2008). This complex has not been assessed using visual survey techniques.

East of Hornby Island (closure #7, 2 reefs)

This complex has not been assessed using visual survey techniques.

Sechelt (closure #8, 1 reef)

The Sechelt complex, also referred to as McCall Bank North, is oriented perpendicular to the bank slope and has distinct wave-form mound morphology with an average reef height of 2 metres and a maximum reef height of 6 metres (Conway et al. 2005). This complex has a depth range of 90 to 210 m, a sedimentation rate of less than 0.75 g/cm²/yr and organic flux of less than 15 mgC/ cm²/yr (Cook et al. 2008).

Brown et al. (2017) assessed the genetic structure of *A. vastus* between individuals within this reef, between reefs, and between populations in and outside the SoG (for details see description of Howe Sound – Queen Charlotte Channel above).

Cook et al. (2008) assessed this reef as 'undamaged' from video transects collected by the Pacific Geoscience Centre in 2003; an undamaged reef was defined as consisting mainly of areas of live reef-building sponges *A. vastus* and *H. calyx* and standing dead sponge growing on a mound of dead sponge skeletons and skeletal fragments in a sediment matrix. Conway et al. (2007) qualitatively described this reef as ‘healthy’ based on ROV video transects by ROPOS in November 2004 and October 2005 and previous reports published in Conway et al. (2004) and Conway et al. (2005); however, the term ‘healthy’ was not defined. Conway et al. (2005) observed large, healthy glass sponges up to 1.2 metres in height from video transects on this complex.

Halibut Bank (closure #9, 3 reefs)

The Halibut bank complex, also known as McCall Bank South, is located in a submarine valley between Halibut and McCall Banks and occurs at depths between 120 to 210 m. This complex is oriented perpendicular to the bank slope and has distinct wave-form mound morphology with an average reef height of 6.4 metres and a maximum reef height of 14 metres (Conway et al. 2005). This complex has sedimentation rate of less than 0.75 $g/cm^2/yr$ and organic flux of less than 15 $mgC/\ cm^2/yr$ (Cook et al. 2008).

Cook et al. (2008) assessed reef 9A as ‘damaged’ from video transects collected by the Pacific Geoscience Centre in 2003; a damaged reef was defined as consisting mainly of areas of broken and fragmented dead sponge with some possible small areas of standing dead sponge and few isolated live reef-building sponges. Reef 9A and Gabriola had the highest relative abundance of demosponges and lyssacine sponges, and were the only reefs where shortspine thornyheads *Sebastolobus alacanus* were observed (Cook et al. 2008). Conway et al. (2007) qualitatively described this reef complex as ‘Largely or Completely Dead’ based on ROV video transects by ROPOS in November 2004 and October 2005 and previous reports published in Conway et al. (2004) and Conway et al. (2005). Conway et al. (2005) noted an absence of healthy, large glass sponges in this complex; they observed few live sponges and some occurrence of broken and dead sponges on the reef surface in contrast to the healthy sponge composition of the Sechelt reef. Continuous, and often parallel, tracks in the seabed were present in sidescan sonar of reef 9A, which may be evidence of damage from mobile fishing gear such as otter trawl doors (Conway et al. 2005).

APPENDIX 3. VIDEO ANNOTATION PROTOCOL.

1.0 Video Miner Software

Video analysis was performed using the software Video Miner (version 3.0), developed by DFO. Video Miner is designed to efficiently record observations from video or still images directly into a Microsoft Access database. One of the software's key features is recording the time with every observation which can be used to link to other data collected such as water quality or positional data. After the time is set, usually from a video overlay, the frame rate and frame count are used to calculate the time for every entry. In addition to time, other header information such as date, project, transect, and on/off bottom are entered once and recorded automatically with every observation until changed. Other information recorded without user input includes file name, review date, review time, and elapsed time of the video file.

Another key feature of the software is that all of the data (with a few exceptions, such as comments or measurements) are entered by choosing from a lookup table. The lookup tables contain standard codes and definitions used widely in DFO which facilitates the exchange and analysis of data between projects. Observations entered using the software fall into two general categories, habitat and species observations.

Observations are entered with buttons, or keys assigned to the buttons, in the habitat, transect, and species button areas. A new record is created in the database for every habitat or species button click. When a habitat button is clicked, the lookup table assigned to that button is displayed and data is entered by selecting the row with the appropriate code (descriptions are also displayed). A comment or screen capture can also be recorded. Species buttons display a detailed species entry window where attributes such as measurements, count, abundance, identification confidence, screen captures, and comments can be entered.

Transect buttons are similar to habitat buttons and differ only in the way data is entered into the database. Buttons can be moved back and forth between the habitat and transect button areas to increase efficiency of data entry. The difference is that when a button in the transect area is used, a record is not created, but that variable is set and is recorded for every subsequent record until changed or cleared. They are called transect buttons since they are generally used for variables that are likely to stay the same for the entire transect such as protocol and image quality. Habitat buttons create a record for every button pressed and will either enter the observation in the database once or can be repeated for every subsequent record if the “repeat habitat data” box is checked at the top of this section (this option should be selected for this project). The preferred method for entering habitat variables is to use the “define all” button which brings up the look up tables for each button in the habitat area in order and creates a single record in the database. This is preferred because it is not only more efficient, it makes the database smaller and easier to use. If individual buttons are used there will be a series of several habitat records for each ten second with one change each which is difficult to interpret and analyse.

In general, every species or habitat button that is pressed creates a record in the database, while data entered using the transect and header buttons do not create records. One exception to this is entering the transect, which is entered using the “Transect Start” button and creates a transect start record and a transect end record is created when the “Transect End” button is used. Every record that is created using a button is assigned a data code that indicates which button was used to create it.

2.0 Protocol Summary

The video analysis protocol is similar to one used by Got et al (2015) to analyse the video from the Cobb Seamount expedition in 2012, with some minor differences (*e.g*. the code for coral rubble as substrate not being used; the full range of relative abundance codes being used). After entering header information, the video is analysed in 10 second segments (bins). At the end of each bin, habitat and species observations are recorded.

3.0 Detailed Protocol

3.1 Database

A database set up specifically for this project and obtained from DFO should be used (not the sample database that is installed with the software). Lookup tables should never be changed without consultation and written agreement with the DFO representative and any changes should be documented. Lookup tables should be retained and included in the database as part of the final deliverable as they are used for quality control.

3.2 Header Information

At the start of each video file, all variables listed on the left side of the screen including date, time, project, and transect are recorded first. Date and time (24 hour format) are recorded in Greenwich Mean Time (GMT) directly from video and may contain two separate dates for one transect if a video file spans midnight. The software should automatically change the date as the time passes midnight, however, since this is a rare event, this feature has not been extensively tested and special attention should be paid in this circumstance to ensure that it functions correctly. The project name corresponds to the DFO Water Properties cruise number (example Pac2012-068) and the Transect name corresponds to the Dive number. Only the dive number should be used, not text. The “Repeat Habitat Data” and “Record Every Second of Video” check boxes should be selected.

The “on bottom” button is near the bottom of the header area and records when the ROV was close enough to the bottom to make habitat and species observations (on bottom = 1, off bottom = 0). It is expected that most video reviewed will be “on bottom” and this is the default setting for this variable, so this button is only occasionally used. There are circumstances, however, including technical difficulties and complex bottom topography, in which the ROV will move too far away to be able to identify organisms and the button is used to set the variable to off bottom. The “on bottom” button toggles the variable between the two states, on and off bottom and creates a record each time it is pressed and like all other buttons in the header area it is recorded with every subsequent record.

The video controls are also at the bottom of the header section. The “play seconds” button in this area is used for this project. After entering “10” in the box beside the button, pressing it will play the video for ten seconds and stop for the annotator to make observations. During the 10 second period the annotator can use the pause/play button or space bar to pause and restart the video and it should still automatically stop after ten seconds. However, once the video has automatically stopped, the “play” button will cause it to play without stopping; in order to play for another 10 second segment the “play seconds” button needs to be pressed again. Because video frame rates are often not round numbers, the interval is subject to small variations and drift. (Note that this has been problematic for some analyses and users of the data so that subsequent protocols have included defined intervals and frequent checking to ensure the data is more consistent).

3.3 Habitat Observations

Habitat observations are made at the end of each 10 second segment of video, for the 10 second segment of video just viewed, before making species observations. Habitat information can be entered with buttons in both the ‘transect’ and ‘habitat’ areas of the software interface. As described earlier, habitat and transect buttons differ only in the way data is entered into the database and for the rest of the document they will be referred to as either just habitat buttons or by the variable they enter into the database. For this project, the “repeat habitat data” option and the “record every second of video” options should be selected and the habitat “define all” button should be used to create a single habitat record for each ten second segment of video.

The order is important when entering data. The header information should always be entered first, once for each video clip. Transect variables should be entered next because, like header information, they are repeated for every record and the most recent information needs to be recorded with the subsequent records. If the “repeat habitat data” option is used, as is the case with this protocol, this needs to be entered first so that the correct habitat data is recorded with the species observations for that segment.

The following habitat variables are to be recorded: protocol, survey mode, image quality, relief, disturbance, dominant substrate, dominant substrate percent cover, subdominant substrate, subdominant substrate percent cover, field of view, and in/out of footprint (2013 only).

Protocol: The Protocol variable specifies the general type of video analysis protocol that was used during video review and should remain consistent across a project. For this project, the Semi-Quantitative (Cobb) protocol is to be used to process both surveys.

Survey Mode: Survey Mode records the current action of the ROV with the codes. Ideally the ROV is in transect mode for most video being analysed, but in some cases the ROV will being doing other things such as stopping to investigate something or having technical difficulties:

Survey mode codes and descriptions (database table name = lu_survey_mode)

SurveyModeId	**Survey Mode**	**Survey Mode Description**
1	Transect	Transecting e.g. moving video survey of area. Video must be suitable for quantitative analysis.
2	Investigation (moving)	In-depth exploration of an area/subject. This is non-transect mode but the survey instrument is still in motion. Good video of the bottom is being collected but the video is not suitable for quantitative analysis
3	Investigation (still)	In-depth exploration of an area/subject. This is non-transect mode and the survey instrument is usually relatively stationary (e.g. examining an organism, bedform, etc). Direct sampling.
4	Sampling	Taking/removing a physical sample from the environment. Equipment is typically stationary. Direct sampling.
5	Transiting	Moving between sampling sites sometimes too fast or too far off the bottom to see clearly. Not in survey mode. Non-directed sampling. Substrate is usually visible.
6	Technical issue	Due to ROV issue not transecting correctly & cannot be annotated
7	Not viewed	Have not yet viewed this video (not priority survey mode conducted)
8	Zoom	Camera has zoomed in significantly, usually, but not always when the ROV has stopped.

Image Quality: Image (video in this case) quality depends mainly on water quality and often does not change during a dive, but camera angle, lighting changes, distance off bottom, etc. can change the quality of the video. The categories and codes are as follows:

Image Quality codes and descriptions (database table name = lu_image_quality)		
ImageQualityId	**Image Quality**	**Image Quality Description**
1	Excellent	National Geographic quality, clear water, perfect lighting, good distance to bottom, camera steady or moving smoothly etc.
2	Good	Very good video, but not quite perfect.
3	Average	Water quality or lighting not good, but still able to see habitat and organisms clearly enough for ID.
4	Poor	Water quality or lighting not good, difficult to see habitat and organisms clearly enough for ID
5	Very Poor	Water quality and or lighting poor very hard to identify even a big object unless it almost hits camera

Relief: Relief is the difference between the high and low points of the substrate within the field of view. Relief can be created by slope, for example a vertical bedrock wall will have steep relief and flat mud or sand will have no relief, but large boulders or bedrock ridges on bottom that is flat on a larger scale can also have high relief. Relief is recorded as follows:

Relief codes and descriptions (database table name = lu_relief)		
ImageQualityId	**Relief**	**Relief Description**
1	None	Flat or rolling
2	Low	Vertical relief 0.5 - 2m
3	High	Vertical relief >2m
4	Steep	Slope or wall

Dominant and Subdominant Substrate and Percent Cover: Substrate is classified by recording the most common substrate as the “dominant substrate” according to the codes in substrate code look up table and the second most common substrate is recorded as the “subdominant substrate” using the same codes:

Substrate codes and descriptions (database table name = lu_substrate)		
SubstrateId	**Substrate Type**	**Substrate Description**
0	Wood	Wood, Bark, or Wood Debris
1	Bedrock, smooth	Bedrock, smooth without crevices
2	Bedrock with crevices	Bedrock with crevices
3	Boulders	Boulders, bigger than a basketball
4	Cobble	Cobble, between 3 inches and basketball size

Substrate codes and descriptions (database table name = lu_substrate)		
SubstrateId	**Substrate Type**	**Substrate Description**
5	Gravel	Gravel, between 3/4 inch and 3 inch
6	Pea Gravel	Pea Gravel, between 1/8 inch and 3/4 inch
7	Sand	Sand
8	Shell	Shell
9	Mud	Mud
10	Crushed Shell	Crushed Shell
11	Whole Shell	Whole Shell
12	Live Reef Sponge	Live reef building sponges
13	Dead Sponge	Dead reef building sponges

In addition, a percent cover category is assigned to each ("Dominant Substrate Percent Cover" and "Subdominant Substrate Percent Cover"):

Percent cover codes and descriptions (database table name = lu_percent)	
Percent	**Percent cover Description**
1	<5%
2	5-25%
3	26-50%
4	51-75%
5	>75%

Field of view: Field of view (FOV) is calculated at the end of each 10 second video segment by measuring the distance between laser dots in the video and the width of the viewable area on screen and using simple cross multiplication for the field of view. The measurement should be in cm and if the laser dots are not in view the video can be played for a short period until they are in view or if the laser dots are not in view for more than half or the video segment, field of view should be left blank (null). The preferred way to measure is with a software on-screen ruler like "A Ruler for Windows" which can measure distances in pixels. A simple way to think about it is that the distance between the laser dots is 10cm so if you divide the distance between the dots by 10 you have the distance for 1cm and then if you divide the width of the video by that measurement you have the width of the FOV in cm. For example, if the distance between the laser dots is 192 pixels then 1cm is 19.2 pixels, and if the video is 1920 pixels wide, 1920/19.2=100cm. Rather than doing the calculation every ten seconds it is acceptable to create "video width" and "laser width" variables, enter the measurements, and calculate FOV after analysing all the video . In this case it is important to use new variable and not enter the laser width in the FOV variable.

In/Out Footprint: In 2013, transects were planned so that they started outside the sponge reef footprint and cross the boundary, sometimes more than once. An additional variable, “In/Out Footprint” (0= out, 1 = in) was used to record when a transect entered or exited a reef as indicated by text displayed on the video as either “Start Footprint” or “Stop Footprint”.

3.4 Species Observations

Species observations are recorded at the end of a 10 second segment of video for the segment viewed, and after the habitat observations have been recorded. For purposes of determining which 10 second segment a species observation should be recorded in, an imaginary line passing horizontally through the laser dots is used as a reference line. If there are no lasers in view, the reference line is approximated in the middle of the area in which the bottom and organisms are visible. The main pair of lasers for the camera should be used if more than one pair is in view. A species observation is recorded if it passes the reference line during the 10 second segment or is on the reference line at the end of the segment. Mobile species that pass the reference line multiple times should only be recorded once.

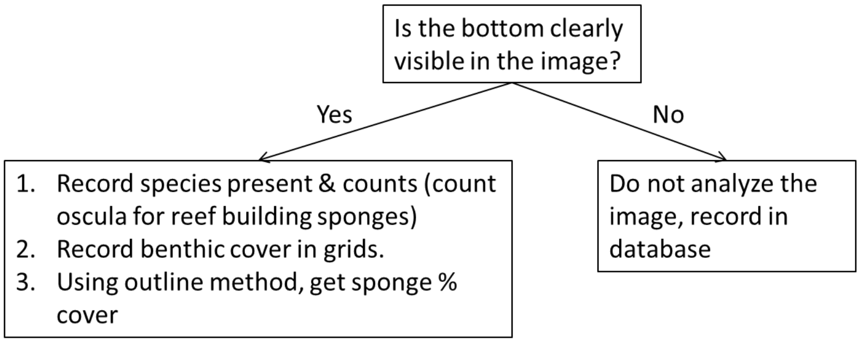
Species observations are recorded using the species buttons which can be customized to increase efficiency. For example, the name that appears on the button can be changed to something that is more easily recognized by the annotator. The species code doesn’t change. Buttons can also be rearranged, given different colours, and assigned keyboard shortcuts according to the preferences of the annotator.

Each organism present in a 10 second segment should be identified to the lowest taxonomic level at which the annotator is confident of the identification. While this should be the default, in some cases it is useful to go to a lower taxonomic level even when the annotator is not confident. In these cases a confidence code should be assigned. Confidence can be assigned in the detailed species entry window using the following codes: (1) High, (2) Medium and (3) Low. In addition, each species record should have a count of the number of individuals present in the video segment when practical and relative abundance recorded using the ACFOR scale adapted from Emmett et al (2007) for species that are too numerous to count or don’t occur as easily distinguishable and countable individuals (*e.g.*, colonial and encrusting organisms):

Relative abundance codes and descriptions (database table name = lu_acfor_scale)

ACFORScaleId	ACFOR Scale	Reef-building glass sponges % cover	Macrofauna density 4-15 cm	Macrofauna density >15 cm
1	A (abundant)	>80	> 50	> 11
2	C (common)	61-80	11-50	6-11
3	F (frequent)	31-60	6-10.9	2-5.9
4	O (occasional)	5-30	2-5	1-1.9
5	R (rare)	<5	1-1.9	<1
6	N/A	N/A	N/A	N/A

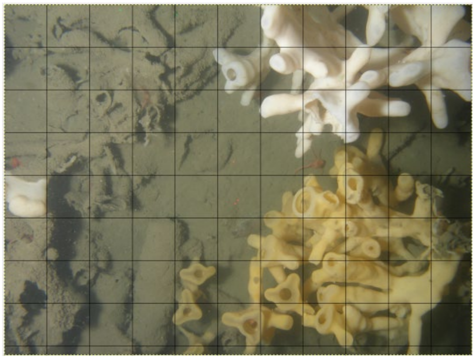
APPENDIX 4. PROTOCOL FOR STILL IMAGE PROCESSING.



1. Record species present in every image:

   Record “species” present in each image, their quantity, and your confidence in your ID in the still images database on the hard drive you received. You do not have to get to species, assign organisms to the lowest taxonomic level you feel comfortable. There are three Photo ID guides in the ID_Guides folder on the hard drive you received to aid with identification. If you need help, make a note of the image number and the approximate location of the organism and we can discuss the ID at a later time. For reef building sponges, do not record a quantity, instead count all live sponge oscula present in the image.

2. Record dominant substrate within a 10 cm grid:
   a. Each transect will have an excel workbook,
      i. For each image create a new sheet. Rename the sheet with the image number. The image number is the very end of the image file name. For example, for image 120513_155212_1.jpg is image number 1.
   b. Open image in image J
      i. Select the straight line tool.
      ii. Draw a line between laser points. There are three laser points. You want to use the two that are in a vertical line. If they are all lined up, use the inner two.
      iii. Press control M or go to Analyze →Measure.
      iv. The length field in the results box is the number of pixels. Retain this number for use in configuring the grid (see c.ii below)
   c. Open image in GIMP
      i. Click on view →show grid



ii. Click on image →configure grid. Set width and height to be the number of pixels between the lasers determined in step b.iv above.

iii. Record the dominant substrate (≥50% of cell) in each grid cell in the appropriate Excel workbook and worksheet using the classifications in Table 1. The substrate observation from the top left grid cell in the image should be entered in cell A1 of the Excel workbook. The remaining observations from the top row should be entered in row A of the Excel workbook in sequential order, row two from the image should be entered in row B of the workbook, continuing until all rows and column of the image have been entered in the workbook.

iv. If the substrate in a cell is exactly split between two or more substrates record each in the appropriate place, separating each ID with a period (e.g. a cell with both wood and bedrock, smooth would be 0.1).

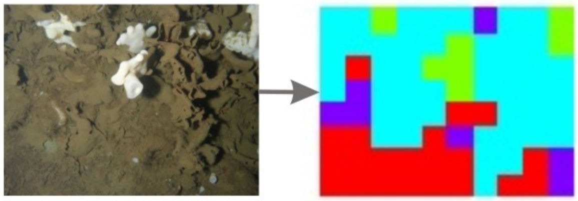
v. Keep image open in GIMP if recording sponge % cover, otherwise move on to next image

Possible substrate classifications

Substrate Id	Substrate Type	Substrate Description
0	Wood	Wood, Bark, or Wood Debris
1	Bedrock, smooth	Bedrock, smooth without crevices
2	Bedrock with crevices	Bedrock with crevices
3	Boulders	Boulders, bigger than a basketball
4	Cobble	Cobble, between 3 inches and basketball size
5	Gravel	Gravel, between 3/4 inch and 3 inch
6	Pea Gravel	Pea Gravel, between 1/8 inch and 3/4 inch
7	Sand	Sand
8	Shell	Shell
9	Mud	Mud
10	Crushed Shell	Crushed Shell (new code 2006)
11	Whole Shell	Whole Shell (new code 2006)
12	Live Reef Sponge	*Heterochone calyx* and *Aphrocallistes vastus*
13	Dead Reef Sponge	Dead reef building sponges
14	Boot sponges	*Rhabdocalyptus* spp.

Possible substrate classifications		
Substrate Id	**Substrate Type**	**Substrate Description**
15	Other sponges	All other sponges
16	Man-made object	Marine debris
17	Non-sponge sessile biota	Corals, anemones, etc
18	Mobile biota	Crabs, sea stars, fish, etc
19	Bottom not visible	
20	Dead sponge rubble	

Example:



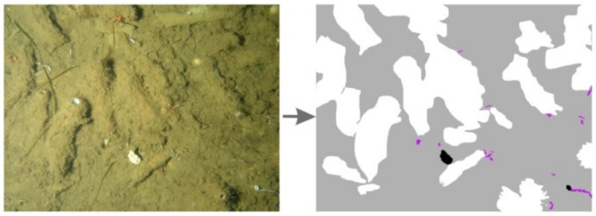
🟦	Dead Reef Sponge
🟪	Boot Sponge
⬛	Other sponges
🟨	Non-sponge sessile biota

⬜	Hard substrate
🟥	Mud
🟩	Live Reef Sponge

3. Obtain sponge % cover:
    a. Working in GIMP
        i. If the toolbox is not on your screen, press control B or go to Windows→toolbox.
        ii. If the tool options dock is not on your screen go to Windows→ Dockable dialogues →tool options
        iii. Outline any sponge (live or dead) either using the foreground select tool (instructions here) or the free select tool.
        iv. Fill in outline using the colors found in Table 2 using the bucket fill option (Shift B).
            1. Make sure to select “fill whole selection” in the “Affected Area” section of the tool options.
            2. Change the color to fill by clicking on the color swath at the bottom of the toolbox.
        v. Once done outlining all sponges, select all filled shapes using the select by color tool (Shift O). Select additional colors by holding the shift button.

Colors for sponge types in percent cover analysis	
Sponge Type	**Color**
Reef building sponges (*Heterochone calyx* and *Aphrocallistes vastus*)- live	Black
Reef building sponges (*Heterochone calyx* and *Aphrocallistes vastus*)- dead	Blue
Rhabdocalyptus spp.	White
Other sponges	Orange

  vi. Once all filled shapes are selected press control I to select the background. Fill the background grey:



  vii. Export file as a .jpg

b. Open exported file in image J

  i. Go to Image→Adjust→Color Threshold

  ii. Unclick the dark background box in dialogue window that opens.

  iii. Use the Wand (tracing) tool to select one filled shape.

  iv. Press sample in the color threshold dialogue window

  1. If all shapes of that color do not automatically turn red adjust the hue, saturation, and brightness until all shapes are red.

  v. Press sample in the color threshold dialogue window

  vi. Press control M or go to Analyze →Measure.

  vii. The area in the Results window should be recorded in the appropriate column on the Percent Cover table in the database.

  viii. Repeat until all sponge types present in the image are measured.

APPENDIX 5. DETAILED SPATIAL PARAMETERS AND SPONGE METRICS.

Table A5-1: Spatial parameters surveyed per transect for Pac2012-068 survey and transect 39 from Pac2011-073 survey (in italics).

Reef complex	Transect	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	Time (mins)	Time (secs)	Speed (m/s)	Mean depth (m)	SD depth (m)	MeanFoV (m)	SD FoV (m)	Count FoV
East of Hornby Island	4	493.72	855.60	0	0	33.93	2036	0.24	127.57	7.58	1.73	0.49	193
	5	559.76	969.98	5.11	8.86	32.20	1932	0.29	130.84	6.71	1.73	0.43	192
	6	544.70	967.26	0	0	38.83	2330	0.23	80.09	10.40	1.78	0.41	228
Foreslope Hills	37	295.69	520.23	0	0	19.57	1174	0.25	165.21	11.14	1.76	0.53	115
	38	139.47	162.08	118.81	138.06	19.37	1162	0.22	180.76	12.73	1.16	0.31	118
Gabriola Island	1	586.89	862.08	37.04	54.41	27.25	1635	0.38	137.25	11.19	1.47	0.50	5597
Halibut Bank	17	525.89	659.93	0	0	40.28	2417	0.22	177.52	1.75	1.25	0.28	242
	18	541.75	665.46	0	0	33.78	2027	0.27	191.62	3.77	1.23	0.28	198
	19	546.26	731.07	0	0	35.80	2148	0.25	227.42	2.78	1.34	0.26	216
Howe Sound (Defence Islands)	21	240.21	432.11	130.03	233.91	22.07	1324	0.28	78.70	14.23	1.80	0.82	112
Howe Sound (QC Channel)	22	505.49	785.33	0	0	37.88	2273	0.22	101.08	2.85	1.55	0.34	224
	23	286.06	449.75	12.25	19.26	21.03	1262	0.24	97.37	21.14	1.57	0.59	126
	24	265.14	363.96	0	0	20.68	1241	0.21	97.85	24.06	1.37	0.46	124
	25	188.25	366.88	1.87	3.64	15.00	900	0.21	68.68	6.35	1.95	0.58	89
	26	279.44	455.77	0	0	20.08	1205	0.23	90.70	7.18	1.63	0.40	120

Reef complex	Transect	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	Time (mins)	Time (secs)	Speed (m/s)	Mean depth (m)	SD depth (m)	MeanFoV (m)	SD FoV (m)	Count FoV
	27	366.58	638.19	0	0	23.07	1384	0.26	77.55	25.27	1.74	0.55	135
	28	433.14	729.91	4.16	7.00	28.18	1691	0.26	92.90	8.06	1.69	0.50	170
	29	160.87	321.02	5.59	11.15	13.50	810	0.21	88.45	5.65	2.00	0.52	80
	30	152.46	206.31	66.13	89.48	15.15	909	0.24	88.35	18.85	1.35	0.31	93
	31	109.10	146.9	98.79	133.02	12.82	769	0.27	83.89	6.68	1.35	0.26	77
Outer Gulf Islands	32	132.36	155.48	52.59	61.78	11.32	679	0.27	94.26	11.22	1.17	0.39	68
	33	125.42	83.84	41.85	27.98	13.48	809	0.21	155.50	8.80	0.67	0.17	79
	34	238.35	179.91	69.67	52.59	18.40	1104	0.28	101.34	2.86	0.75	0.18	77
	35	91.73	65.08	57.89	41.07	9.82	589	0.25	132.54	4.70	0.71	0.18	60
	36	337.53	274.11	0	0	23.80	1428	0.24	125.83	7.16	0.81	0.23	143
	39	*117.33*	*152.53*	*156.37*	*203.28*	*21.00*	*1260*	*0.22*	*110.23*	*15.99*	*1.30*	*0.15*	*100*
Parksville	2	559.29	915.32	0	0	34.67	2080	0.27	68.55	4.42	1.64	0.46	187
	3	427.13	802.42	0	0	24.42	1465	0.29	62.96	7.89	1.88	0.36	141
Sechelt	7	501.95	568.50	0	0	34.25	2055	0.24	178.87	7.38	1.13	0.21	189
	8	1070.74	1650.46	0	0	65.77	3946	0.27	160.93	8.62	1.54	0.46	392
	9	546.92	643.19	0	0	37.93	2276	0.24	149.24	8.61	1.18	0.41	255
	10	512.84	771.40	0	0	30.83	1850	0.28	153.76	3.96	1.50	0.40	183
	11	523.38	665.44	0	0	28.97	1738	0.30	115.53	7.04	1.27	0.47	172
	12	506.71	703.25	0	0	28.67	1720	0.29	99.30	16.89	1.39	0.30	162

Reef complex	Transect	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	Time (mins)	Time (secs)	Speed (m/s)	Mean depth (m)	SD depth (m)	MeanFoV (m)	SD FoV (m)	Count FoV
	13	514.85	813.12	0	0	24.80	1488	0.35	98.49	7.78	1.58	0.30	147
	14	523.82	808.03	0	0	26.98	1619	0.32	107.03	6.57	1.54	0.29	160
	15	523.93	886.23	0	0	23.67	1420	0.37	102.61	16.62	1.69	0.29	144
	16	536.74	855.86	0	0	30.85	1851	0.29	120.43	19.05	1.59	0.33	186

Table A5-2: Spatial parameters per transect for Pac2013-070 survey.

Reef complex	Transect	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	Time (mins)	Time (secs)	Speed (m/s)	Mean depth (m)	SD depth (m)	Mean FoV (m)	SD FoV (m)	Count FoV
East of Hornby Island	3	522.21	579.72	137.78	152.95	38.73	2324	0.28	95.33	17.65	1.11	0.25	247
	4	451.11	388.02	272.32	234.23	33.65	2019	0.36	108.13	8.37	0.86	0.24	212
	5	1371.97	1275.66	374	347.74	89.80	5388	0.32	130.00	6.05	0.93	0.23	565
Foreslope Hills	38	100.81	122.46	106.38	129.23	15.30	918	0.23	177.63	11.13	1.21	0.24	92
	39	132.84	152.63	25.85	29.70	15.23	914	0.17	176.37	8.27	1.15	0.41	92
Gabriola Island	24	460.92	451.86	438.89	430.27	51.38	3083	0.29	127.92	18.28	0.98	0.19	315
Halibut Bank	13	38	47.65	180.65	226.49	20.32	1219	0.18	186.94	0.68	1.25	0.25	102
	14	505.92	649.24	151.04	193.83	42.53	2552	0.26	173.56	2.29	1.28	0.24	252
	15	512.2	648.79	171.85	217.67	43.23	2594	0.26	193.98	9.12	1.27	0.30	262
	16	73.75	110.26	103.14	154.21	15.03	902	0.20	230.03	0.90	1.5	0.44	84
	17	563.71	804.69	380.57	543.25	53.62	3217	0.29	205.97	15.30	1.43	0.34	318

Reef complex	**Transect**	**On-reef length (m)**	**On-reef area (m^2)**	**Off-reef length (m)**	**Off-reef area (m^2)**	**Time (mins)**	**Time (secs)**	**Speed (m/s)**	**Mean depth (m)**	**SD depth (m)**	**Mean FoV (m)**	**SD FoV (m)**	**Count FoV**
	18	495.56	575.67	0	0	30.45	1827	0.27	178.77	5.84	1.16	0.25	187
Howe Sound (Defence Islands)	28	209.01	190.95	94.09	85.96	18.75	1125	0.27	85.11	11.41	0.91	0.32	115
	29	136.93	155.01	70.7	80.03	15.67	940	0.22	70.67	9.97	1.13	0.27	96
Howe Sound (QC Channel)	25	288.31	270.67	133.16	125.02	31.43	1886	0.22	102.97	6.32	0.94	0.33	193
	26	267.49	256.64	72.52	69.58	24.78	1487	0.23	86.59	29.24	0.96	0.42	145
	27	243.79	175.22	138.63	99.64	26.9	1614	0.24	99.12	26.03	0.72	0.33	153
	31	109.79	127.90	127.56	148.60	18.82	1129	0.21	76.68	6.10	1.17	0.40	115
	32	207.72	204.53	130.94	128.93	27.37	1642	0.21	79.39	17.68	0.98	0.32	168
	33	261.85	297.05	145.98	165.6	37.77	2266	0.18	98.93	14.06	1.13	0.41	228
	34	372.48	426.61	140.74	161.19	38.43	2306	0.22	92.43	3.81	1.15	0.42	233
	35	553.23	600.95	234.3	254.51	57.58	3455	0.23	97.33	6.24	1.09	0.35	334
	36	4.35	6.23	43.3	62.03	3.68	221	0.22	85.31	2.46	1.43	0.50	23
Outer Gulf Islands	40	137.61	168.77	128.7	157.84	22.12	1327	0.20	98.20	21.72	1.23	0.45	135
	41	138.76	219.86	132.13	209.35	18.17	1090	0.25	99.30	3.29	1.58	0.39	109
	42	162.15	184.69	58.32	66.43	18.92	1135	0.19	102.71	20.57	1.14	0.40	110
	44	120.06	197.3	134.67	221.31	22.9	1374	0.19	125.99	4.00	1.64	0.39	117
	45	95.64	149.45	122.3	191.11	13.5	810	0.27	141.07	10.27	1.56	0.44	65
	46	255.84	391.20	329.14	503.27	41.77	2506	0.23	113.09	27.17	1.53	0.44	222
Parksville	2	757.38	1347.14	436.17	775.82	65.95	3957	0.30	73.36	1.95	1.78	0.40	403

Reef complex	Transect	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	Time (mins)	Time (secs)	Speed (m/s)	Mean depth (m)	SD depth (m)	Mean FoV (m)	SD FoV (m)	Count FoV
Sechelt	6	497.34	649.15	241.18	314.8	56.27	3376	0.22	122.60	2.20	1.31	0.29	349
	7	507.38	717.08	192.1	271.49	46.58	2795	0.25	158.63	8.16	1.41	0.32	276
	8	549.92	690.31	183.46	230.3	45.58	2735	0.27	175.44	17.22	1.26	0.38	261
	9	529.12	722.52	149.04	203.52	43.58	2615	0.26	178.74	14.81	1.37	0.36	252
	10	517.68	593.45	198.3	227.33	45.83	2750	0.26	180.16	16.25	1.15	0.36	286
	11	472.82	649.37	168.71	231.71	39.47	2368	0.27	185.13	10.64	1.37	0.24	232
	12	501.05	601.45	137.49	165.04	34.43	2066	0.31	144.36	7.69	1.20	0.25	206
	19	492.18	545.27	177.97	197.16	35.38	2123	0.32	115.73	3.66	1.11	0.23	220
	20	486.51	531	234.77	256.23	45.72	2743	0.26	99.06	3.85	1.09	0.29	278
	21	515.19	520.39	138.28	139.67	34.12	2047	0.32	126.64	8.01	1.01	0.24	211
	23	86.92	94.09	0	0	8.15	489	0.18	155.44	0.82	1.08	0.27	29

Table A5-3: Summary of spatial parameters per reef closure for Pac2012-068 survey and transect 39 incorporated from Survey Pac2011-073 to provide additional coverage of the Outer Gulf Islands Reef Complex.

Reef Complex	Total area (m²)	N transects	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	% area of reef surveyed	Time (mins)	FoV (m)	SD FoV (m)	Count FoV
East of Hornby Island	1097694.94	3	1598.18	2792.84	5.11	8.86	0.25	104.97	1.75	0.44	613
Foreslope Hills	176760.98	2	435.17	682.31	118.81	138.06	0.39	38.93	1.46	0.52	233
Gabriola Island	168114.38	1	586.89	862.10	37.04	54.41	0.51	27.25	1.47	0.50	5597

Reef Complex	Total area (m²)	N transects	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	% area of reef surveyed	Time (mins)	FoV (m)	SD FoV (m)	Count FoV
Halibut Bank	2004965.87	3	1613.90	2056.45	0	0	0.10	109.87	1.27	0.28	656
Howe Sound (Defence Islands)	99793.89	1	240.21	432.11	130.03	233.91	0.43	22.07	1.80	0.82	112
Howe Sound (QC Channel)	894785.47	10	2746.53	4464.02	188.78	263.56	0.50	207.40	1.61	0.50	1238
Outer Gulf Islands	859046.56	6	1042.73	910.94	378.38	386.69	0.11	97.82	0.90	0.22	527
Parksville	614240.40	2	986.42	1717.74	0	0	0.28	59.08	1.74	0.44	328
Sechelt	4999438.20	10	5761.89	8365.47	0	0	0.17	332.72	1.44	0.41	1990

Table A5-4: Summary of spatial parameters per reef closure for Pac2013-070 survey.

Reef Complex	Total area (m²)	N transects	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	% area of reef surveyed	Time (mins)	FoV (m)	SD FoV (m)	Count FoV
East of Hornby Island	1097694.94	3	2345.29	2243.40	784.09	734.92	0.20	162.18	0.96	0.25	1024
Foreslope Hills	176760.98	2	233.65	275.10	132.22	158.92	0.16	30.53	1.18	0.34	184
Gabriola Island	168114.38	1	460.92	451.86	438.89	430.27	0.27	51.38	0.98	0.19	315
Halibut Bank	2004965.87	6	2189.14	2836.30	987.25	1335.46	0.14	205.18	1.31	0.32	1205
Howe Sound (Defence Islands)	99793.89	2	345.94	345.96	164.79	165.99	0.35	34.42	1.01	0.31	211
Howe Sound (QC Channel)	894785.47	9	2309.01	2365.81	1167.13	1215.10	0.26	266.77	1.04	0.40	1592

Reef Complex	Total area (m²)	N transects	On-reef length (m)	On-reef area (m²)	Off-reef length (m)	Off-reef area (m²)	% area of reef surveyed	Time (mins)	FoV (m)	SD FoV (m)	Count FoV
Outer Gulf Islands	859046.56	6	910.07	1311.26	905.26	1349.29	0.15	137.37	1.45	0.46	758
Parksville	614240.40	1	757.38	1347.14	436.17	775.82	0.22	65.95	1.78	0.40	403
Sechelt	4999438.20	11	5156.11	6314.08	1821.30	2237.26	0.13	435.12	1.23	0.33	2600

Table A5-5: Summary of values, by transect, used to estimate live and dead reef-building sponge abundance using the Video Bin method for Pac2012-068 survey. Note transect 39 was incorporated from Pac2011-073 to provide additional coverage of the Outer Gulf Islands Reef complex (included in italics).

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
East of Hornby Island	4	On Reef	204	130	0	68	0	119	74
	4	Off Reef	0	0	0	0	0	0	0
	5	On Reef	193	149	0	84	0	86	44
	5	Off Reef	1	1	0	0	0	0	0
	6	On Reef	235	226	0	94	0	70	9
	6	Off Reef	0	0	0	0	0	0	0
Foreslope Hills	37	On Reef	118	49	0	26	0	72	69
	37	Off Reef	0	0	0	0	0	0	0
	38	On Reef	70	49	0	40	0	26	21
	38	Off Reef	47	2	0	1	0	15	45

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
Gabriola Island	1	On Reef	142	77	0	0	0	123	65
	1	Off Reef	9	2	0	0	0	4	7
Halibut Bank	17	On Reef	244	45	0	0	0	241	199
	17	Off Reef	0	0	0	0	0	0	0
	18	On Reef	204	83	0	0	0	201	121
	18	Off Reef	0	0	0	0	0	0	0
	19	On Reef	215	13	0	0	0	141	202
	19	Off Reef	0	0	0	0	0	0	0
Howe Sound (Defence Islands)	21	On Reef	89	51	0	0	0	86	38
	21	Off Reef	39	5	0	0	0	11	34
Howe Sound (QC Channel)	22	On Reef	228	210	0	117	0	111	18
	22	Off Reef	0	0	0	0	0	0	0
	23	On Reef	123	76	0	47	0	71	47
	23	Off Reef	4	0	0	0	0	0	4
	24	On Reef	123	68	0	29	0	54	55
	24	Off Reef	0	0	0	0	0	0	0
	25	On Reef	91	81	0	61	0	29	10
	25	Off Reef	1	1	0	0	0	1	0

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	26	On Reef	121	97	0	46	0	74	24
	26	Off Reef	0	0	0	0	0	0	0
	27	On Reef	139	67	0	46	0	73	72
	27	Off Reef	0	0	0	0	0	0	0
	28	On Reef	169	82	0	55	0	63	87
	28	Off Reef	1	0	0	0	0	0	1
	29	On Reef	79	71	0	40	0	37	8
	29	Off Reef	3	3	0	3	0	0	0
	30	On Reef	65	34	0	0	0	46	31
	30	Off Reef	27	12	0	1	0	20	15
	31	On Reef	46	25	0	7	0	27	21
	31	Off Reef	31	0	0	0	0	0	31
Outer Gulf Islands	32	On Reef	48	12	0	0	0	0	36
	32	Off Reef	21	3	0	2	0	0	18
	33	On Reef	63	4	0	0	0	53	59
	33	Off Reef	19	0	0	0	0	0	19
	34	On Reef	90	5	0	0	0	34	85
	34	Off Reef	21	0	0	0	0	0	21

Reef Complex	**Transect**	**Section**	**Total N bins**	**N Bins with sponges as species**	**N Bins per sponge substrate classification**				**N Bins with no sponge present***
					Dominant Live	**Dominant Dead**	**Subdom. Live**	**Subdom. Dead**	
	35	On Reef	36	5	0	2	0	23	31
	35	Off Reef	24	1	0	0	0	0	23
	36	On Reef	144	24	0	3	0	112	120
	36	Off Reef	0	0	0	0	0	0	0
	39	*On Reef*	*47*	*1*	*5*	*35*	*19*	*5*	*22*
	39	*Off Reef*	*58*	*28*	*0*	*23*	*10*	*0*	*20*
Parksville	2	On Reef	211	0	0	0	0	7	211
	2	Off Reef	0	0	0	0	0	0	0
	3	On Reef	146	3	0	0	0	0	143
	3	Off Reef	0	0	0	0	0	0	0
Sechelt	7	On Reef	193	3	0	0	0	149	190
	7	Off Reef	0	0	0	0	0	0	0
	8	On Reef	395	167	0	122	3	237	225
	8	Off Reef	0	0	0	0	0	0	0
	9	On Reef	228	59	0	43	0	164	169
	9	Off Reef	0	0	0	0	0	0	0
	10	On Reef	186	76	0	50	0	115	110
	10	Off Reef	0	0	0	0	0	0	0

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	11	On Reef	175	15	0	54	0	89	160
	11	Off Reef	0	0	0	0	0	0	0
	12	On Reef	174	40	0	6	0	120	134
	12	Off Reef	0	0	0	0	0	0	0
	13	On Reef	151	41	0	22	0	66	110
	13	Off Reef	0	0	0	0	0	0	0
	14	On Reef	164	20	0	0	0	152	144
	14	Off Reef	0	0	0	0	0	0	0
	15	On Reef	143	44	0	0	0	81	99
	15	Off Reef	0	0	0	0	0	0	0
	16	On Reef	186	72	0	9	0	136	114
	16	Off Reef	0	0	0	0	0	0	0

*The number of bins with no sponge present was calculated as the number of bins with live sponge observations (as species counts and/or dominant or subdominant substrate) subtracted from the total bin count.

Table A5-6: Summary of values, by transect, used to estimate live and dead reef-building sponge abundance using the Video Bin method for Pac2013-070 survey.

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
East of Hornby Island	3	On Reef	181	171	0	86	0	88	10
	3	Off Reef	55	54	0	16	0	23	1
	4	On Reef	126	116	0	46	0	23	10
	4	Off Reef	79	78	0	64	0	13	1
	5	On Reef	424	325	0	121	0	242	99
	5	Off Reef	116	110	0	12	0	93	6
Foreslope Hills	38	On Reef	47	0	0	0	0	0	47
	38	Off Reef	43	0	0	0	0	0	43
	39	On Reef	80	36	0	16	0	37	44
	39	Off Reef	11	1	0	0	0	0	10
Gabriola Island	24	On Reef	166	144	0	0	0	165	22
	24	Off Reef	145	125	0	0	0	145	20
Halibut Bank	13	On Reef	10	1	0	0	0	5	9
	13	Off Reef	90	1	0	0	0	5	89
	14	On Reef	198	94	0	0	0	126	104
	14	Off Reef	48	3	0	0	0	0	45
	15	On Reef	200	85	0	0	0	153	115

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	15	Off Reef	59	10	0	0	0	22	49
	16	On Reef	33	0	0	0	0	0	33
	16	Off Reef	50	0	0	0	0	0	50
	17	On Reef	192	21	0	6	0	70	171
	17	Off Reef	118	1	0	0	0	1	117
	18	On Reef	183	19	0	0	0	150	164
	18	Off Reef	0	0	0	0	0	0	0
Howe Sound (Defence Islands)	28	On Reef	79	54	0	0	0	54	25
	28	Off Reef	35	1	0	0	0	0	34
	29	On Reef	64	21	0	0	0	64	43
	29	Off Reef	31	7	0	0	0	14	24
Howe Sound (QC Channel)	25	On Reef	137	110	0	0	0	114	27
	25	Off Reef	54	12	0	0	0	10	42
	26	On Reef	114	35	0	0	0	42	79
	26	Off Reef	30	0	0	0	0	0	30
	27	On Reef	101	52	0	0	0	62	49
	27	Off Reef	50	10	0	0	0	4	40
	31	On Reef	59	42	0	0	0	50	17

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	31	Off Reef	55	0	0	0	0	0	55
	32	On Reef	110	91	0	0	0	102	19
	32	Off Reef	56	1	0	0	0	1	55
	33	On Reef	149	98	0	0	0	122	51
	33	Off Reef	75	2	0	0	0	0	73
	34	On Reef	172	97	0	34	0	71	75
	34	Off Reef	57	0	0	0	0	0	57
	35	On Reef	254	156	0	0	0	207	98
	35	Off Reef	75	1	0	0	0	2	74
	36	On Reef	5	0	0	0	0	0	5
	36	Off Reef	18	0	0	0	0	0	18
Outer Gulf Islands	40	On Reef	68	27	0	0	0	61	41
	40	Off Reef	65	27	0	15	0	19	38
	41	On Reef	66	22	0	5	0	44	44
	41	Off Reef	40	8	0	0	0	0	32
	42	On Reef	89	43	0	13	0	7	46
	42	Off Reef	19	3	0	0	0	0	16
	44	On Reef	57	23	0	7	0	40	34

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	44	Off Reef	57	5	0	0	0	0	52
	45	On Reef	30	12	0	0	0	30	18
	45	Off Reef	34	10	0	0	0	2	24
	46	On Reef	88	19	0	3	0	1	69
	46	Off Reef	139	30	0	6	0	0	109
Parksville	2	On Reef	250	50	0	0	0	221	200
	2	Off Reef	147	1	0	0	0	11	146
Sechelt	6	On Reef	209	25	0	11	0	103	184
	6	Off Reef	129	18	0	0	0	75	111
	7	On Reef	195	27	0	0	0	69	168
	7	Off Reef	75	0	0	0	0	0	75
	8	On Reef	184	64	0	0	0	96	120
	8	Off Reef	75	0	0	0	0	0	75
	9	On Reef	187	54	0	0	0	97	133
	9	Off Reef	60	0	0	0	0	0	60
	10	On Reef	192	120	0	0	0	152	72
	10	Off Reef	79	2	0	0	0	6	77
	11	On Reef	169	69	0	0	0	68	100

Reef Complex	Transect	Section	Total N bins	N Bins with sponges as species	N Bins per sponge substrate classification				N Bins with no sponge present*
					Dominant Live	Dominant Dead	Subdom. Live	Subdom. Dead	
	11	Off Reef	60	0	0	0	0	0	60
	12	On Reef	156	8	0	0	0	0	148
	12	Off Reef	46	5	0	0	0	2	41
	19	On Reef	157	25	0	27	0	105	132
	19	Off Reef	57	5	0	0	0	8	52
	20	On Reef	188	76	0	59	0	80	112
	20	Off Reef	87	31	0	0	0	68	56
	21	On Reef	160	43	0	0	0	114	117
	21	Off Reef	46	2	0	0	0	16	44
	23	On Reef	29	7	0	0	0	0	22
	23	Off Reef	0	0	0	0	0	0	0

*The number of bins with no sponge present was calculated as the number of bins with live sponge observations (as species counts and/or dominant or subdominant substrate) subtracted from the total bin count.

Table A5-7: Summary of live and dead reef-building sponge abundance (bin method) by reef closure for Pac2012-068 survey; transect 39 was incorporated from Pac2011-073 to provide additional coverage of the Outer Gulf Islands Reef complex.

Reef Complex	**Transect**	**Section**	**Total N bins**	**N Bins with sponges as species**	**N Bins per sponge substrate classification**				**N Bins with no sponge present***
					Dominant Live	**Dominant Dead**	**Subdom. Live**	**Subdom. Dead**	
East of Hornby Island	3	On Reef	632	505	0	246	0	275	127
		Off Reef	1	1	0	0	0	0	0
Foreslope Hills	2	On Reef	188	98	0	66	0	98	90
		Off Reef	47	2	0	1	0	15	45
Gabriola Island	1	On Reef	142	77	0	0	0	123	65
		Off Reef	9	2	0	0	0	4	7
Halibut Bank	3	On Reef	663	141	0	0	0	583	522
		Off Reef	0	0	0	0	0	0	0
Howe Sound (Defence Islands)	1	On Reef	89	51	0	0	0	86	38
		Off Reef	39	5	0	0	0	11	34
Howe Sound (QC Channel)	10	On Reef	1184	811	0	448	0	585	373
		Off Reef	67	16	0	4	0	21	51
Outer Gulf Islands	6	On Reef	428	51	5	40	19	227	353
		Off Reef	143	32	0	25	10	0	101
Parksville	2	On Reef	357	3	0	0	0	7	354
		Off Reef	0	0	0	0	0	0	0
Sechelt	10	On Reef	1995	537	0	306	3	1309	1455
		Off Reef	0	0	0	0	0	0	0

*The number of bins with no sponge present was calculated as the number of bins with live sponge observations (as species counts and/or dominant or subdominant substrate) subtracted from the total bin count.

Table A5-8: Summary of live and dead reef-building sponge abundance (bin method) by reef closure for Pac2013-070 survey.

Reef Complex	**Transect**	**Section**	**Total N bins**	**N Bins with sponges as species**	**N Bins per sponge substrate classification**				**N Bins with no sponge present***
					Dominant Live	**Dominant Dead**	**Subdom. Live**	**Subdom. Dead**	
East of Hornby Island	3	On Reef	731	612	0	253	0	353	119
		Off Reef	250	242	0	92	0	129	8
Foreslope Hills	2	On Reef	127	36	0	16	0	37	91
		Off Reef	54	1	0	0	0	0	53
Gabriola Island	1	On Reef	166	144	0	0	0	165	22
		Off Reef	145	125	0	0	0	145	20
Halibut Bank	6	On Reef	816	220	0	6	0	504	596
		Off Reef	365	15	0	0	0	28	350
Howe Sound (Defence Islands)	2	On Reef	143	75	0	0	0	118	68
		Off Reef	66	8	0	0	0	14	58
Howe Sound (QC Channel)	9	On Reef	1101	681	0	34	0	770	420
		Off Reef	470	26	0	0	0	17	444
Outer Gulf Islands	6	On Reef	398	146	0	28	0	183	252
		Off Reef	354	83	0	21	0	21	271
Parksville	1	On Reef	250	50	0	0	0	221	200
		Off Reef	147	1	0	0	0	11	146
Sechelt	11	On Reef	1826	518	0	97	0	884	1308
		Off Reef	714	63	0	0	0	175	651

*The number of bins with no sponge present was calculated as the number of bins with live sponge observations (as species counts and/or dominant or subdominant substrate) subtracted from the total bin count.

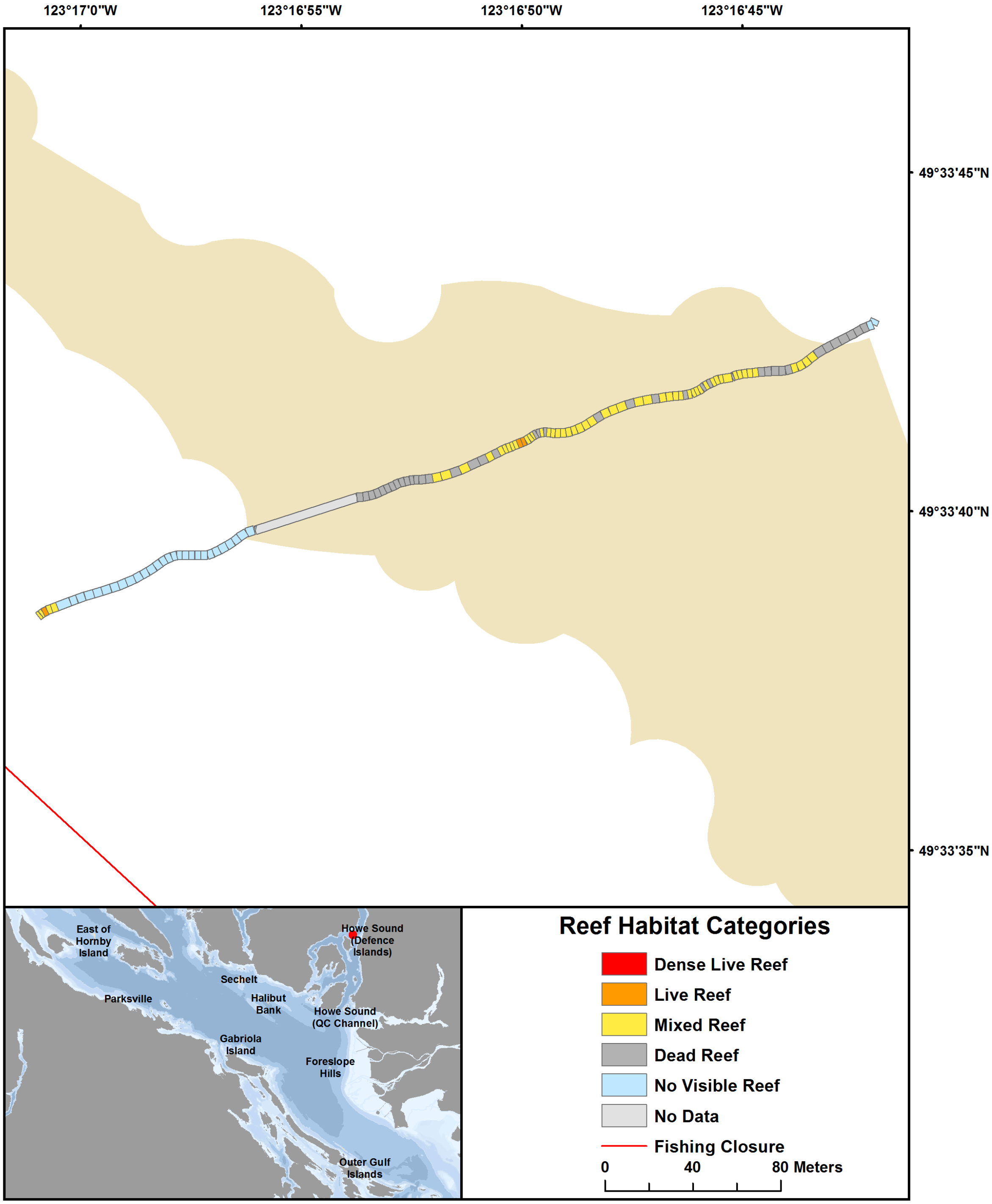
APPENDIX 6. FREQUENCIES OF OCCURRENCE OF HABITAT CATEGORIES WITHIN AND ADJACENT TO EACH OF THE NINE REEF COMPLEXES.

Table A6-1: Frequencies of occurrence of habitat categories within and adjacent to each of the nine reef complexes.

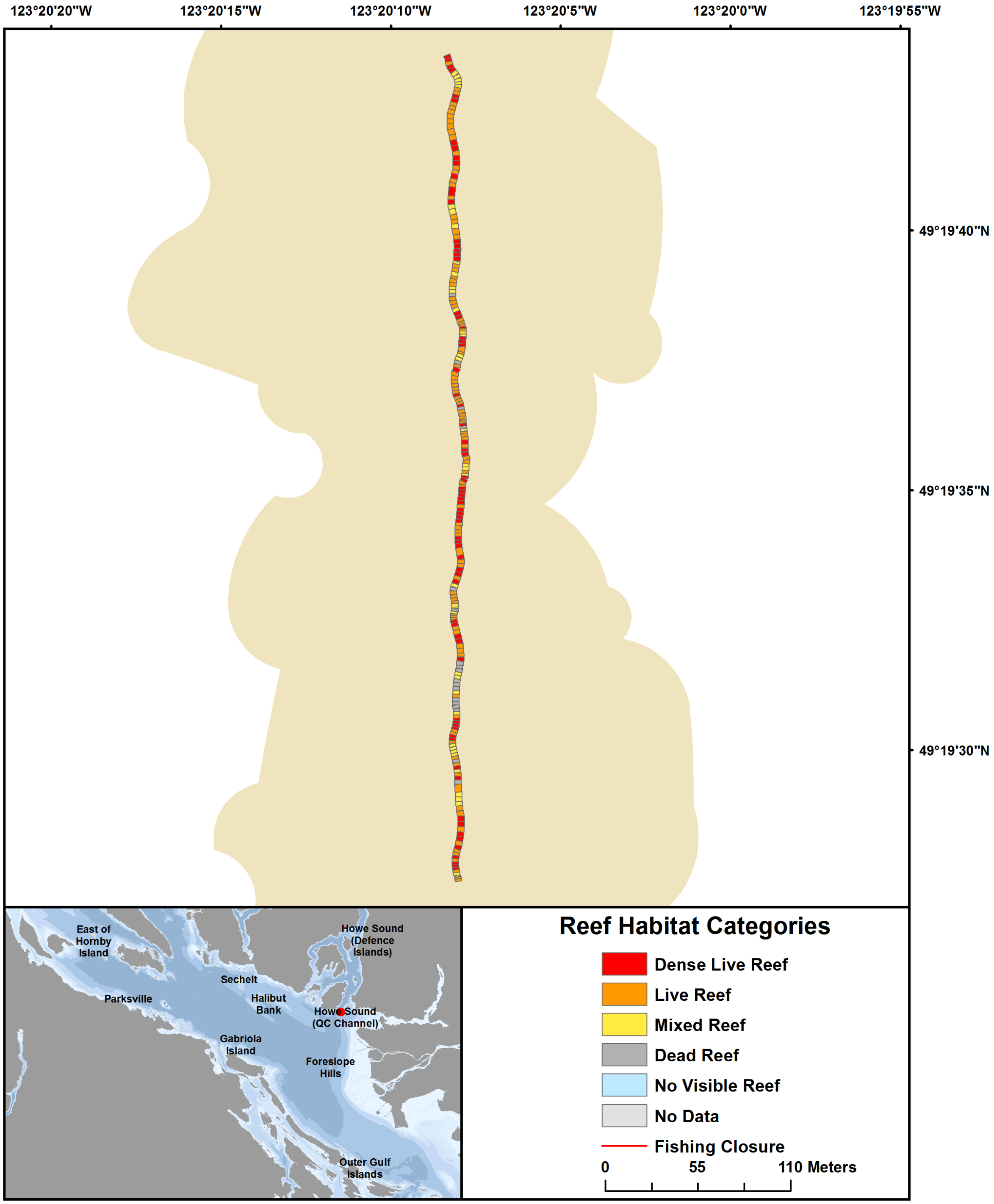
		Adjacent to reef complex					Within reef complex				
#	**Reef complex name**	**Other**	**Dead Reef**	**Mixed Reef**	**Live Reef**	**Dense Live Reef**	**Other**	**Dead Reef**	**Mixed Reef**	**Live Reef**	**Dense Live Reef**
1	Howe Sound (Defence Islands)	72.38	15.24	11.43	0.95	0	10.78	34.91	43.53	8.19	2.59
2	Howe Sound (QC Channel)	89.76	2.61	4.66	2.05	0.93	18.07	16.67	27.79	24.33	13.13
3	Foreslope Hills	83.17	13.86	1.98	0.99	0	31.11	26.35	16.19	9.84	16.51
4	Outer Gulf Islands	71.23	5.43	17.71	4.83	0.8	34.62	38.62	21.31	4.48	0.97
5	Gabriola Island	2.6	14.94	61.69	16.88	3.9	5.19	23.05	68.18	3.57	0
6	Parksville	92.52	6.8	0.68	0	0	61.78	29.82	8.24	0.16	0
7	East of Hornby Island	0	3.19	59.76	34.26	2.79	3.15	14.97	54.22	18.64	9.02
8	Sechelt	72.27	18.91	8.82	0	0	27.45	44.91	19.13	6.75	1.75
9	Halibut Bank	90.41	5.48	3.84	0.27	0	24.61	50.98	23.46	0.95	0

APPENDIX 7. POLYGON-BASED HEAT MAPS SHOWING HABITAT CATEGORIES DISTRIBUTION ALONG EACH TRANSECT.

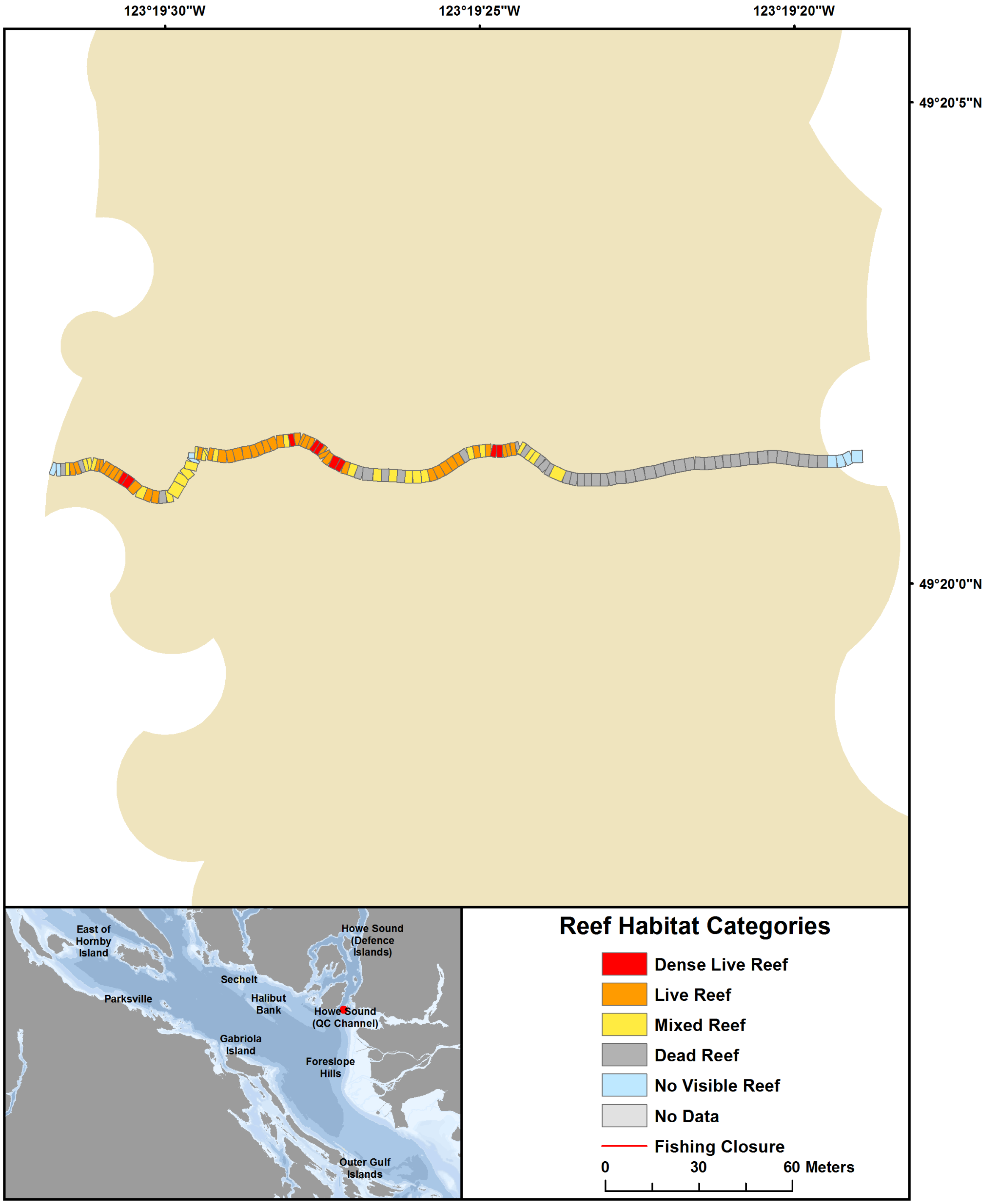
Howe Sound (Defence Islands) - Pac2012-068 Transect 21



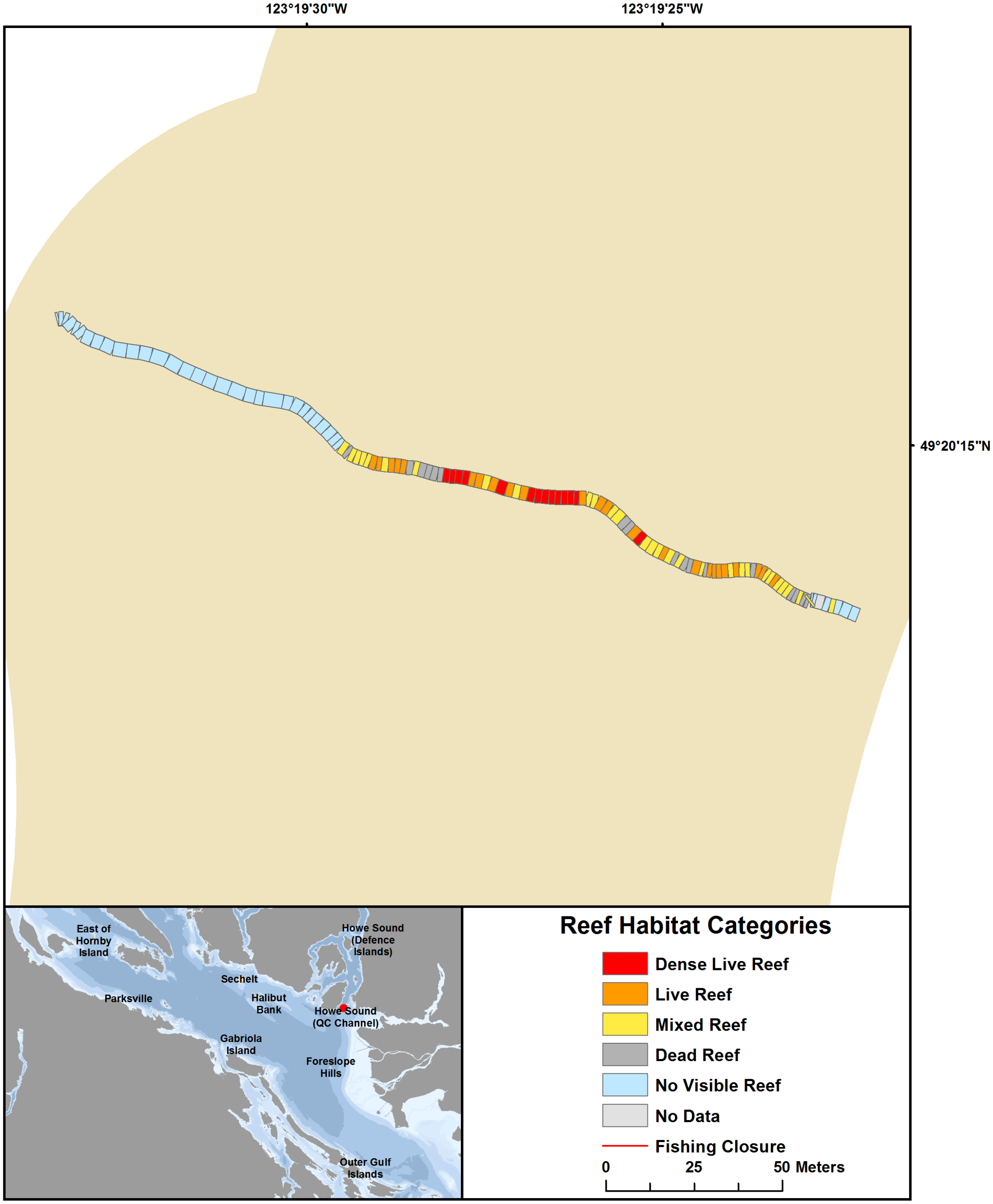
Howe Sound (QC Channel) - Pac2012-068 Transect 22



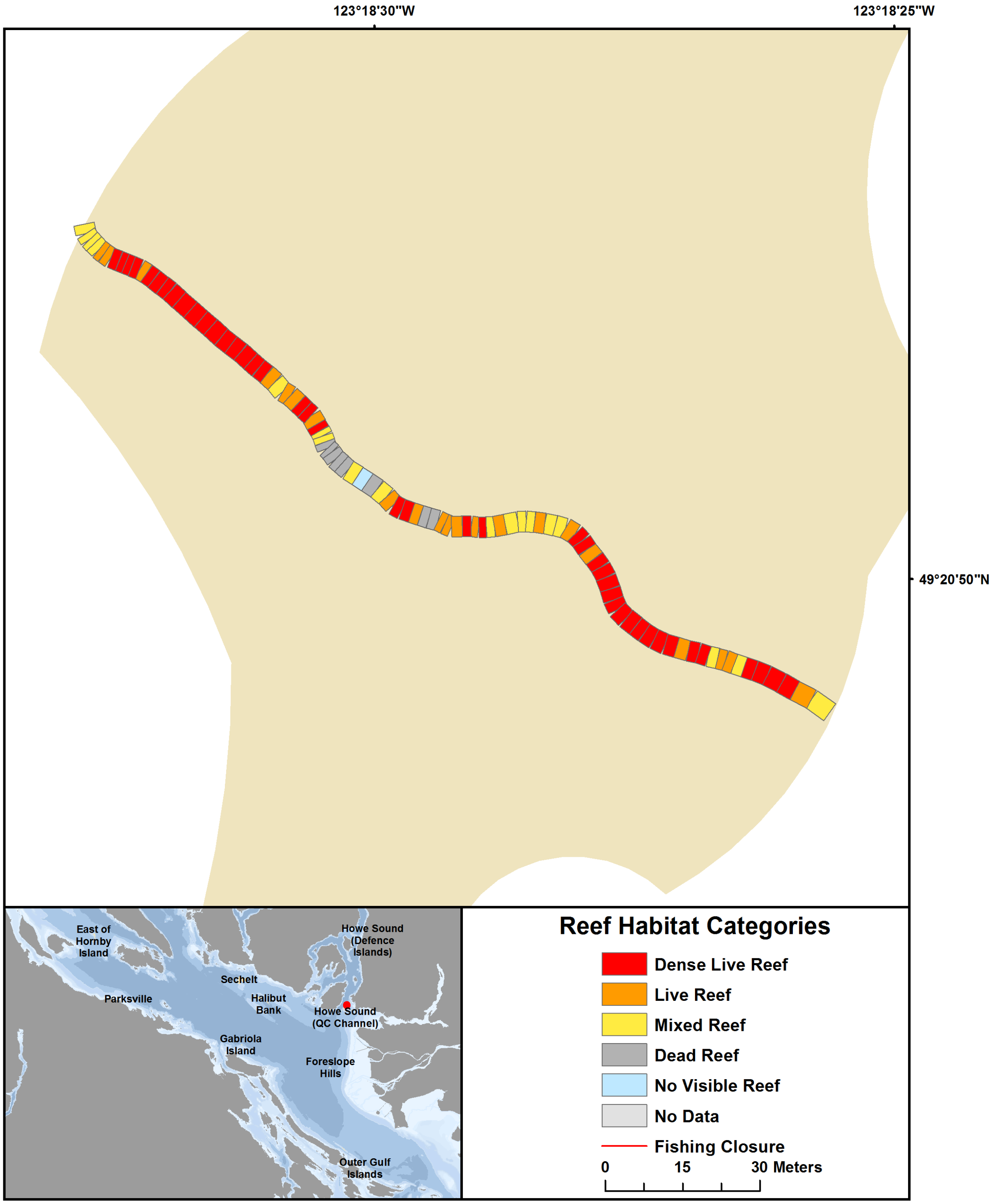
Howe Sound (QC Channel) - Pac2012-068 Transect 23



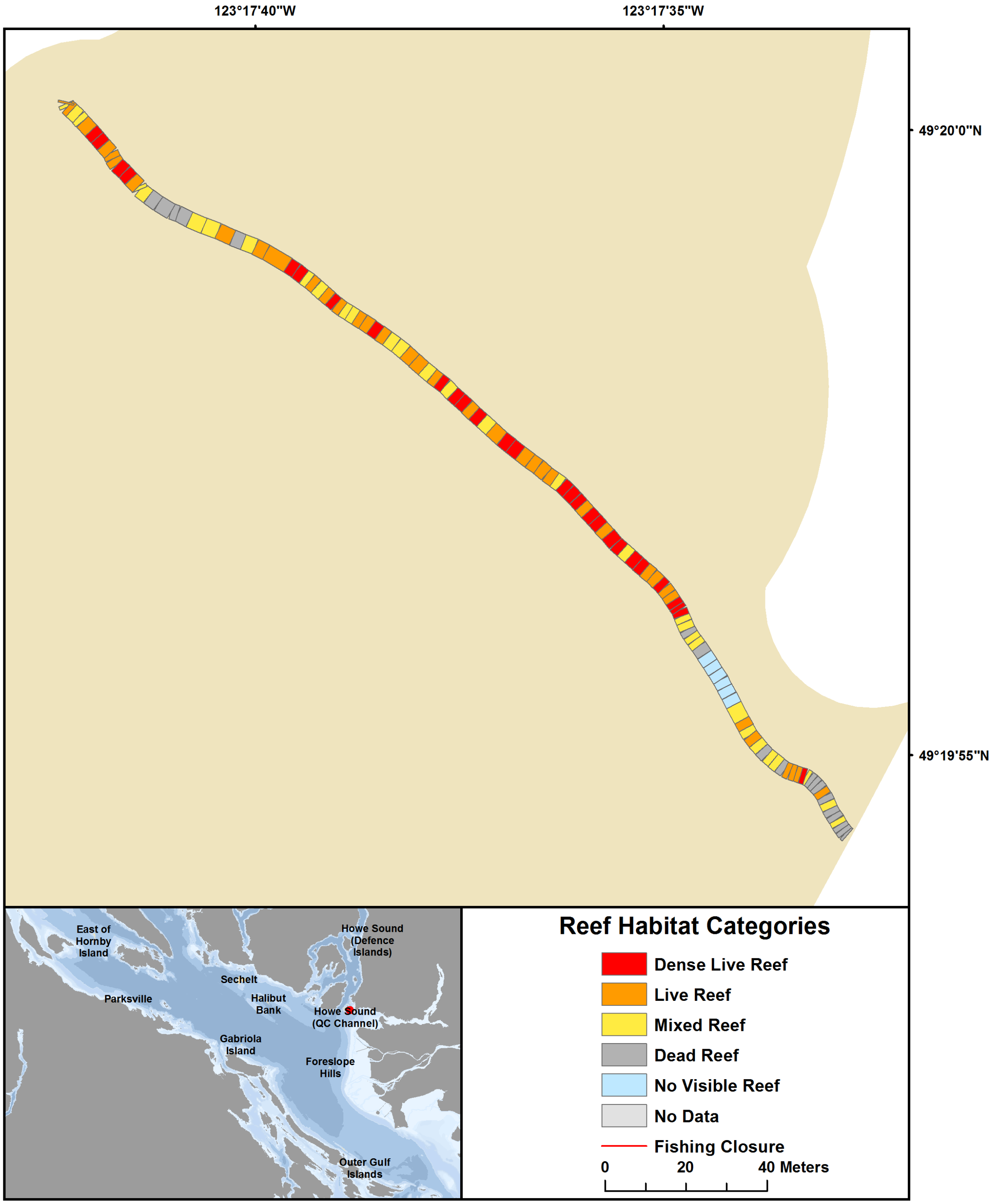
Howe Sound (QC Channel) - Pac2012-068 Transect 24



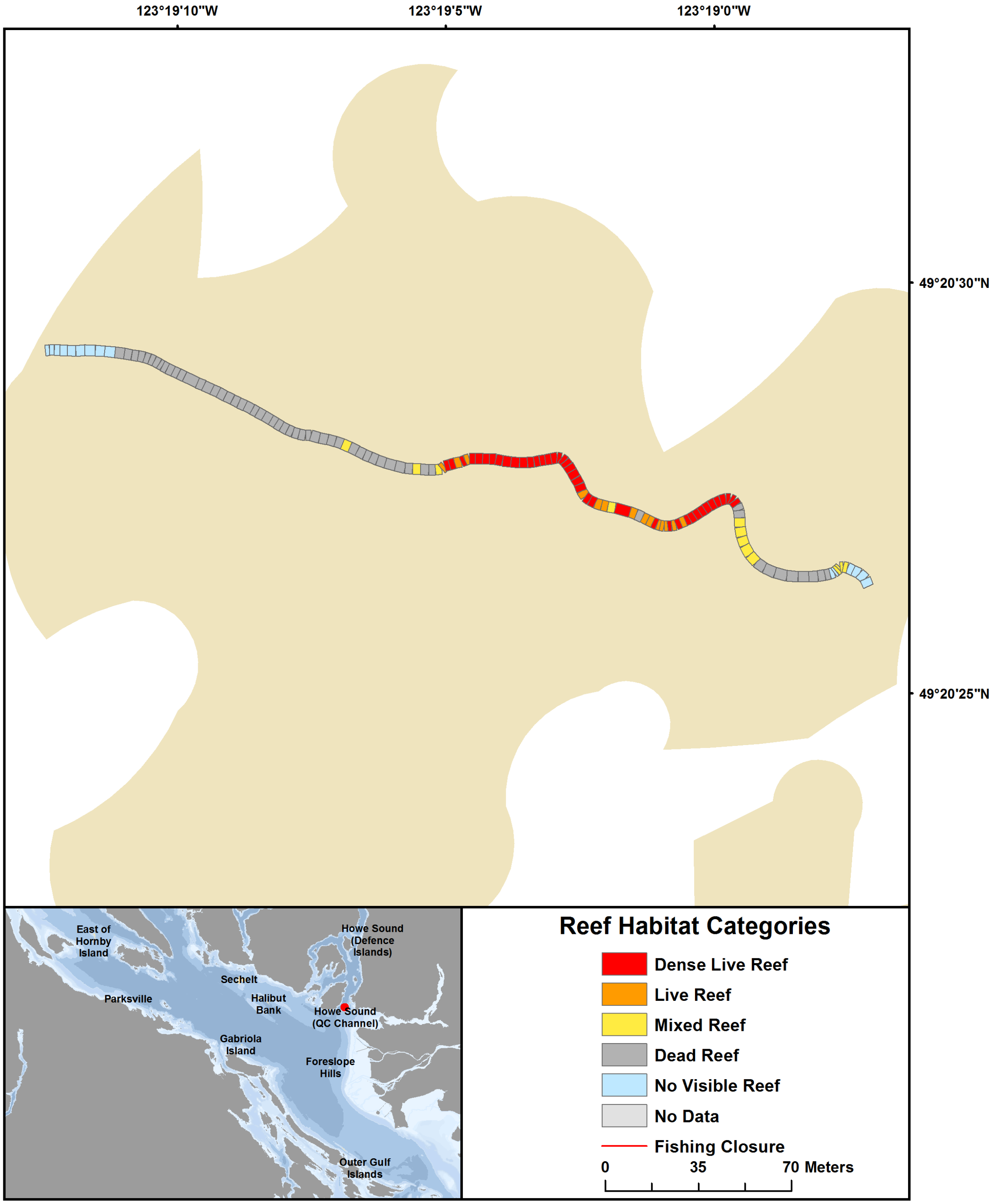
Howe Sound (QC Channel) - Pac2012-068 Transect 25



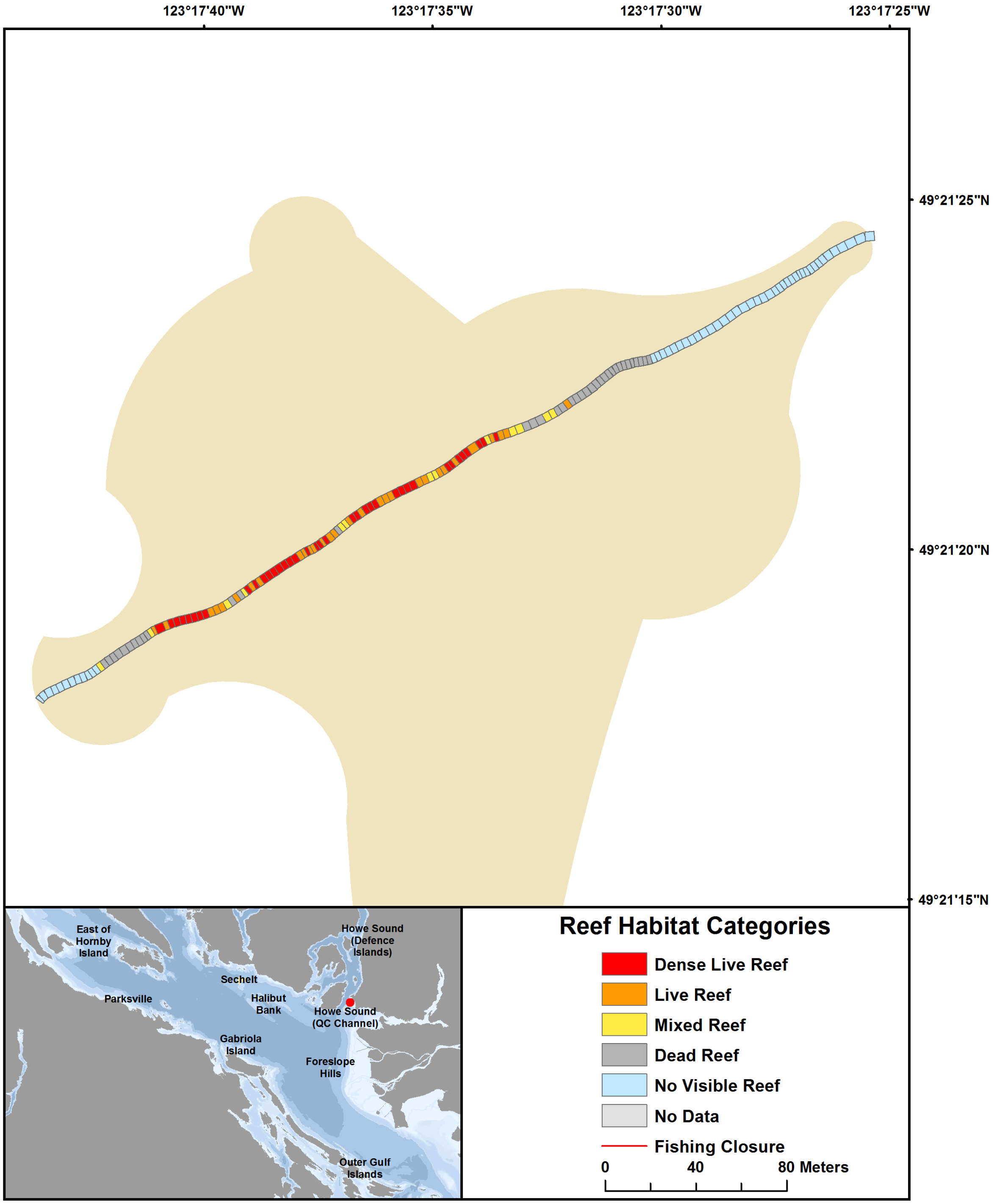
Howe Sound (QC Channel) - Pac2012-068 Transect 26



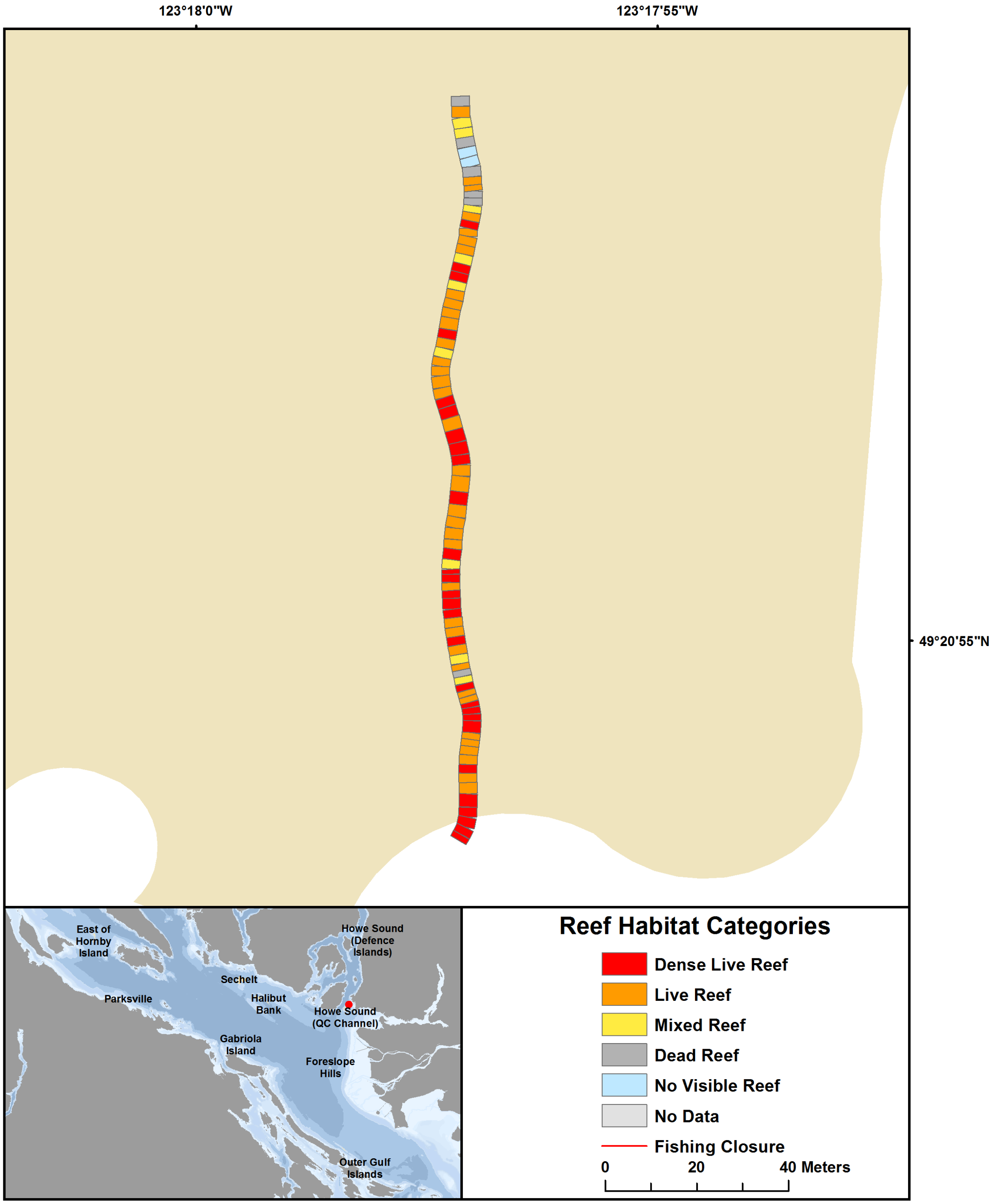
Howe Sound (QC Channel) - Pac2012-068 Transect 27



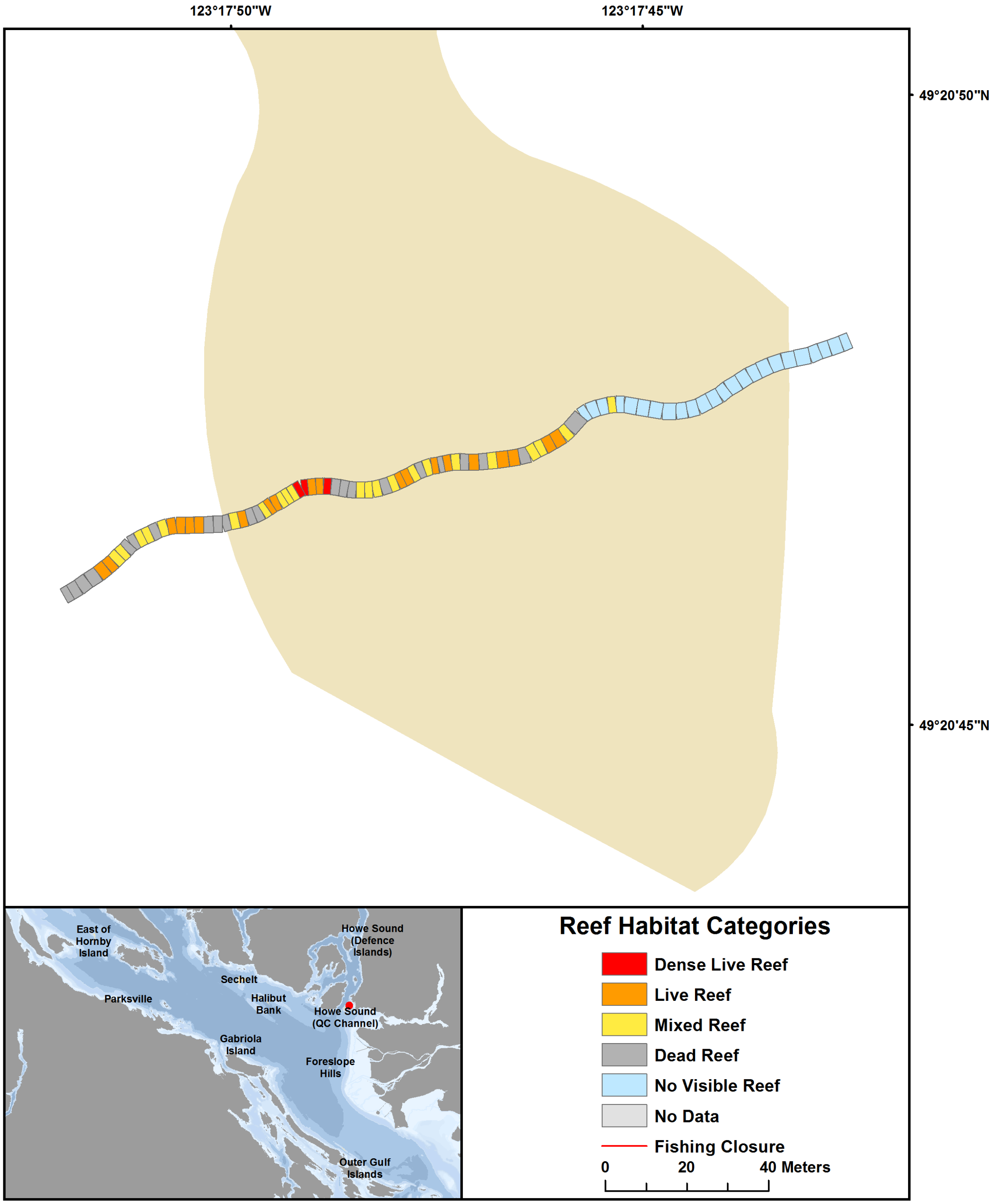
Howe Sound (QC Channel) - Pac2012-068 Transect 28



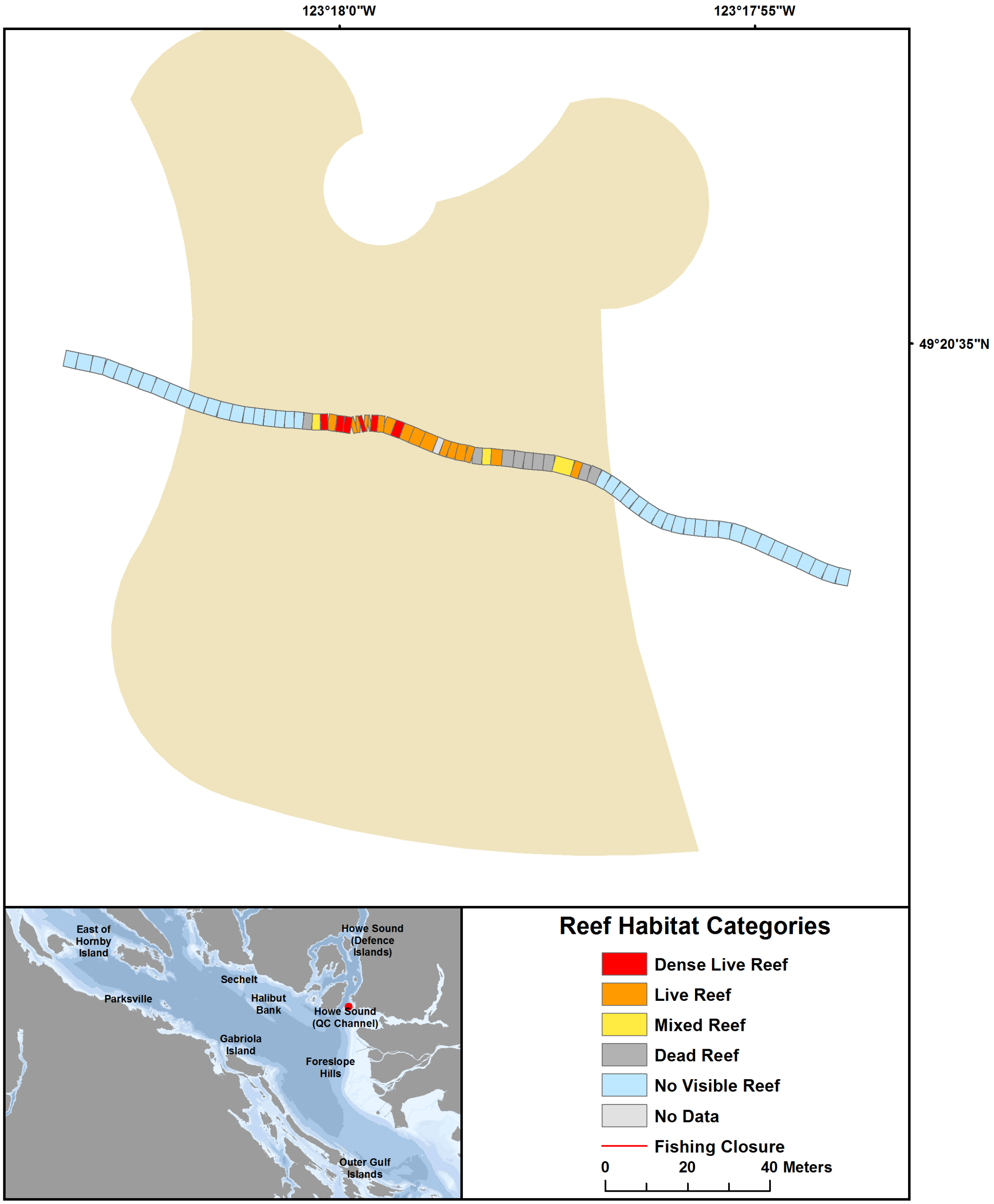
Howe Sound (QC Channel) - Pac2012-068 Transect 29



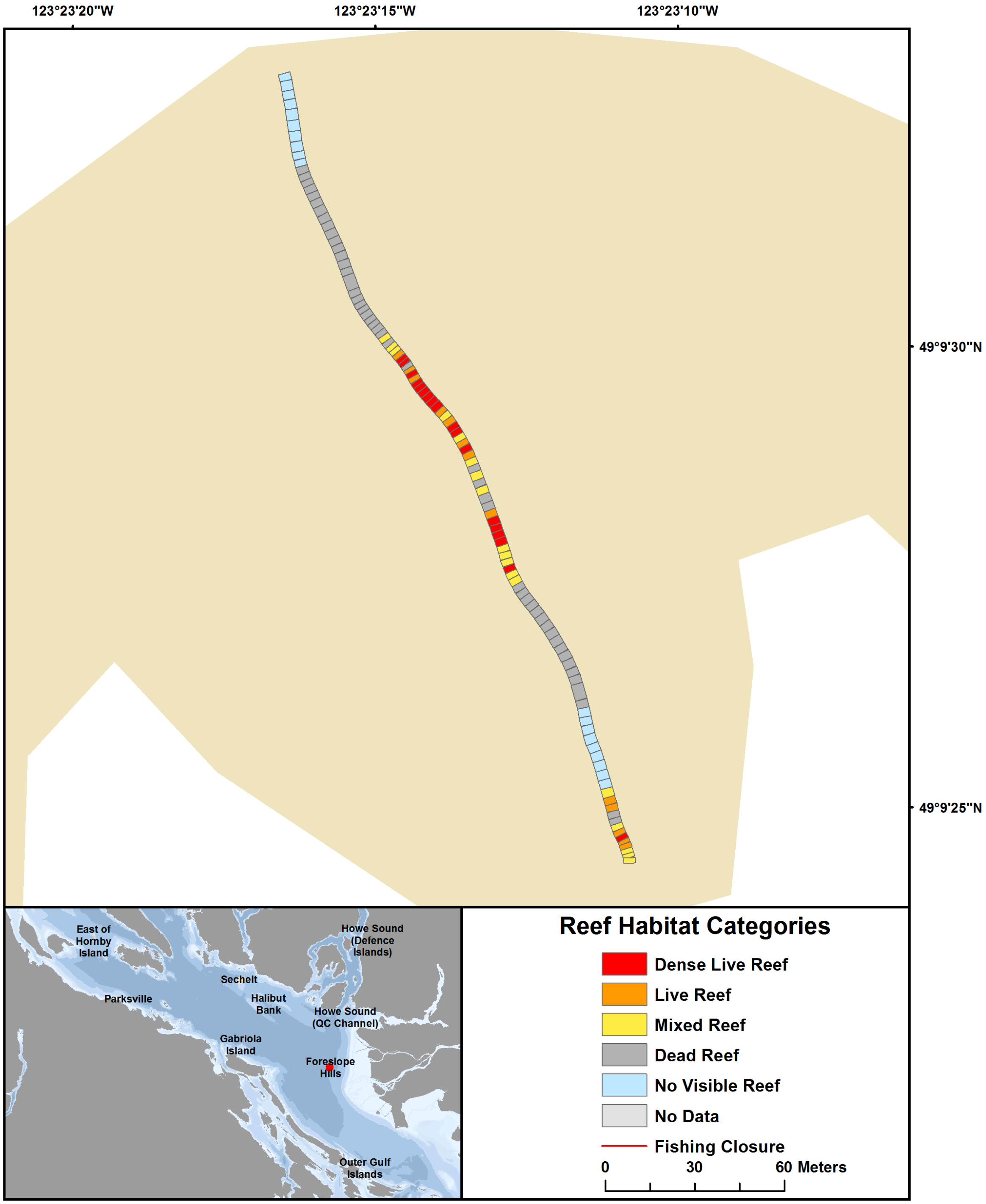
Howe Sound (QC Channel) - Pac2012-068 Transect 30



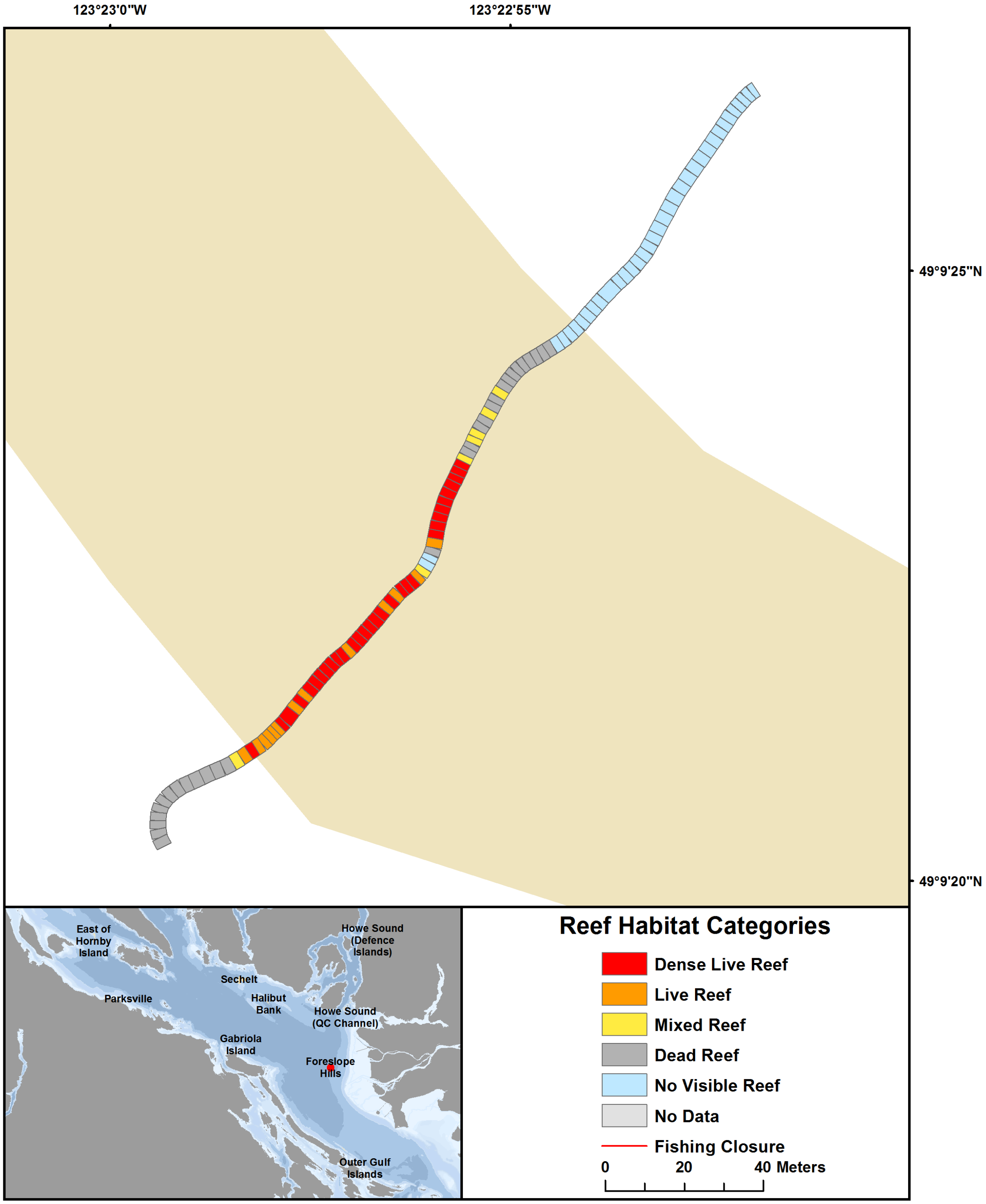
Howe Sound (QC Channel) - Pac2012-068 Transect 31



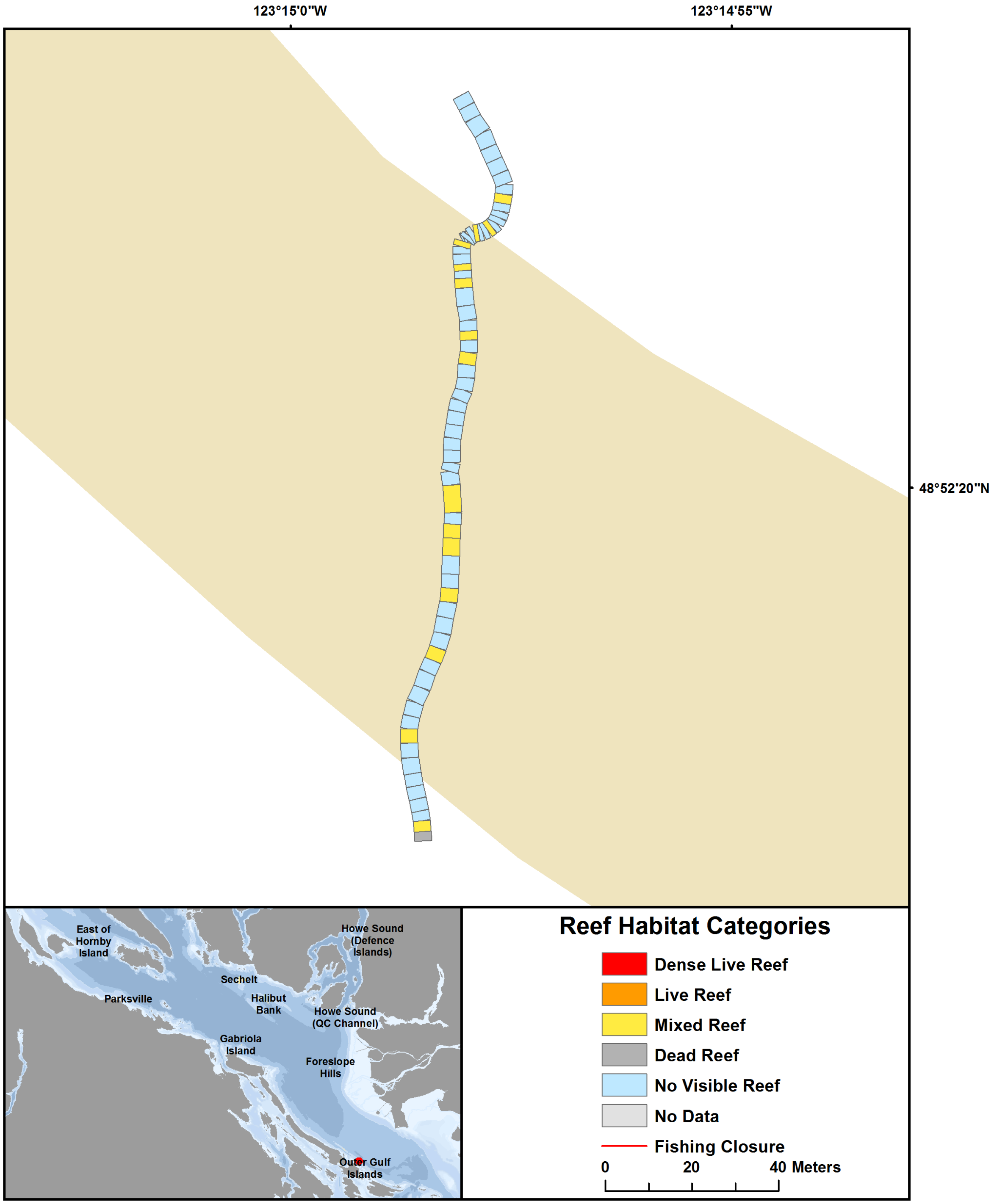
Foreslope Hills - Pac2012-068 Transect 37



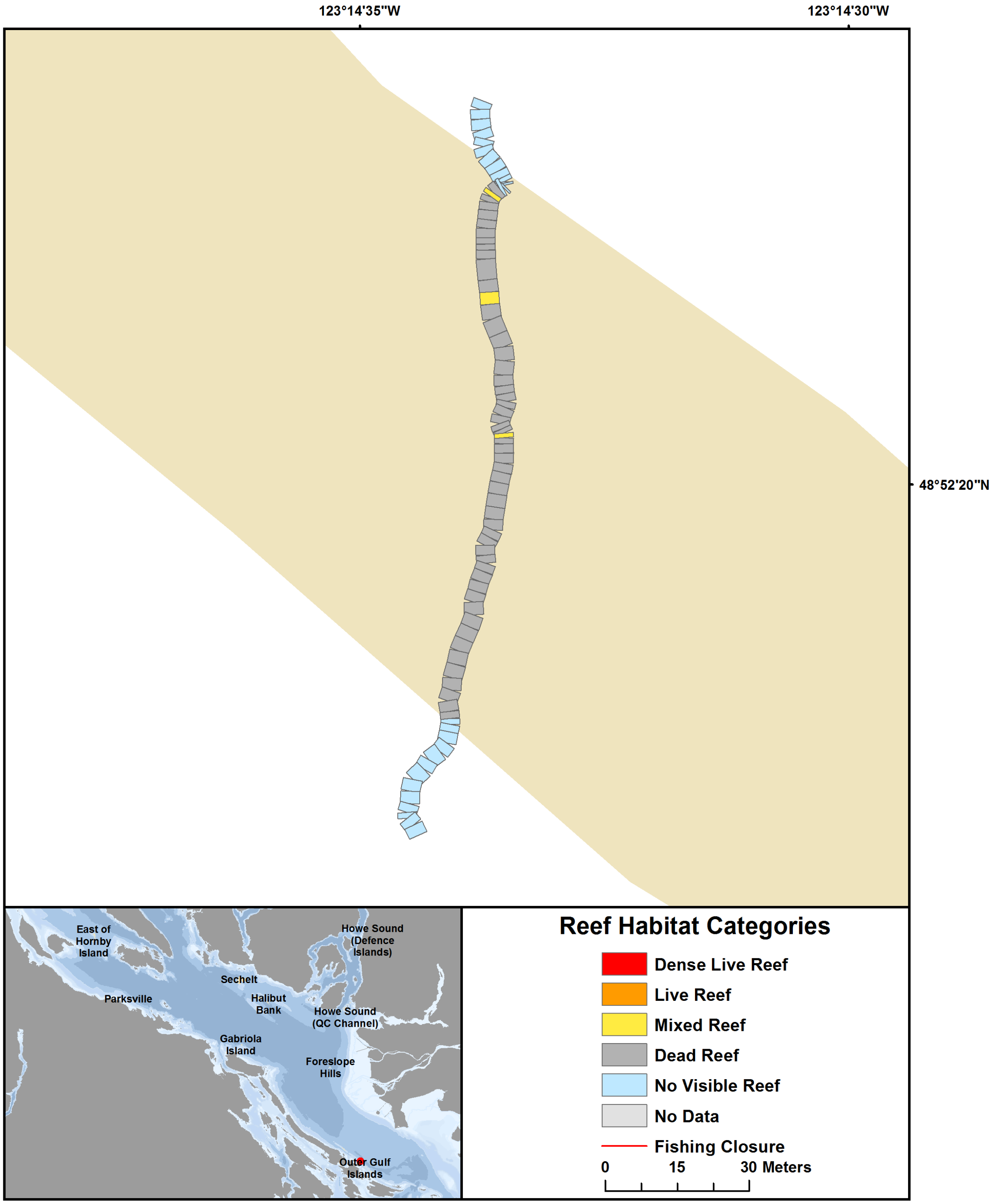
Foreslope Hills - Pac2012-068 Transect 38



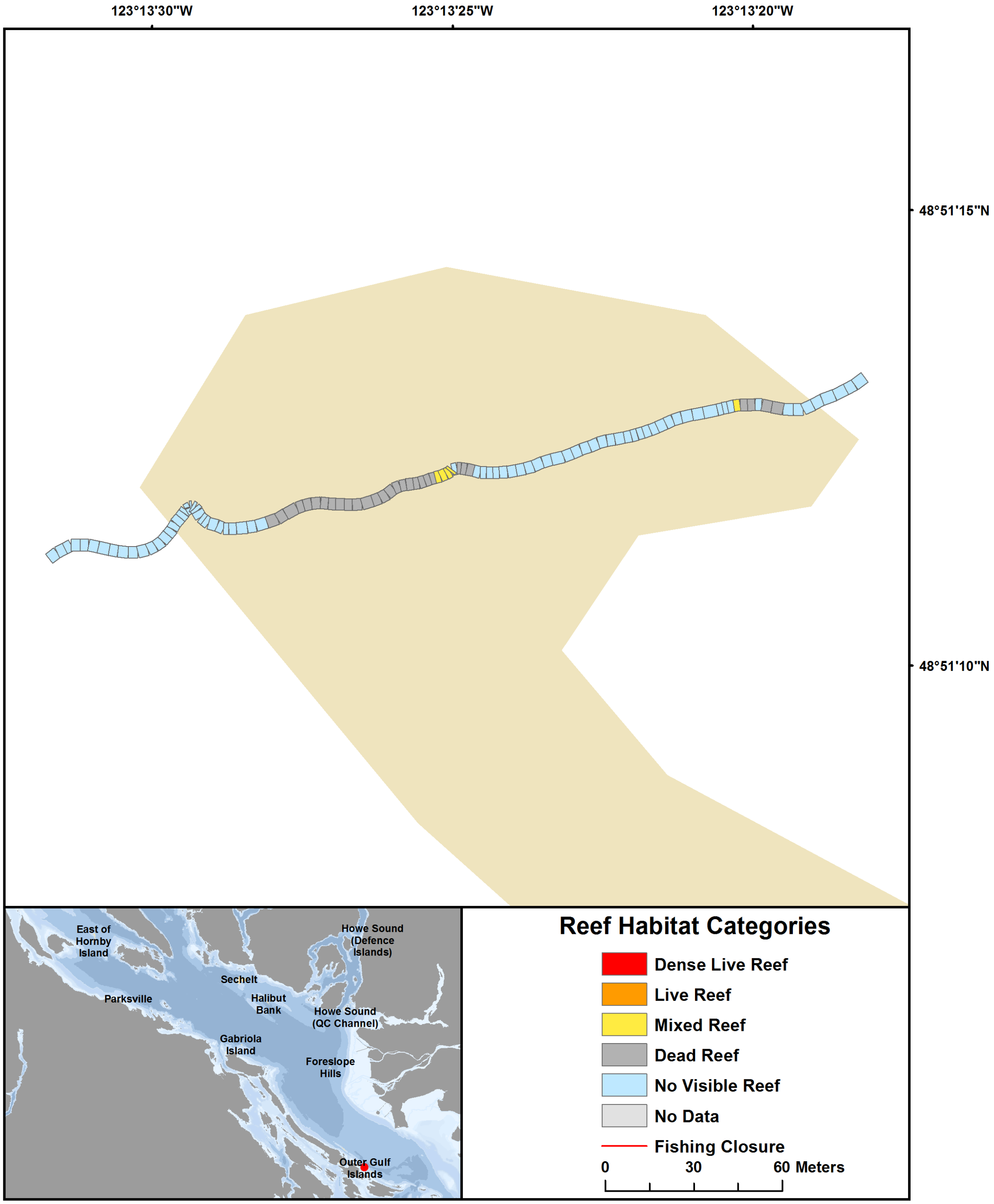
Outer Gulf Islands - Pac2012-068 Transect 32



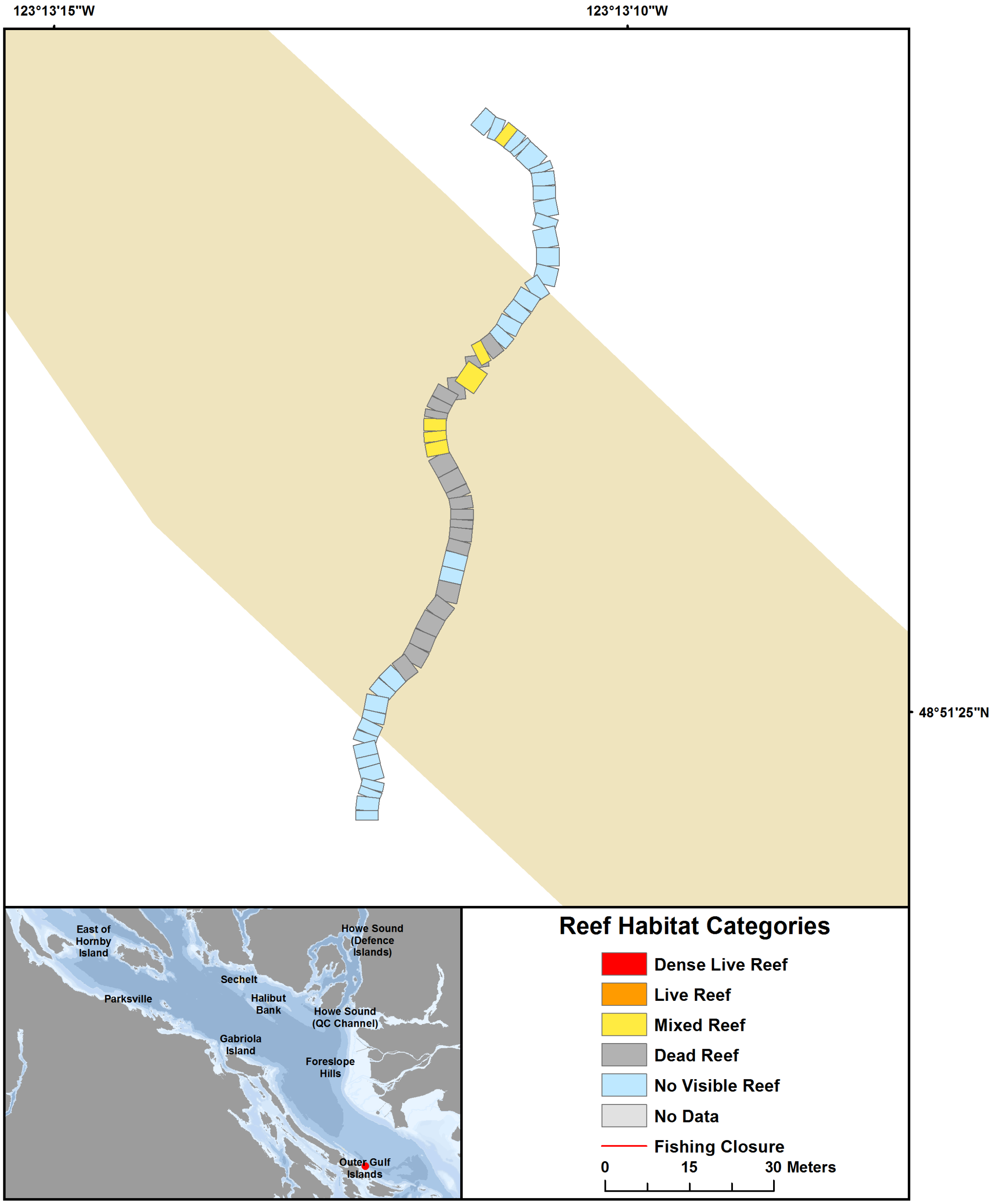
Outer Gulf Islands - Pac2012-068 Transect 33



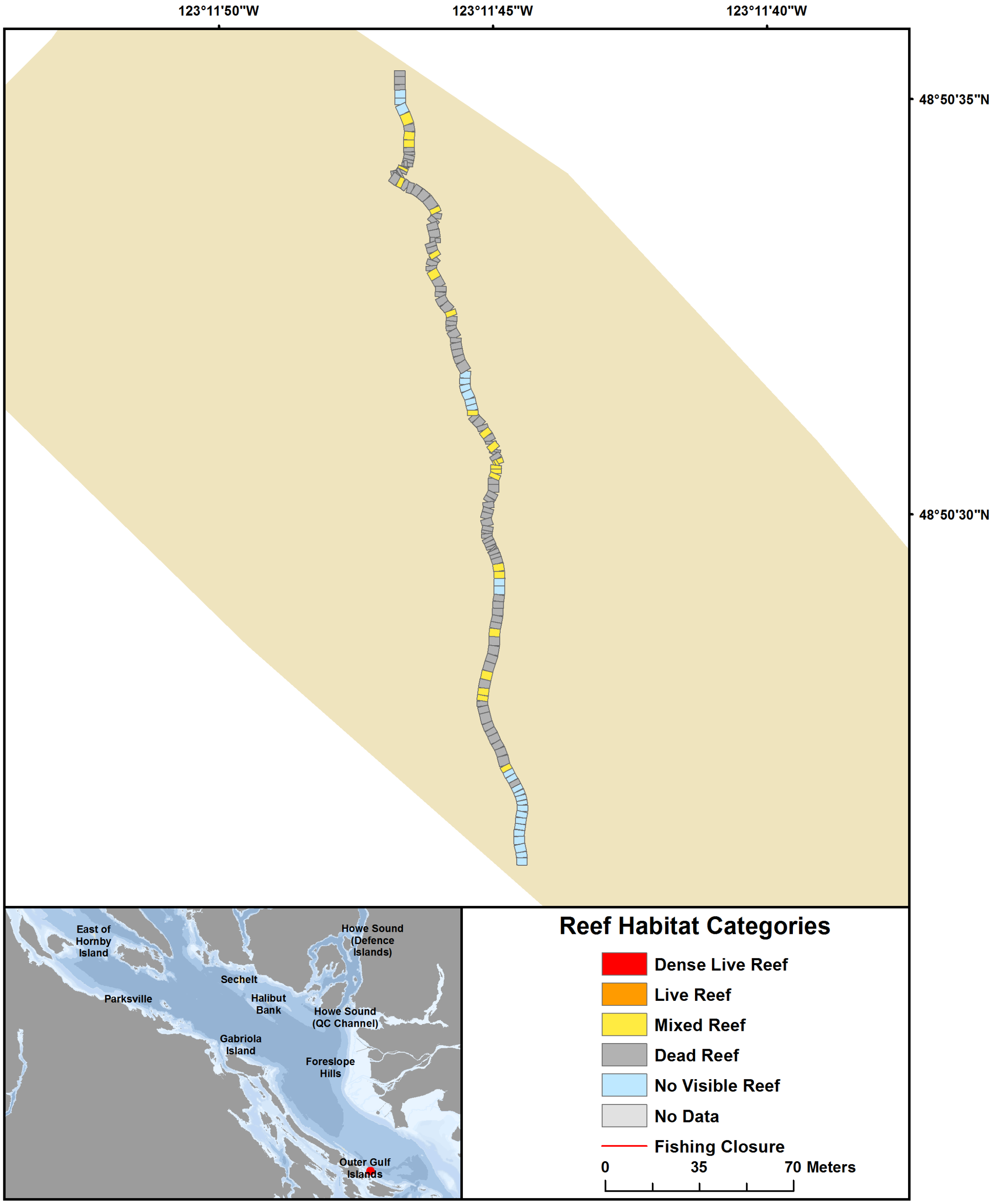
Outer Gulf Islands - Pac2012-068 Transect 34



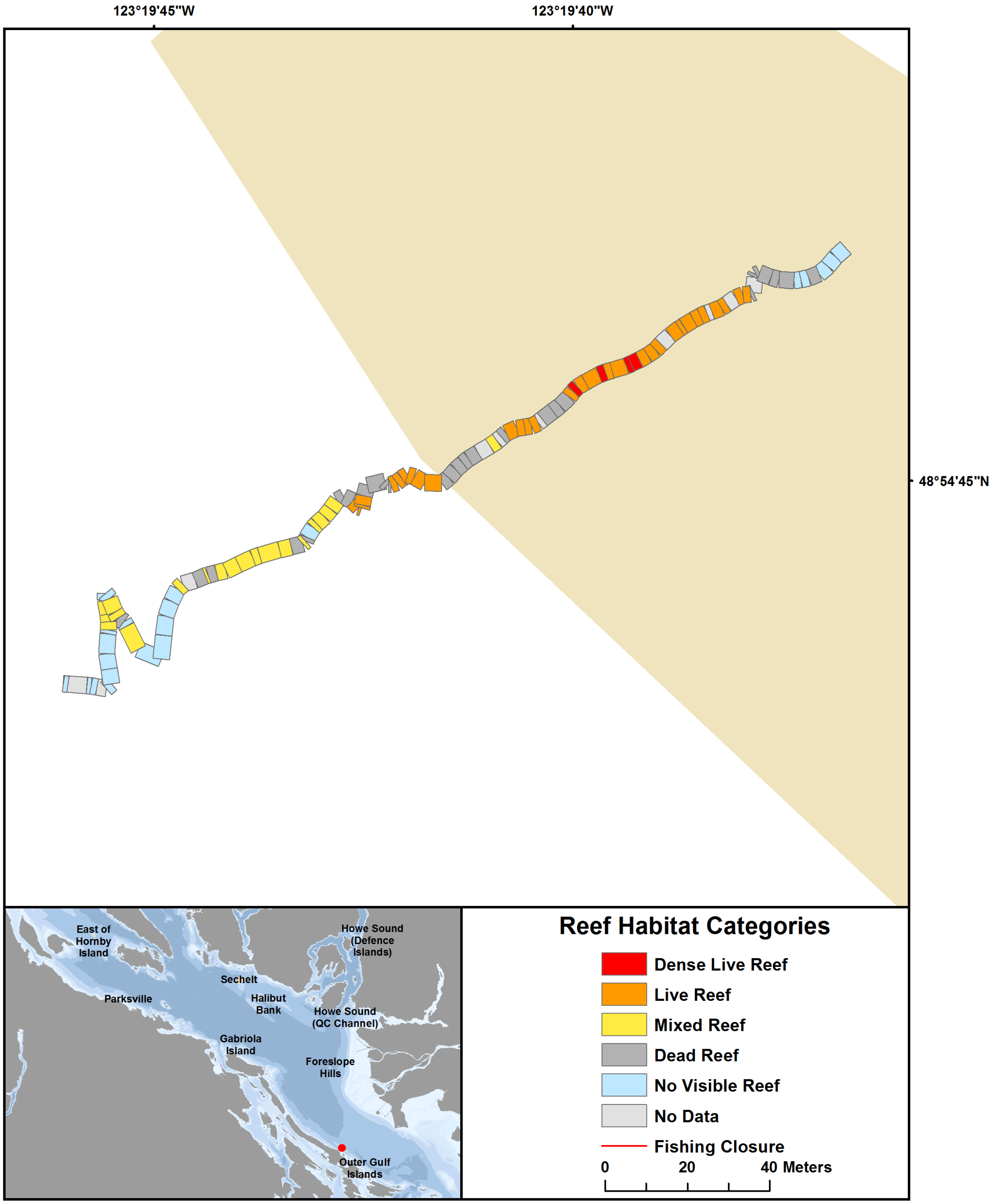
Outer Gulf Islands - Pac2012-068 Transect 35



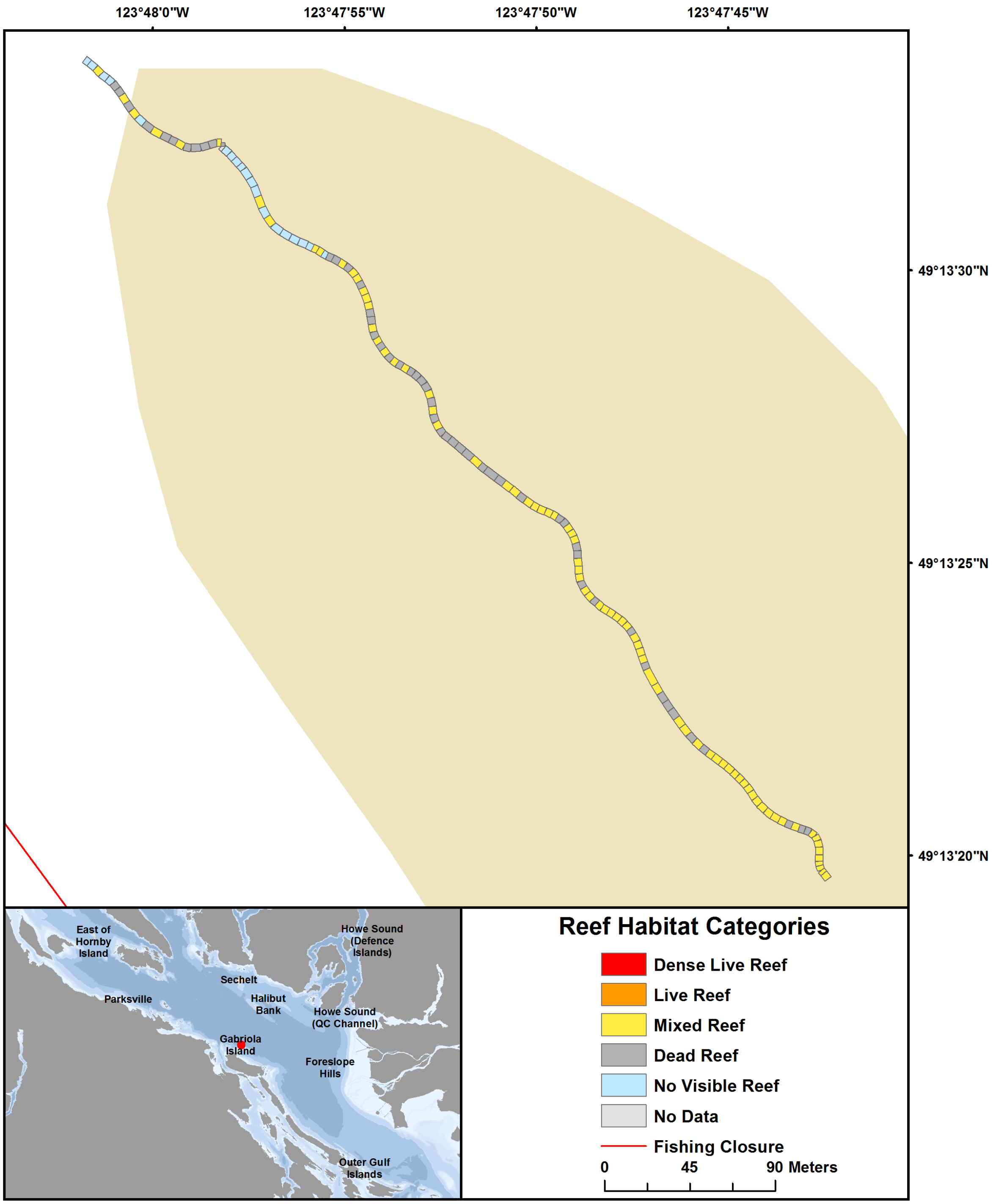
Outer Gulf Islands - Pac2012-068 Transect 36



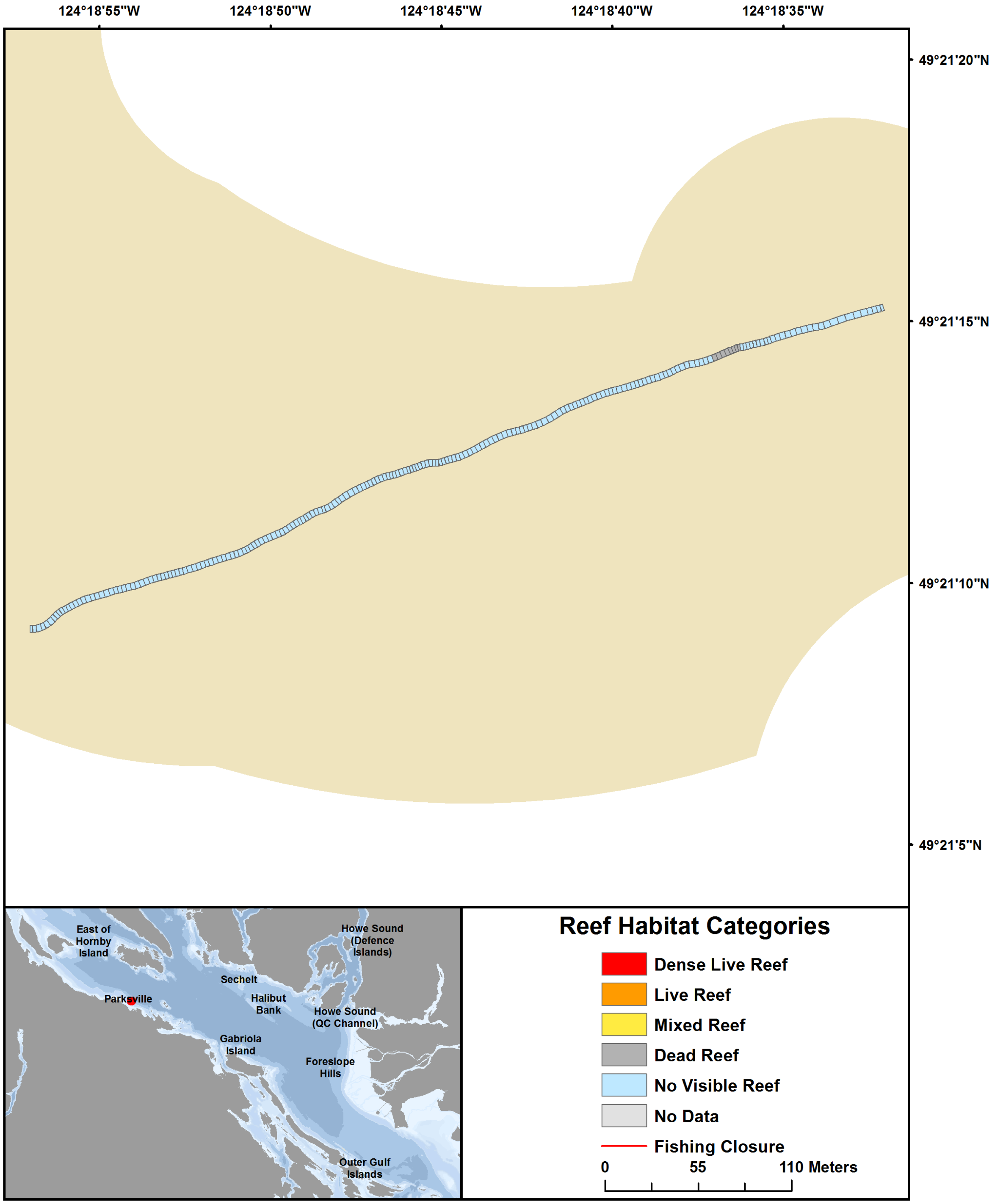
Outer Gulf Islands - Pac2012-068 Transect 39



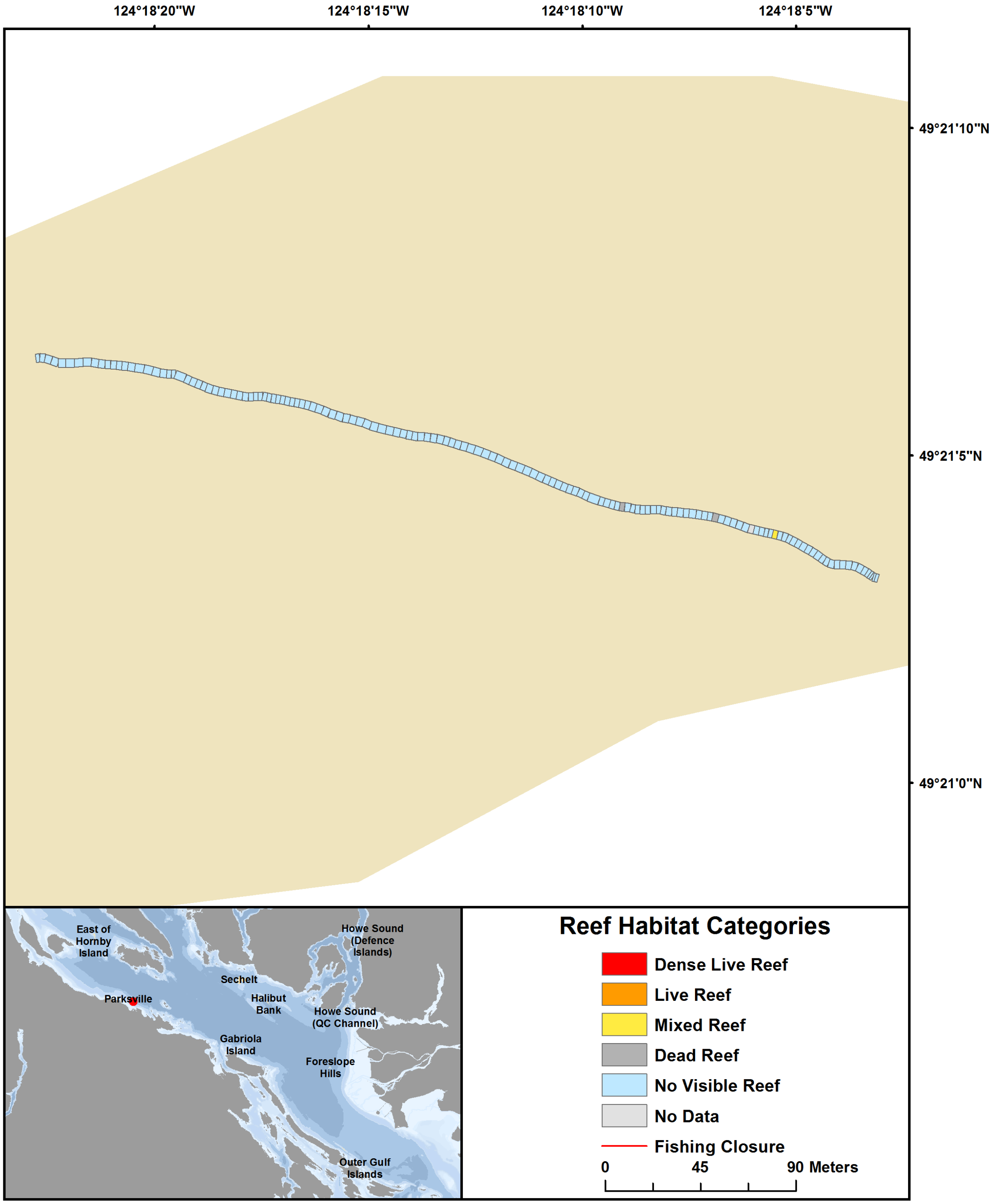
Gabriola Island - Pac2012-068 Transect 1



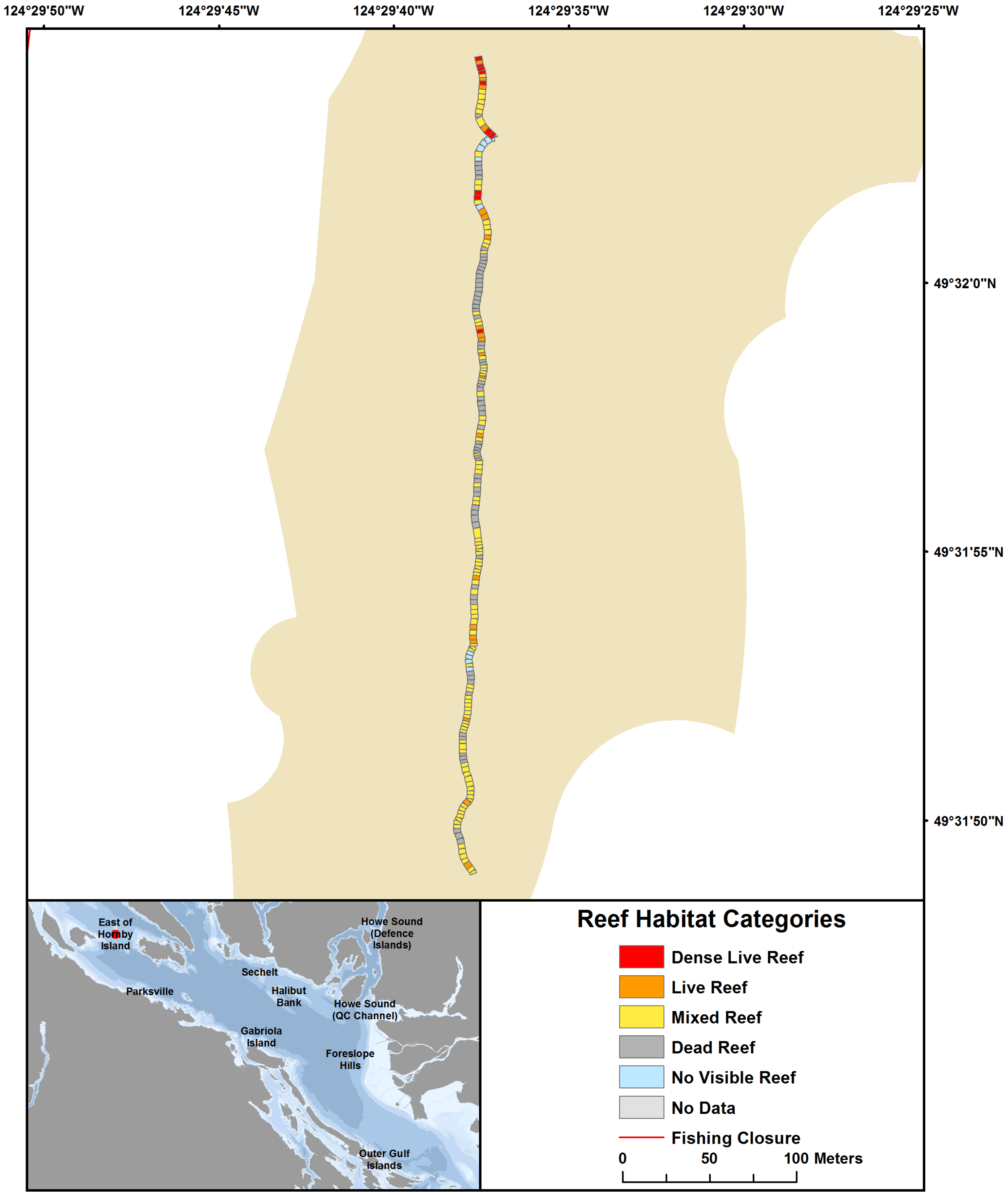
Parksville - Pac2012-068 Transect 2



Parksville - Pac2012-068 Transect 3



East of Hornby Island - Pac2012-068 Transect 4



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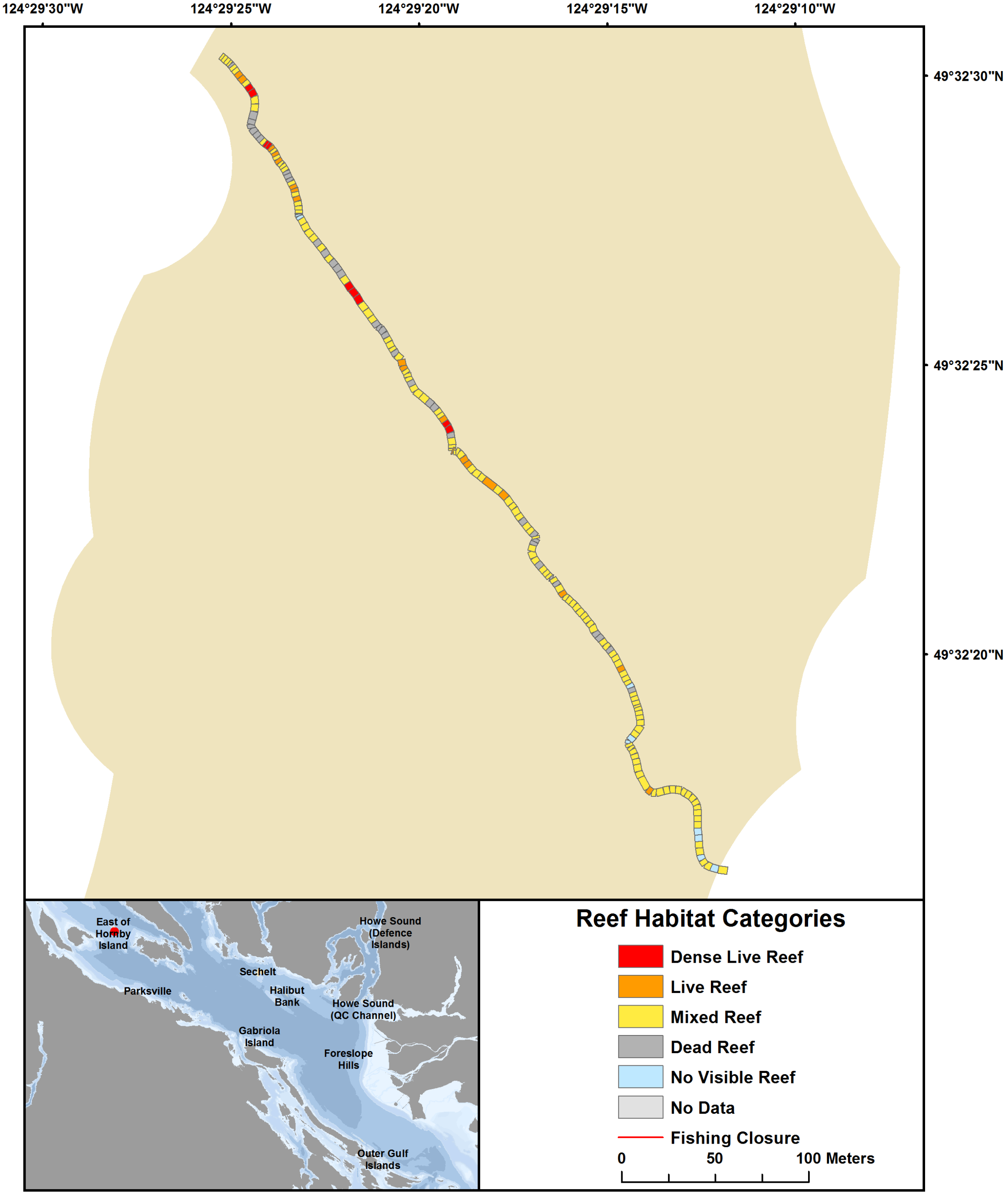

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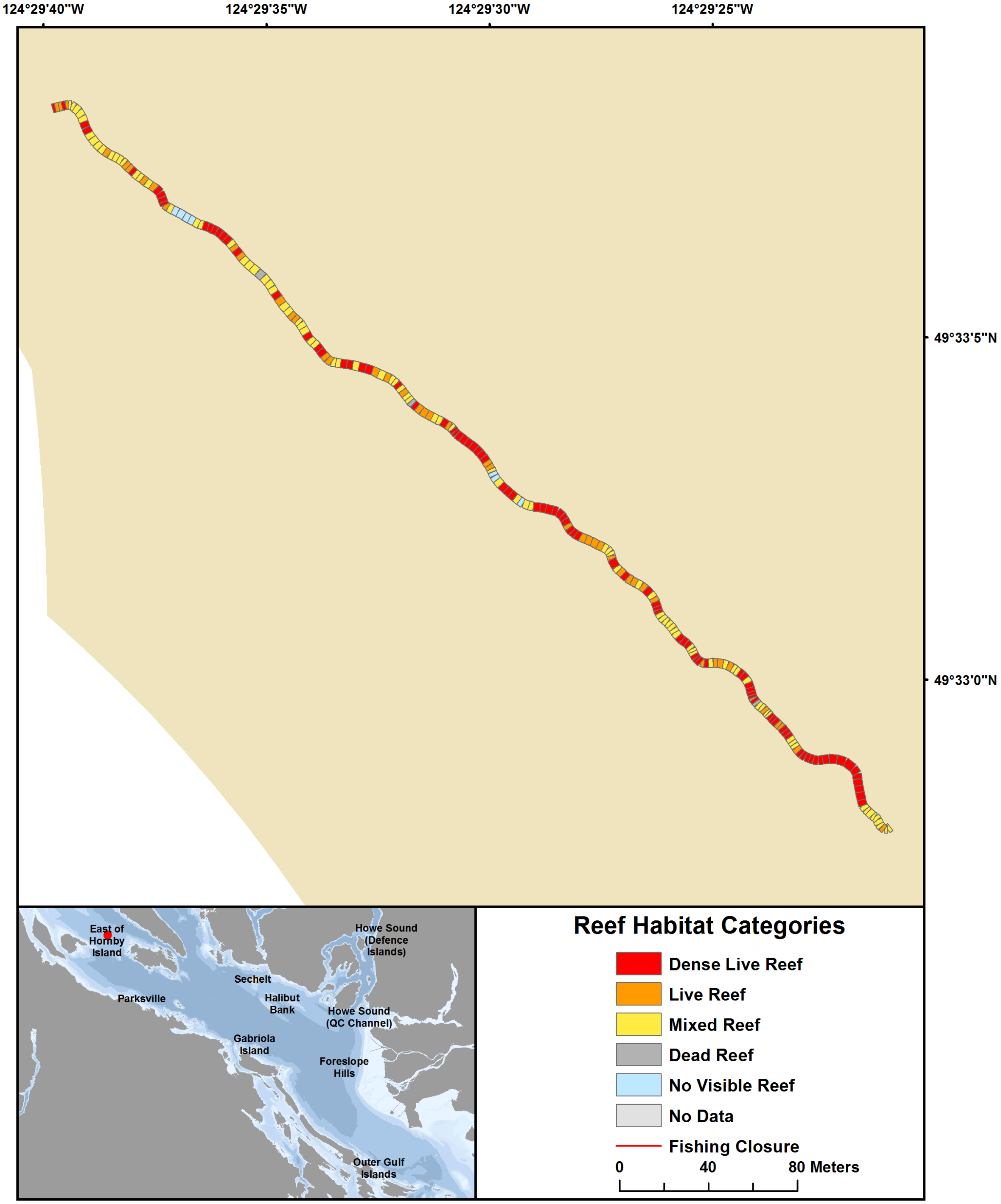
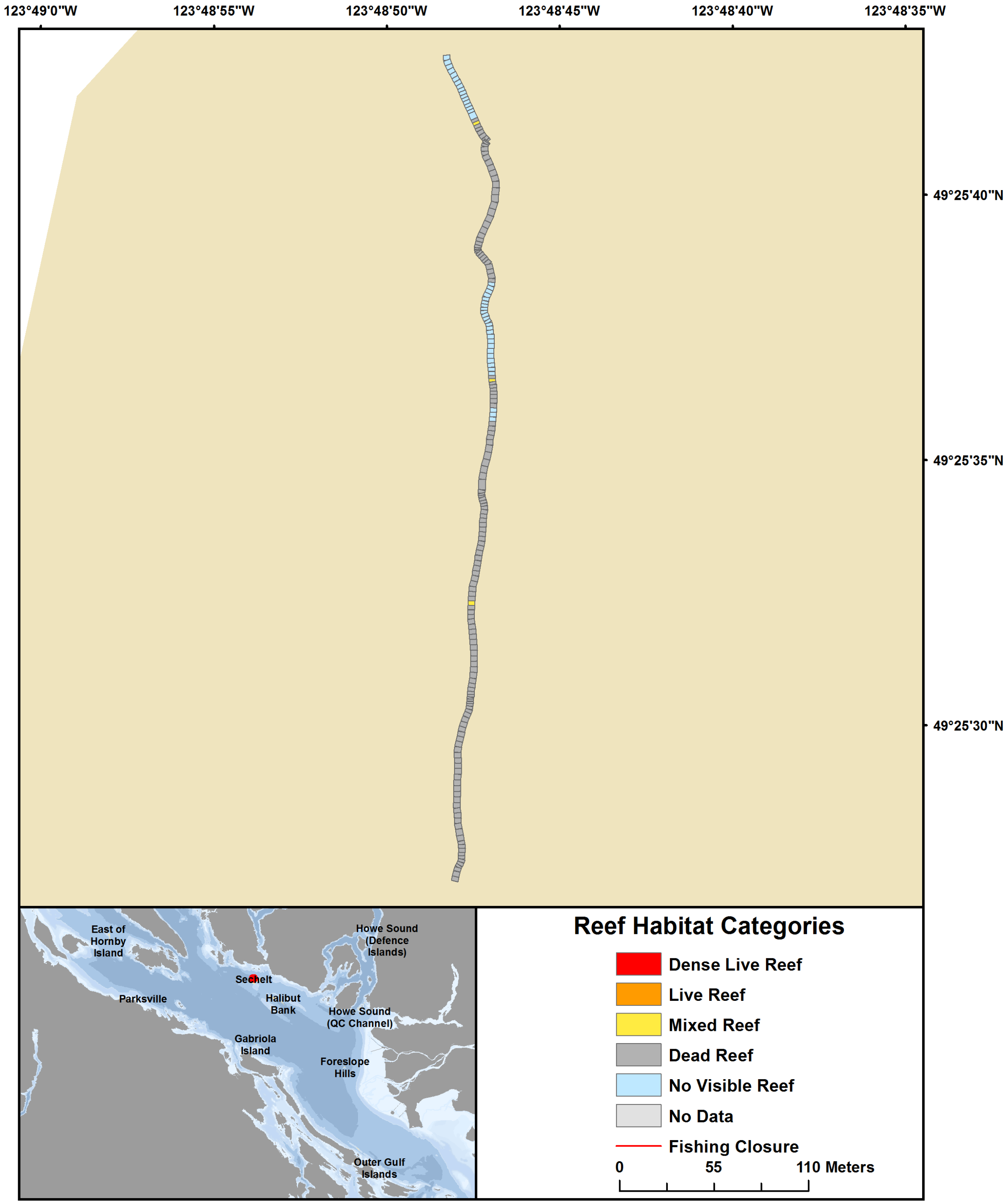


Sechelt - Pac2012-068 Transect 7



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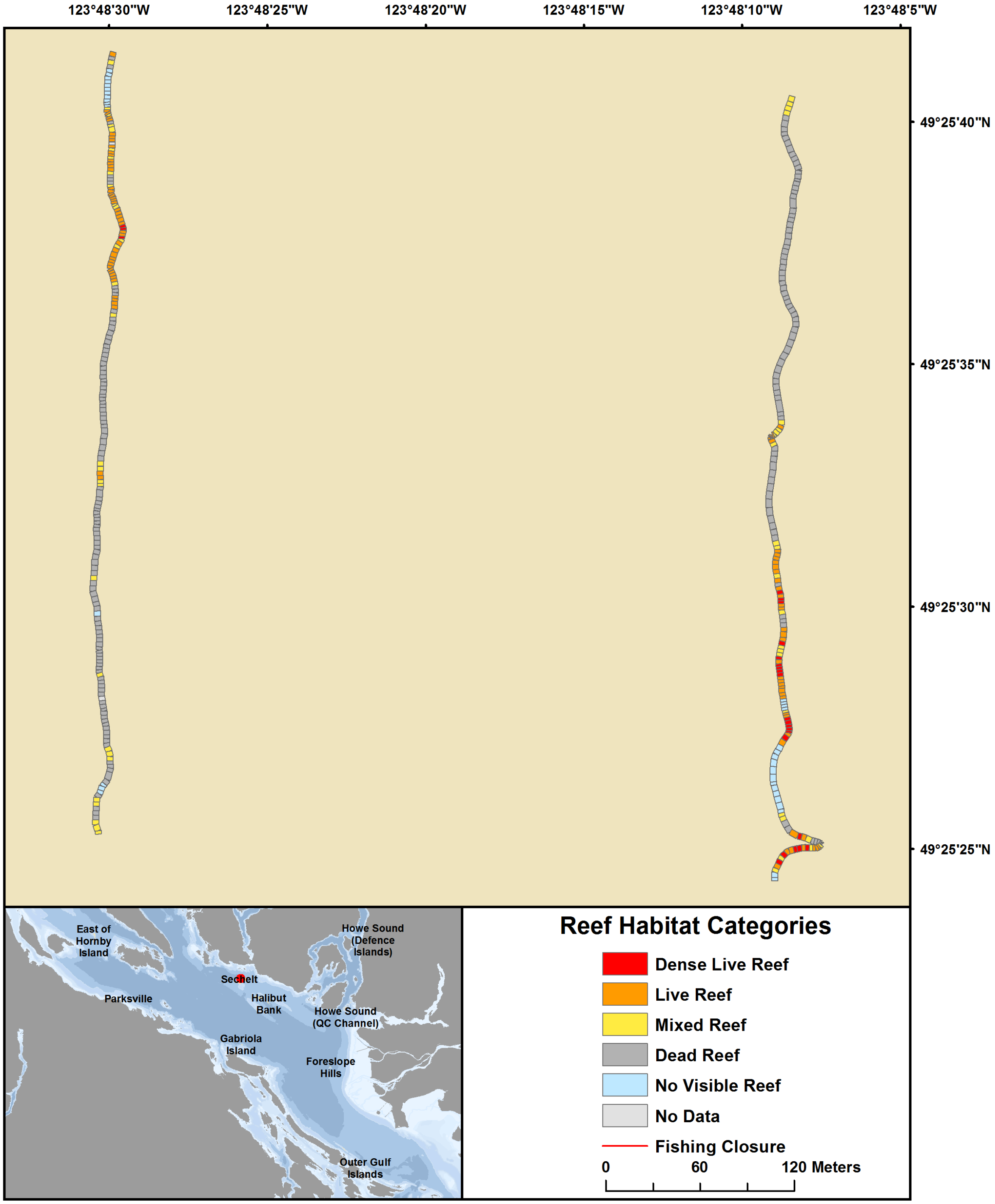

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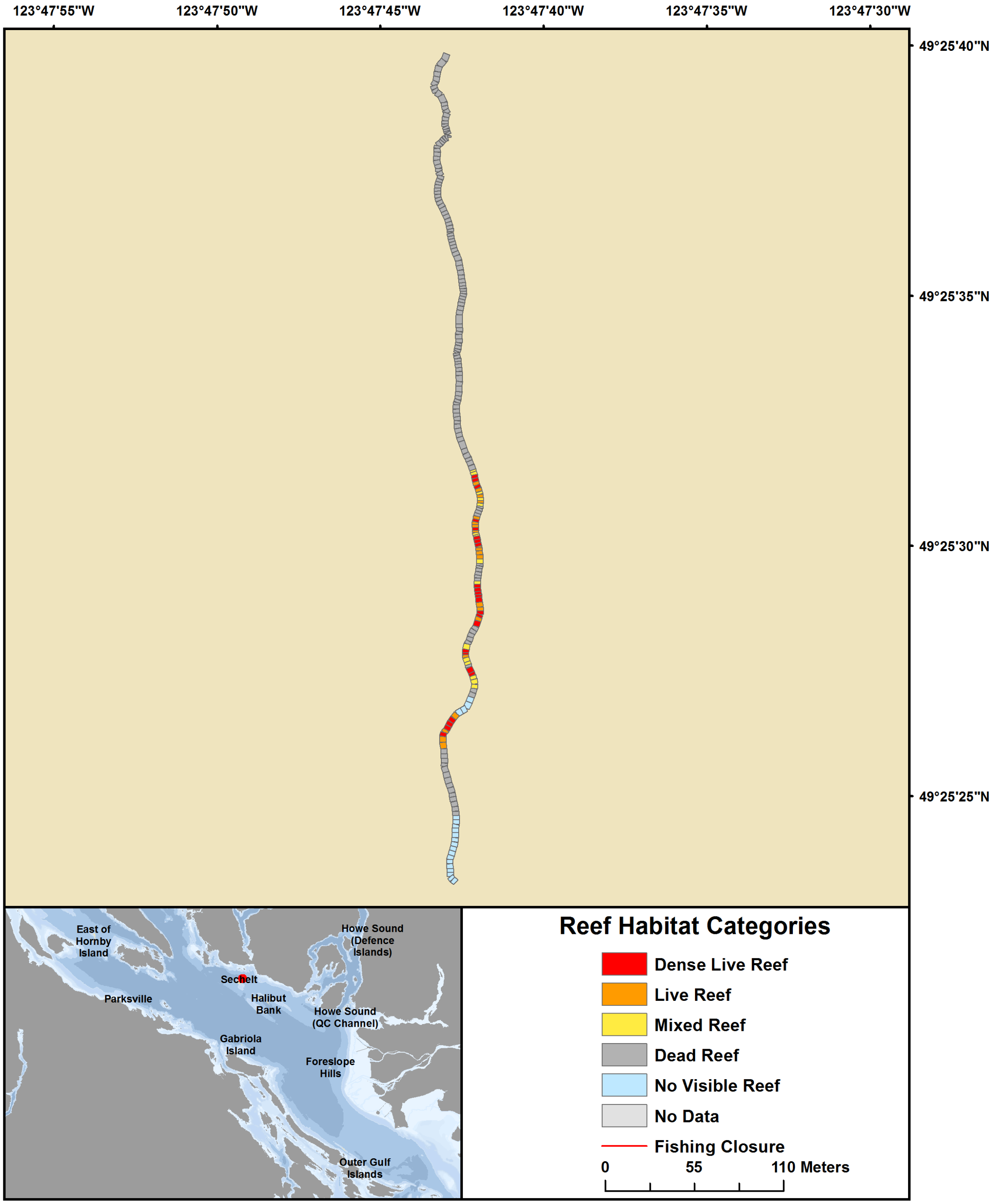

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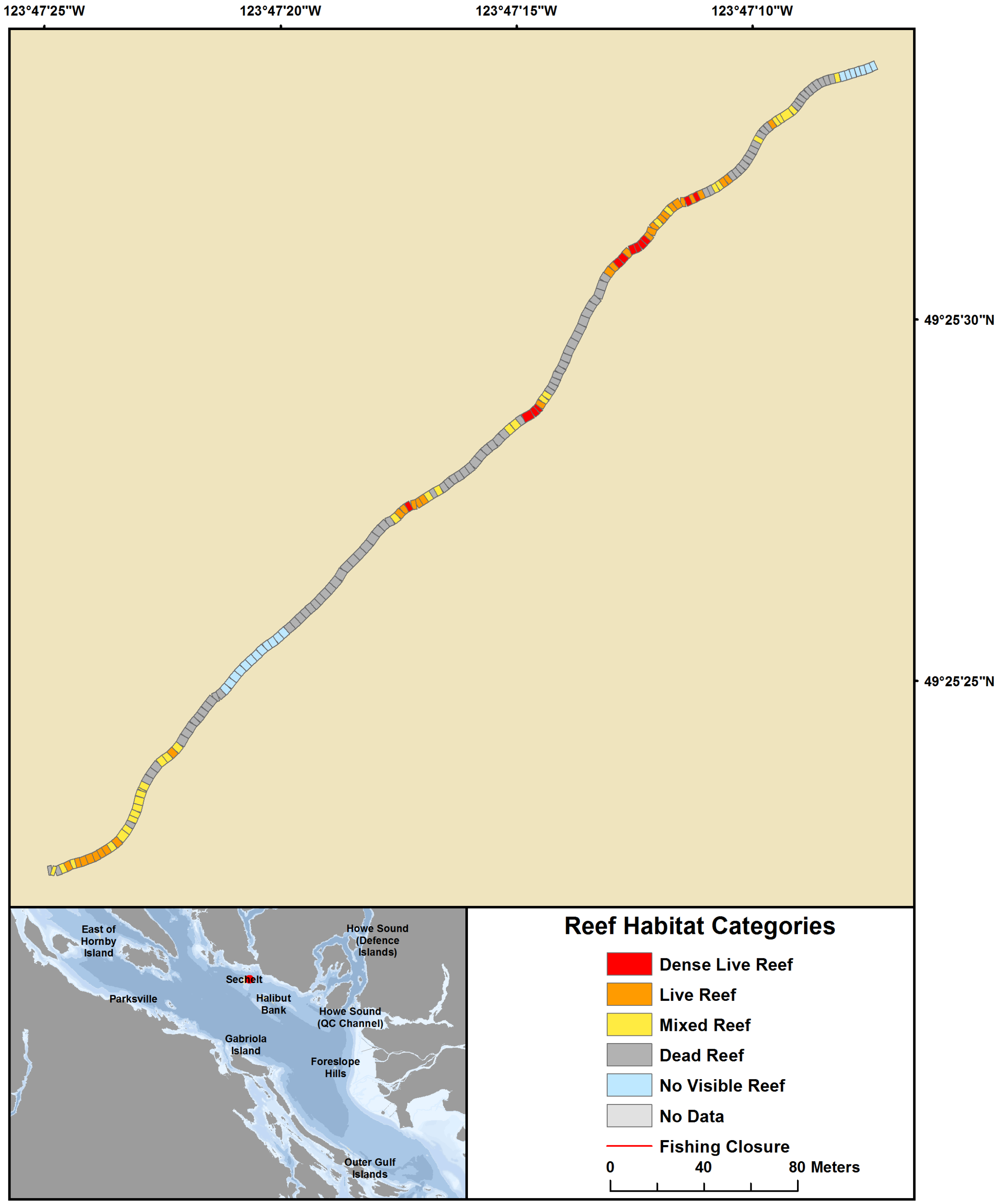

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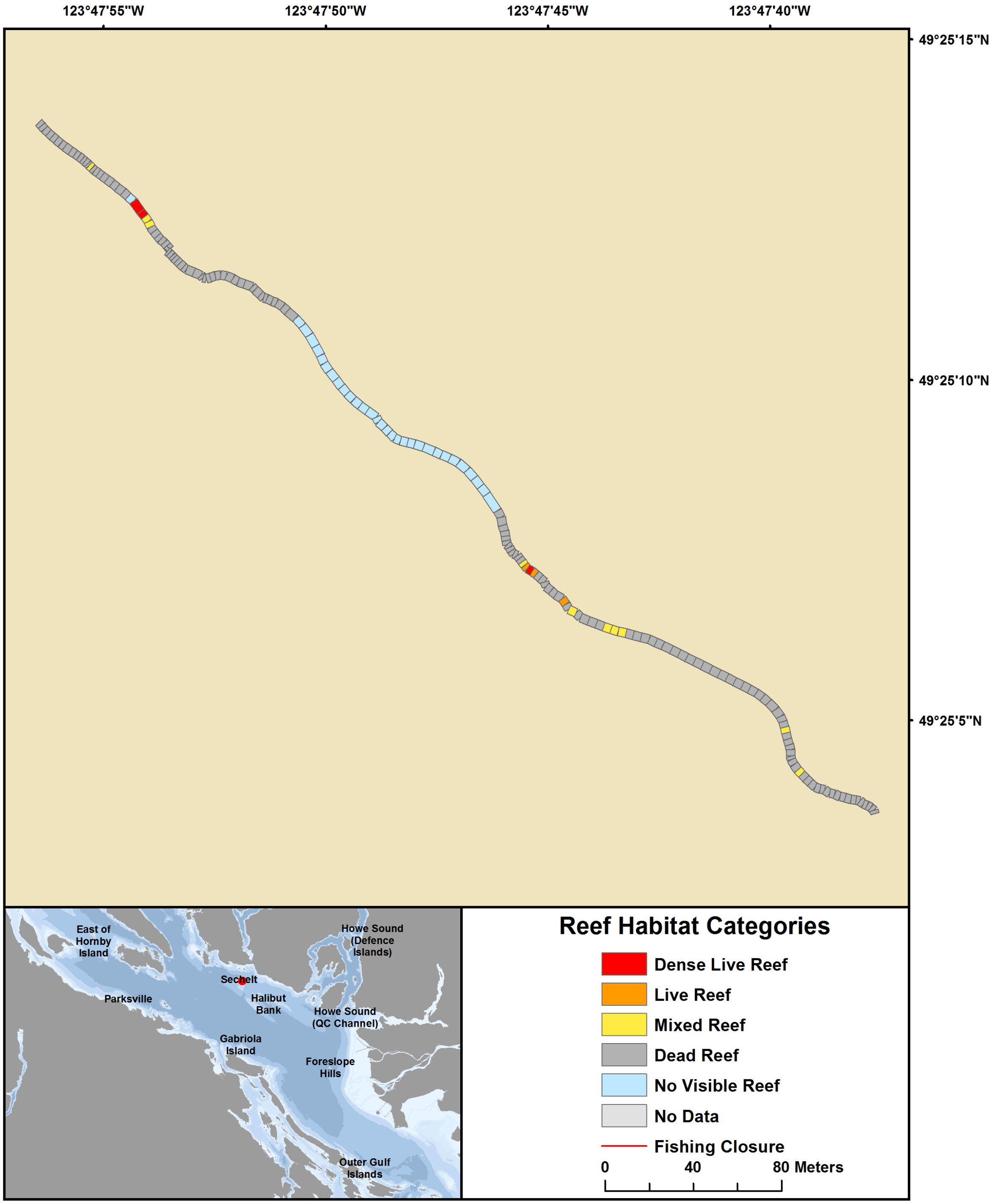

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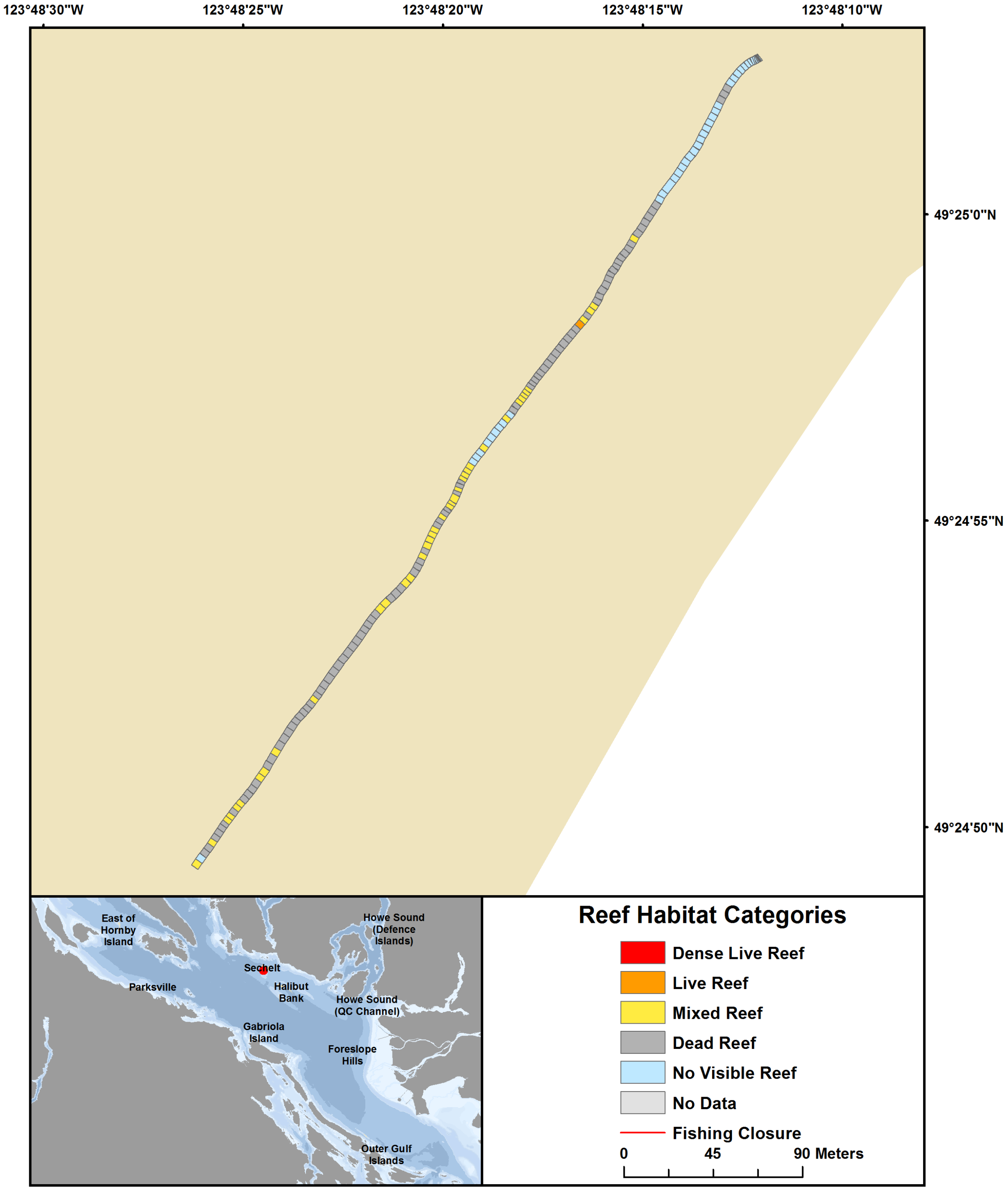

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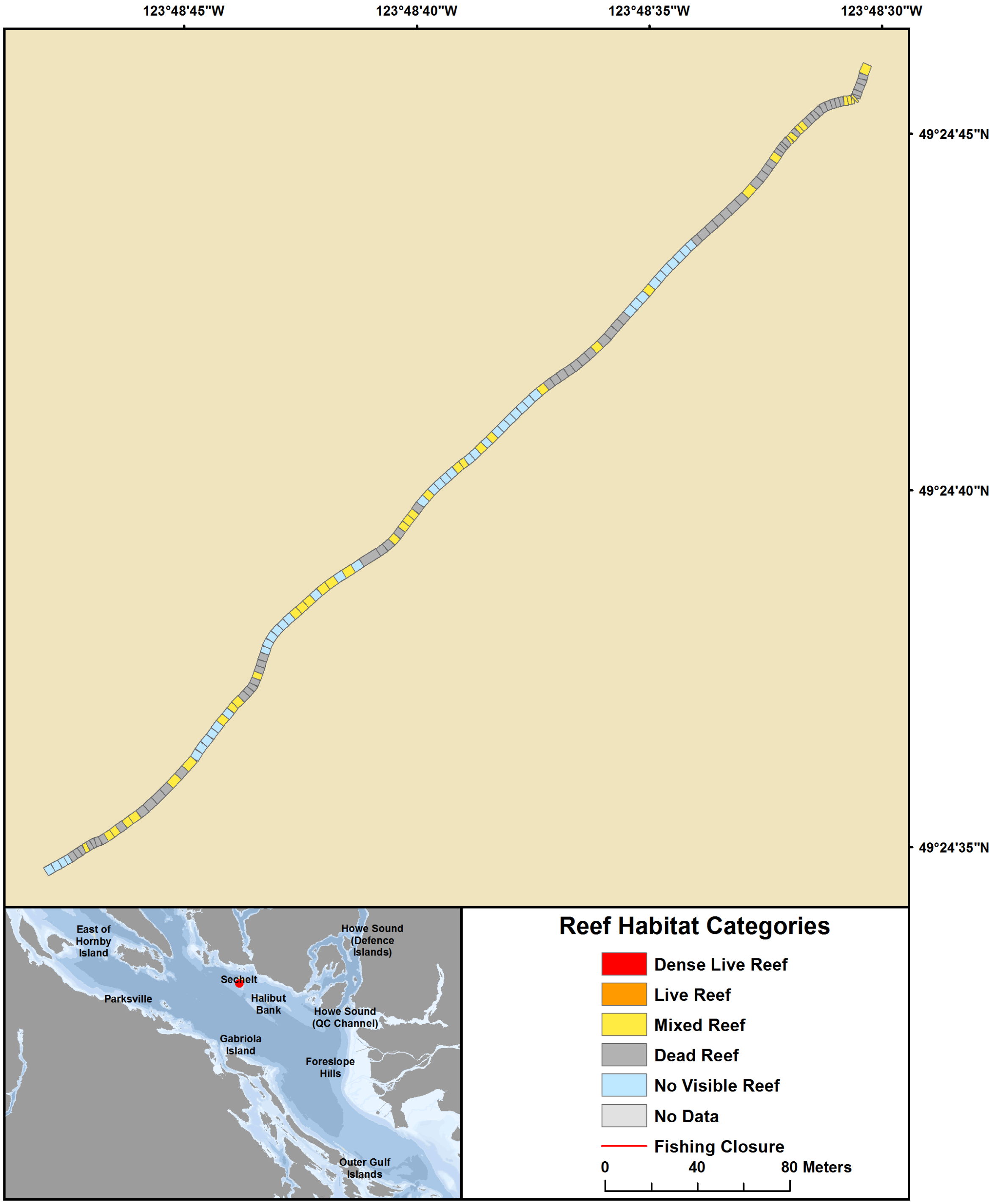

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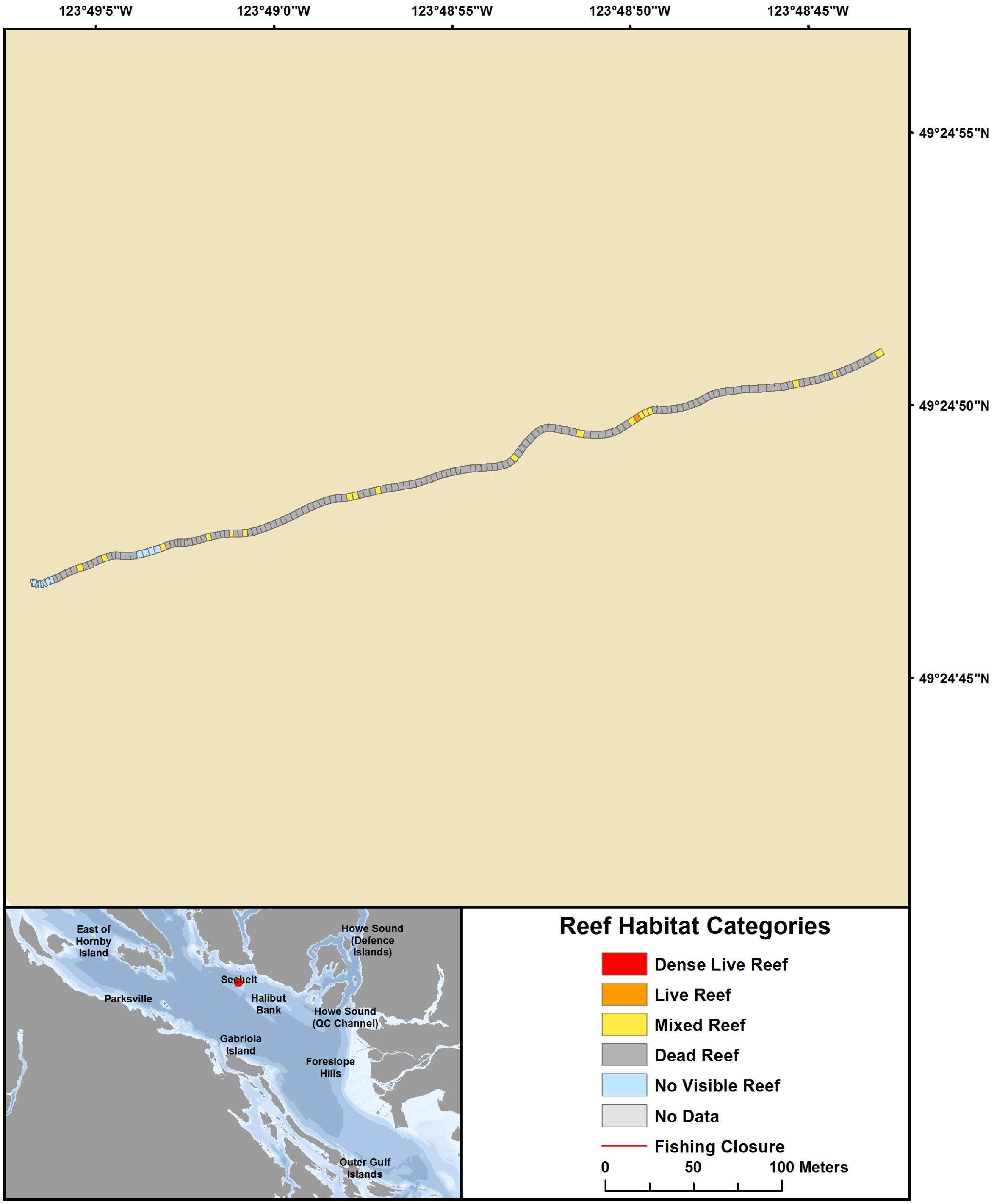

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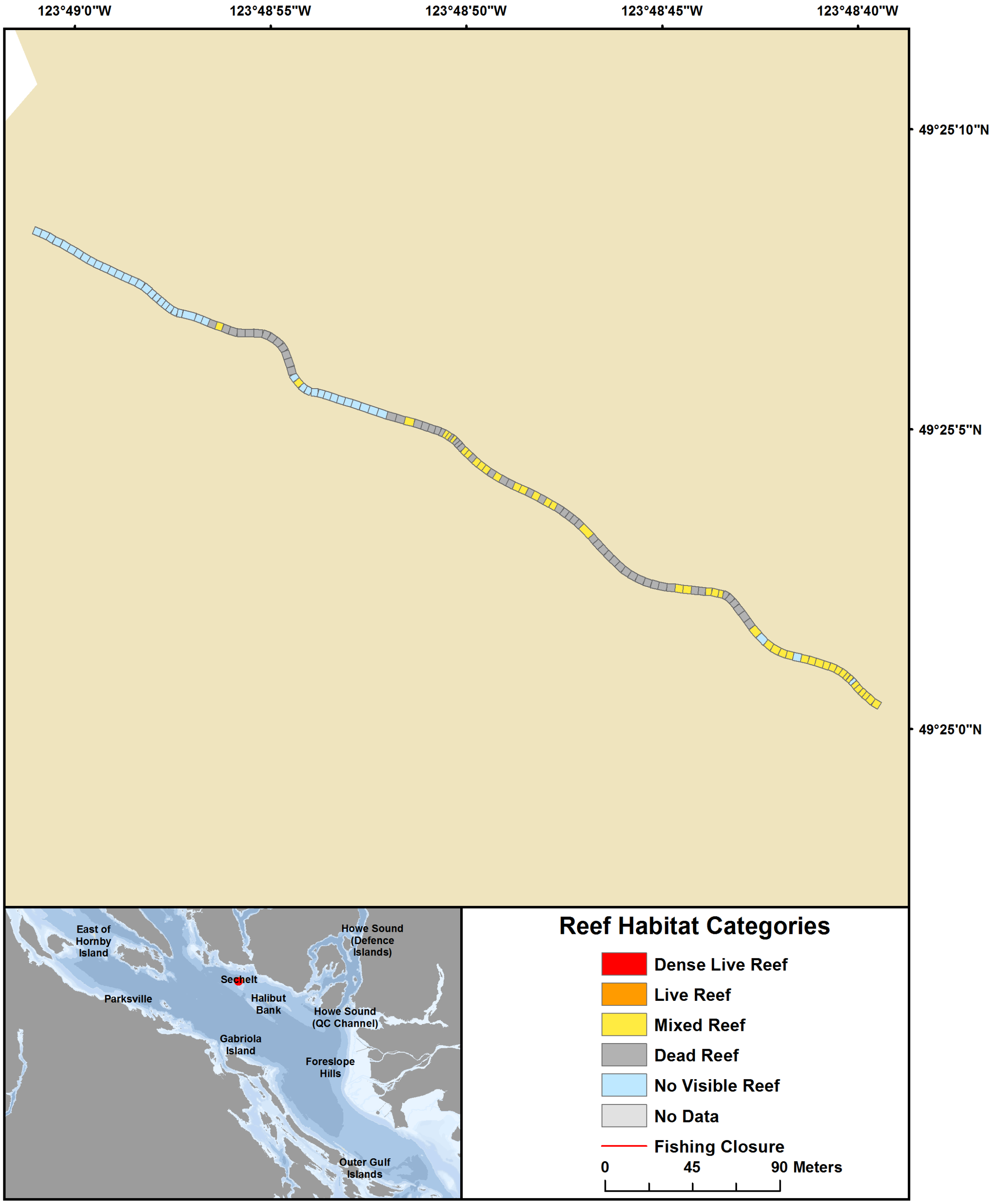

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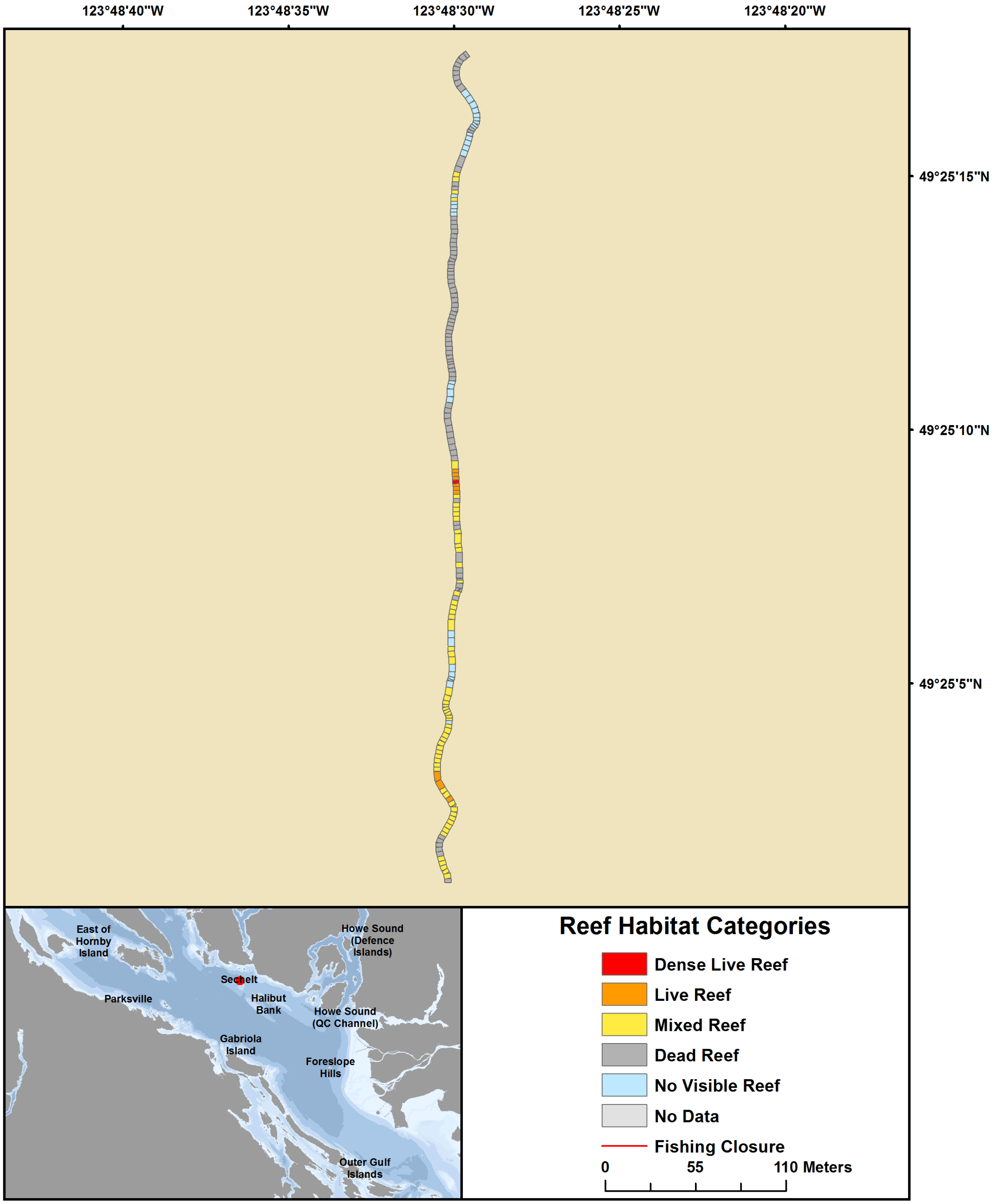
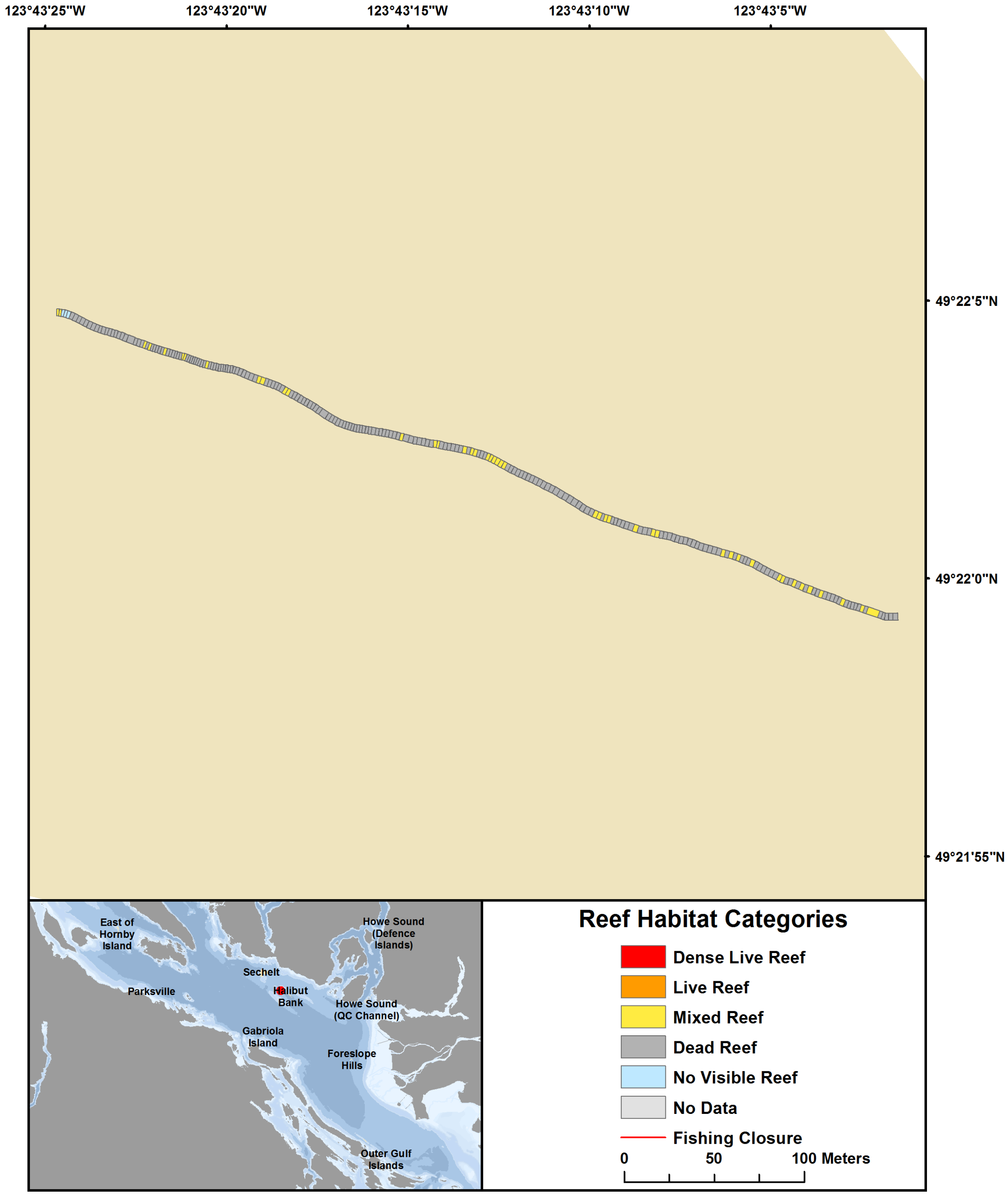
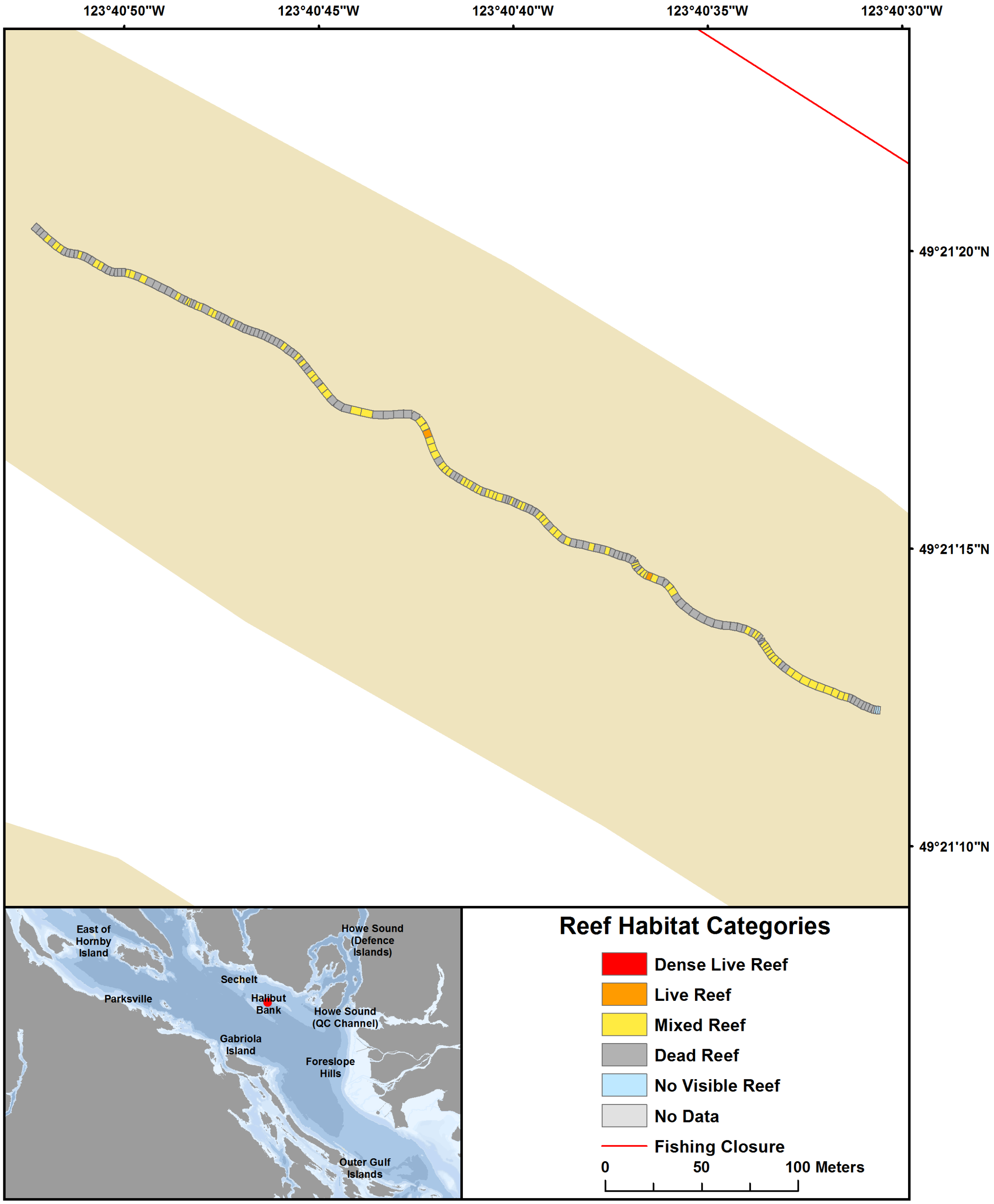


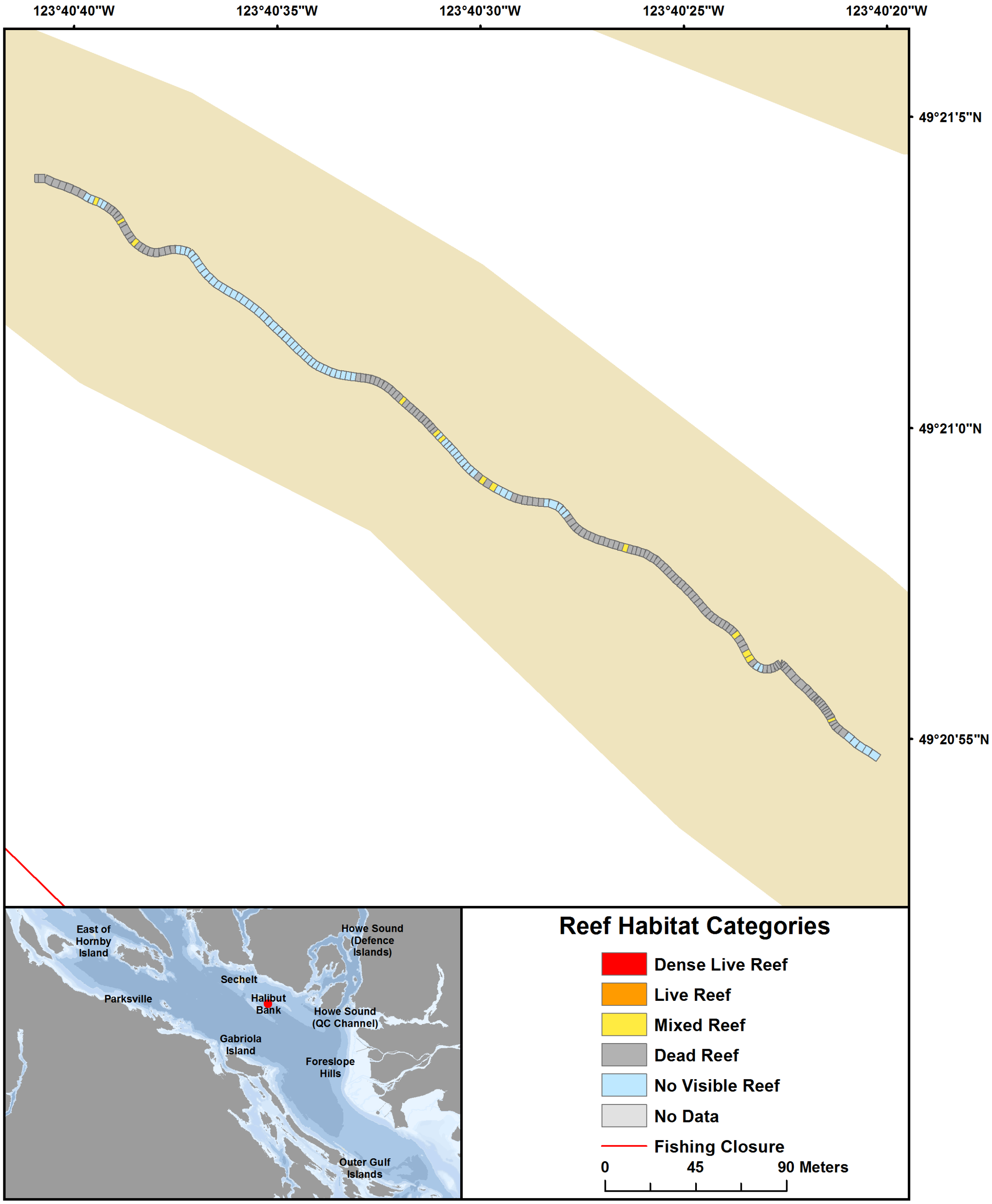
Halibut Bank - Pac2012-068 Transect 17



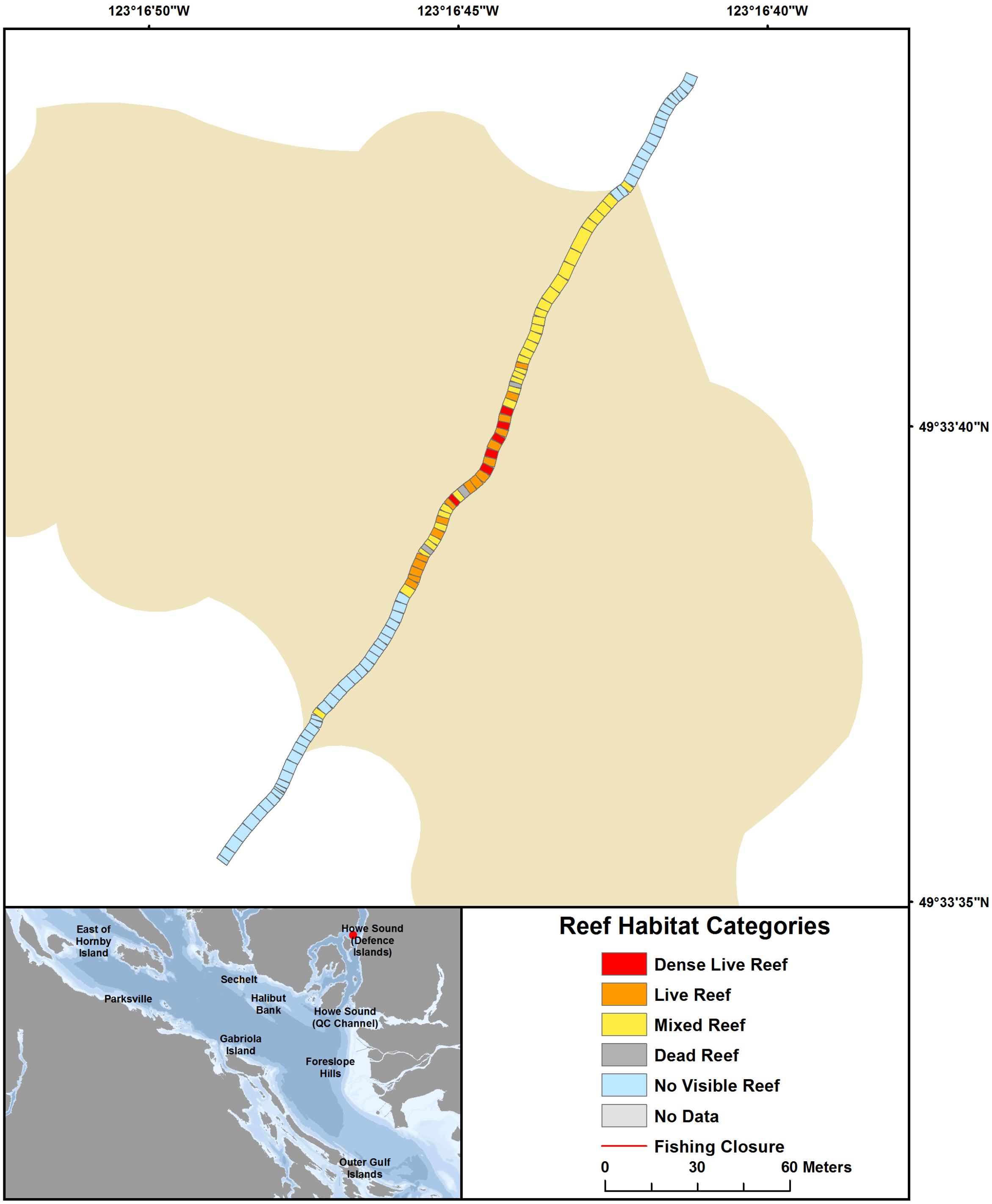
Halibut Bank - Pac2012-068 Transect 18



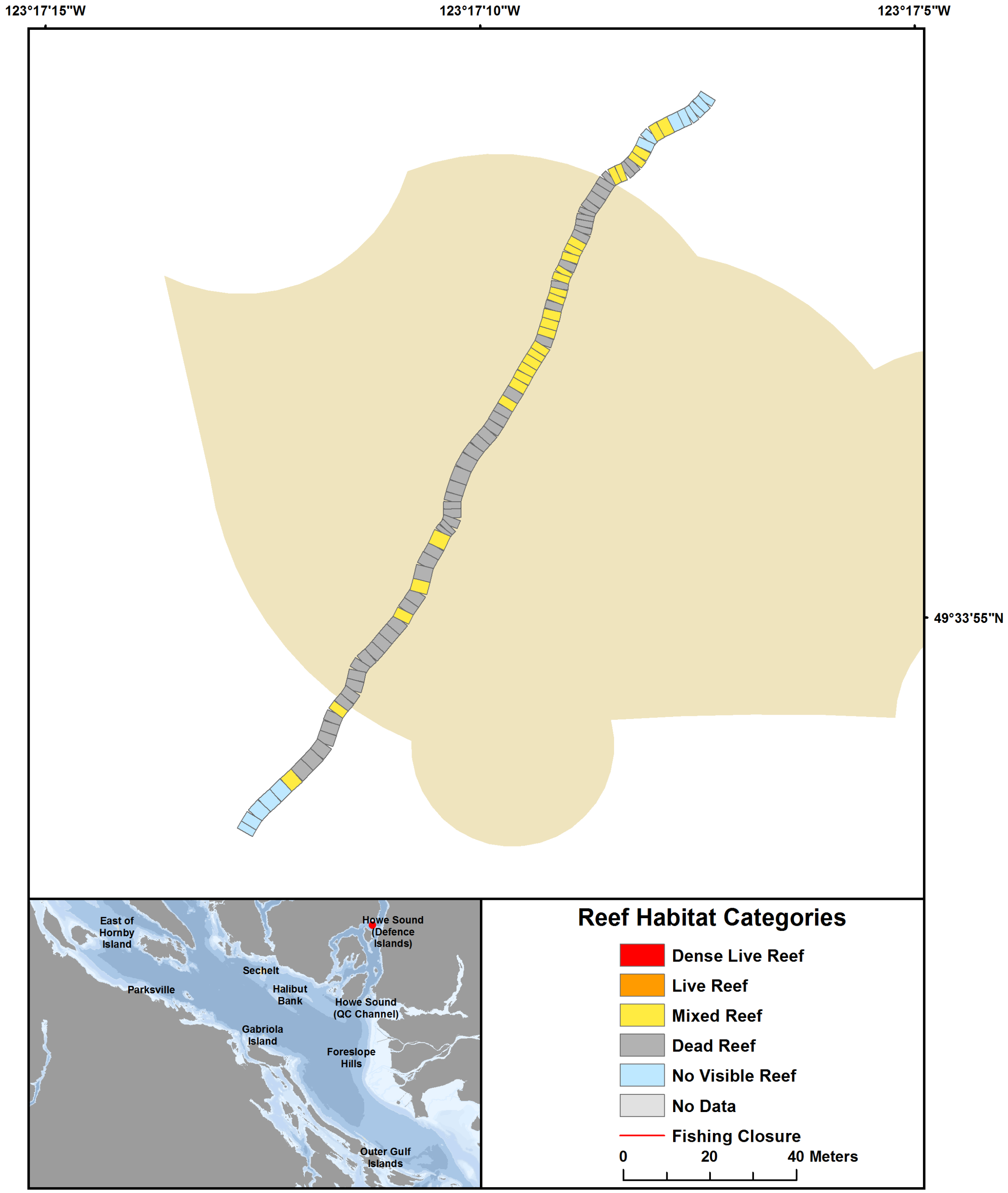
Halibut Bank - Pac2012-068 Transect 19



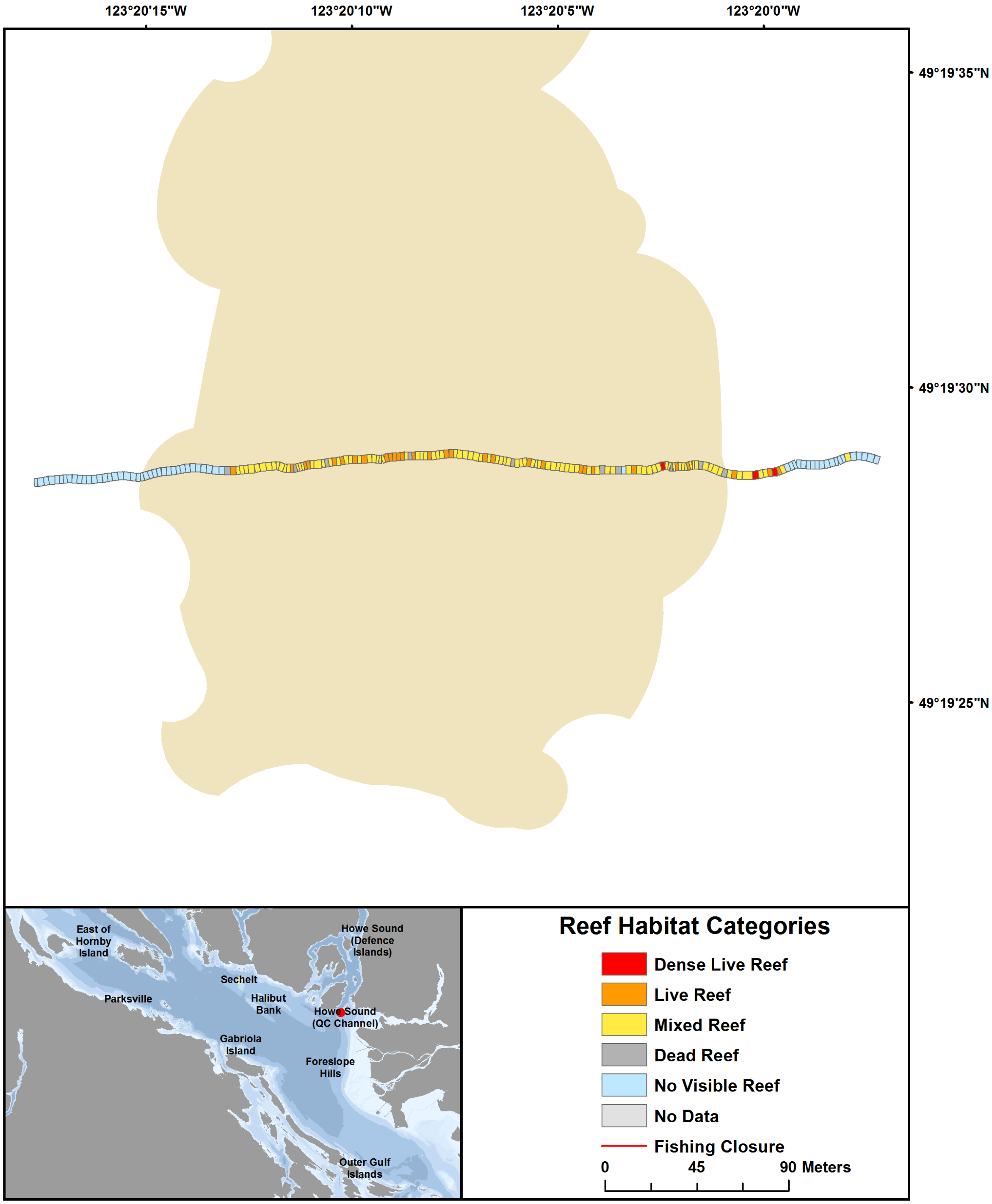
Howe Sound (Defence Islands) - Pac2013-070 Transect 28



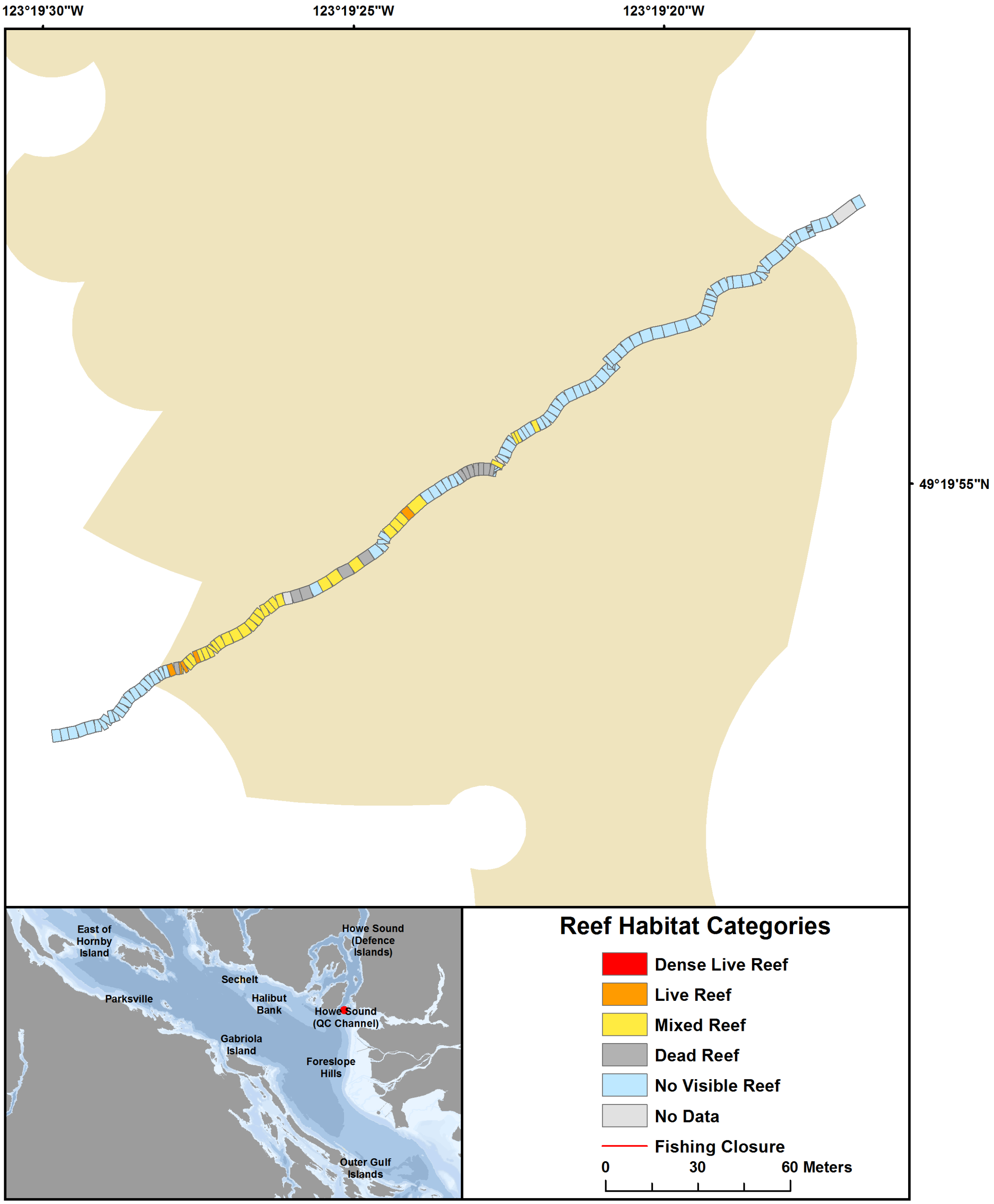
Howe Sound (Defence Islands) - Pac2013-070 Transect 29



Howe Sound (QC Channel) - Pac2013-070 Transect 25



Howe Sound (QC Channel) - Pac2013-070 Transect 26



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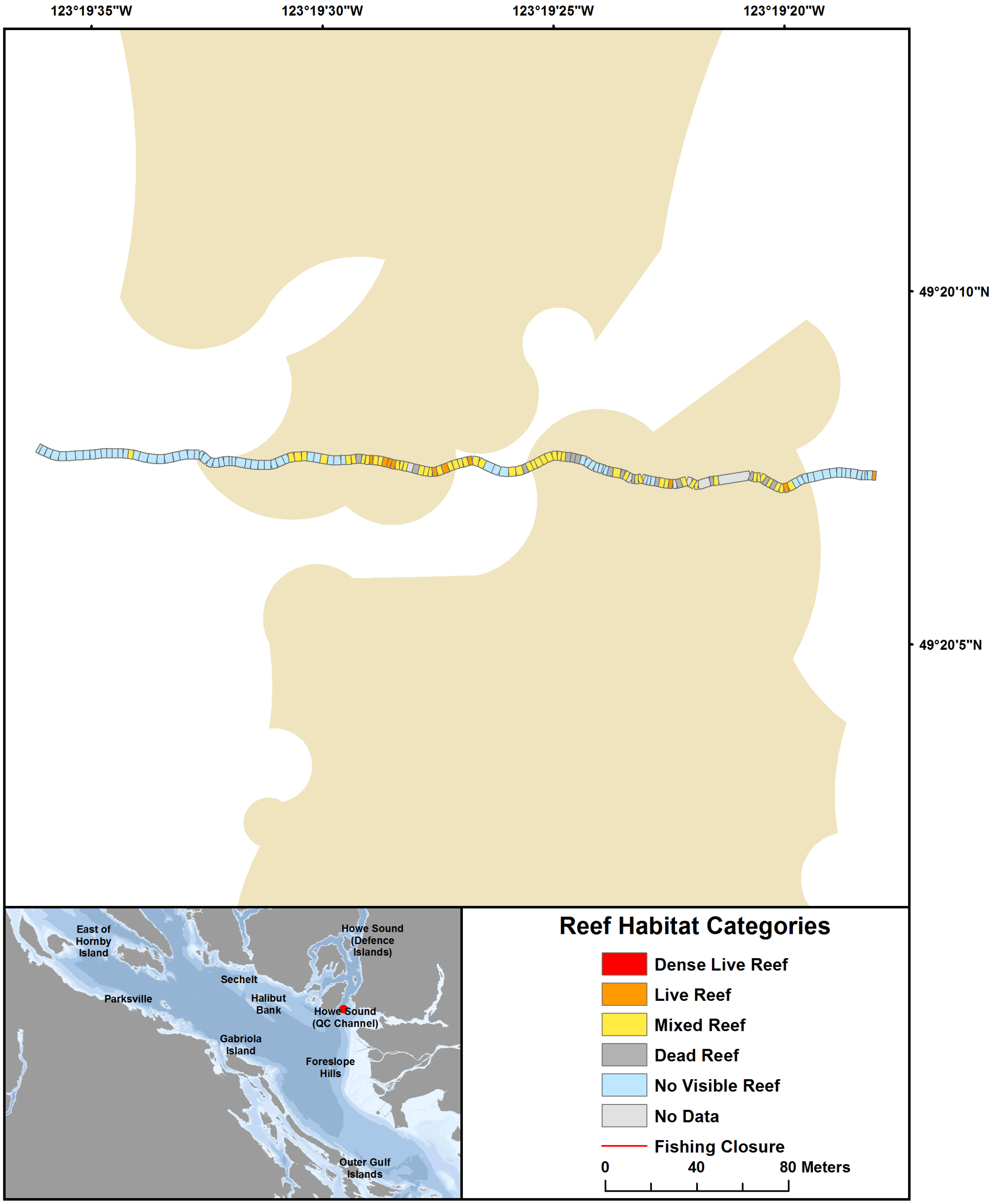
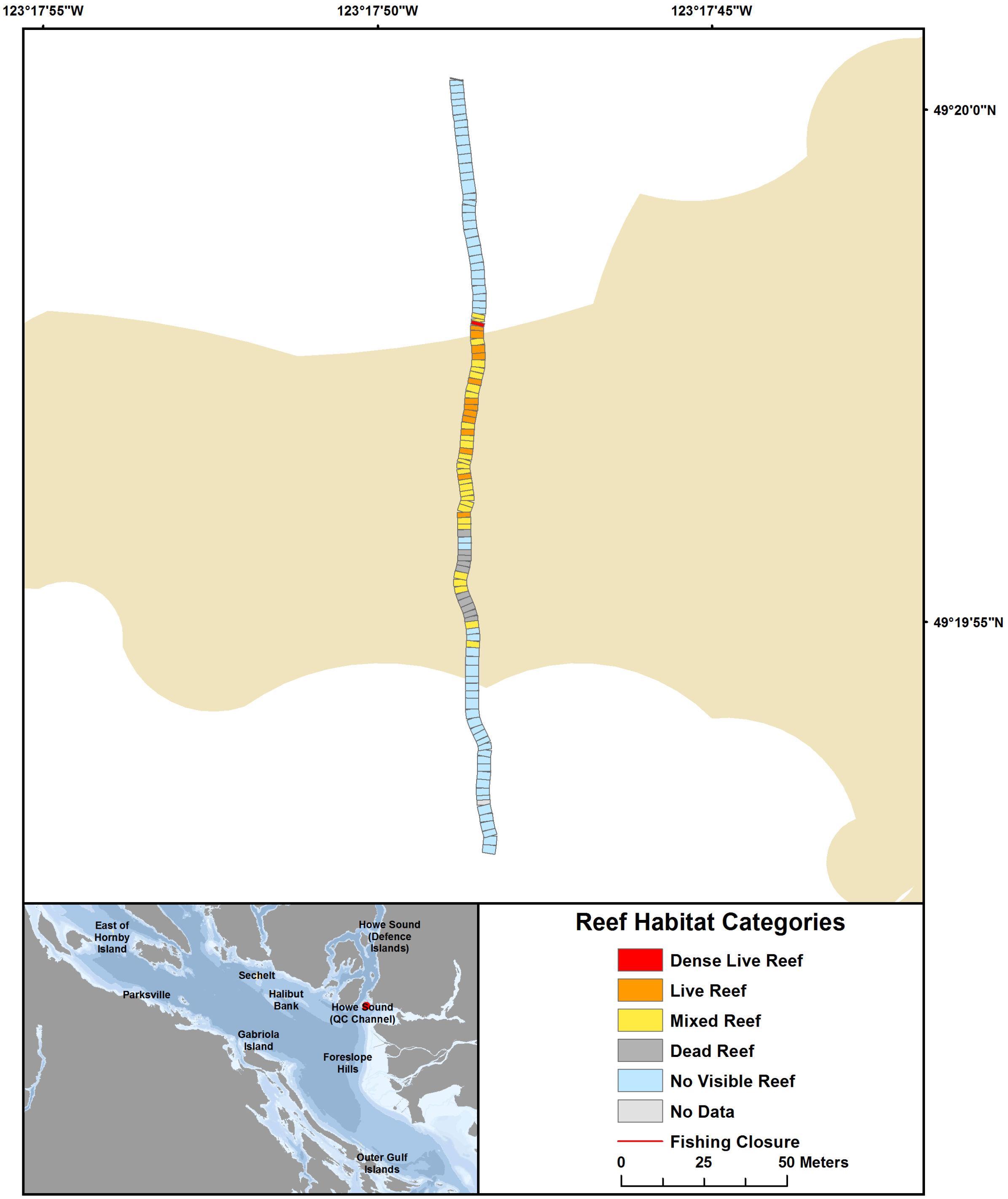


Howe Sound (QC Channel) - Pac2013-070 Transect 31



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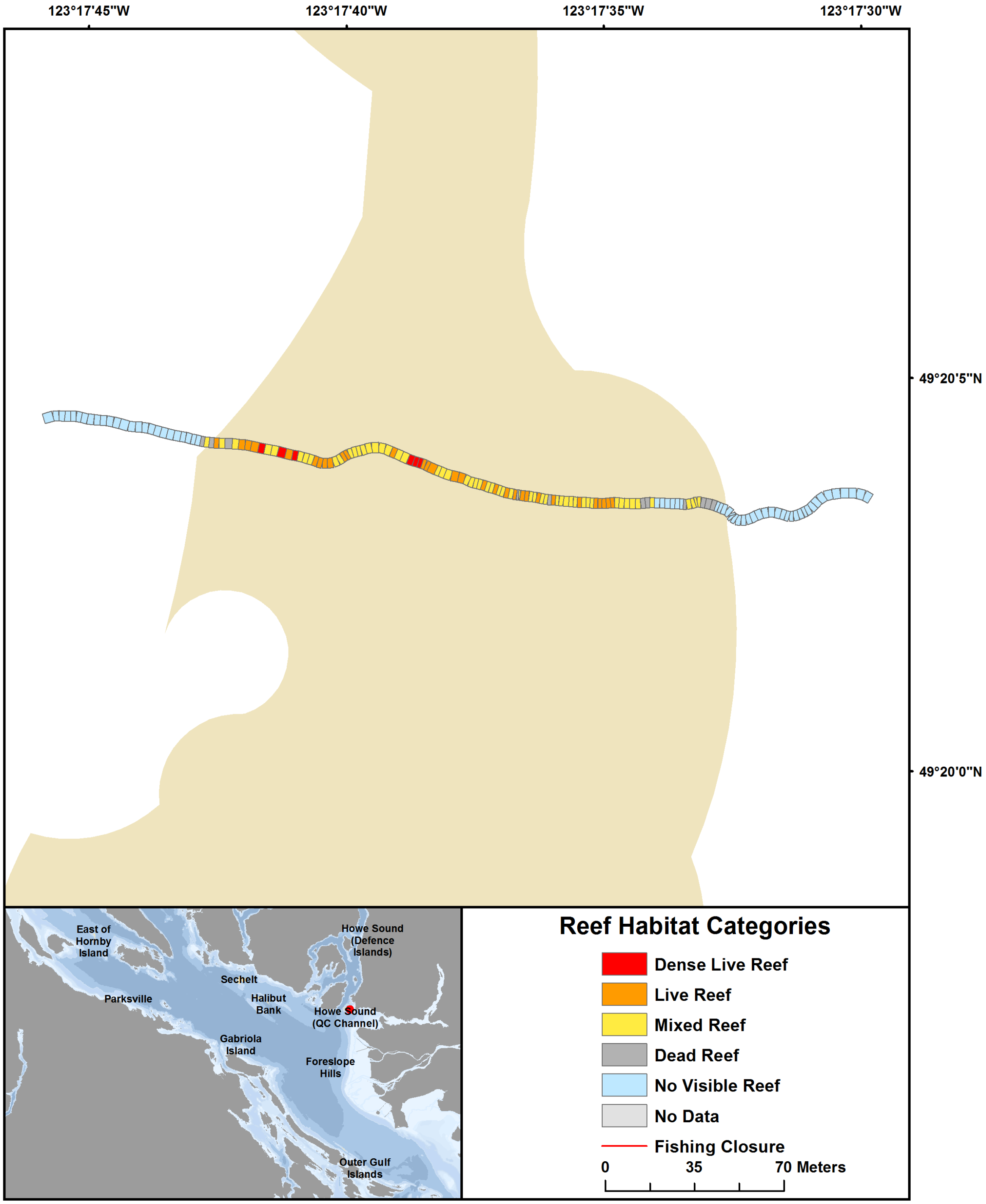
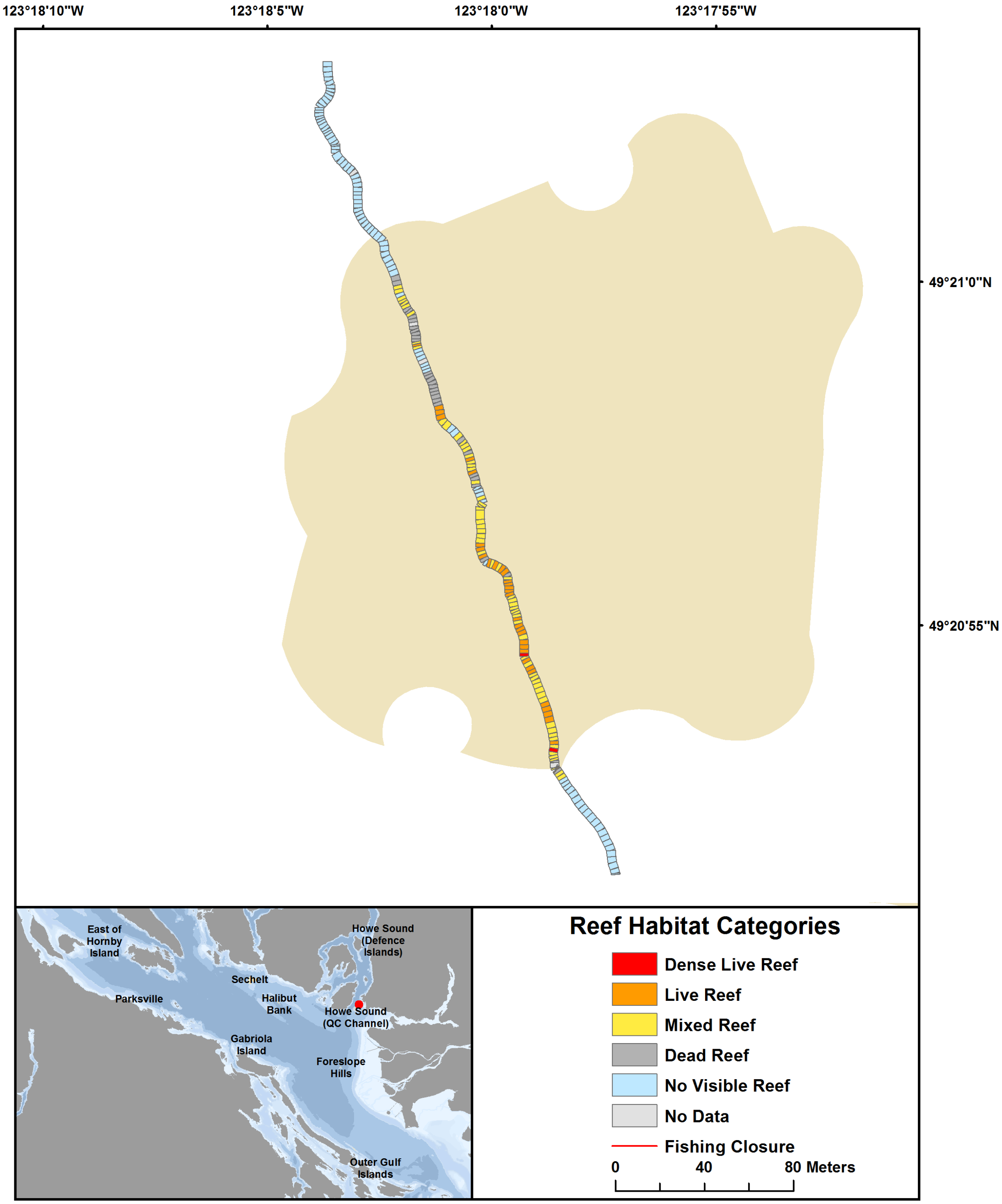
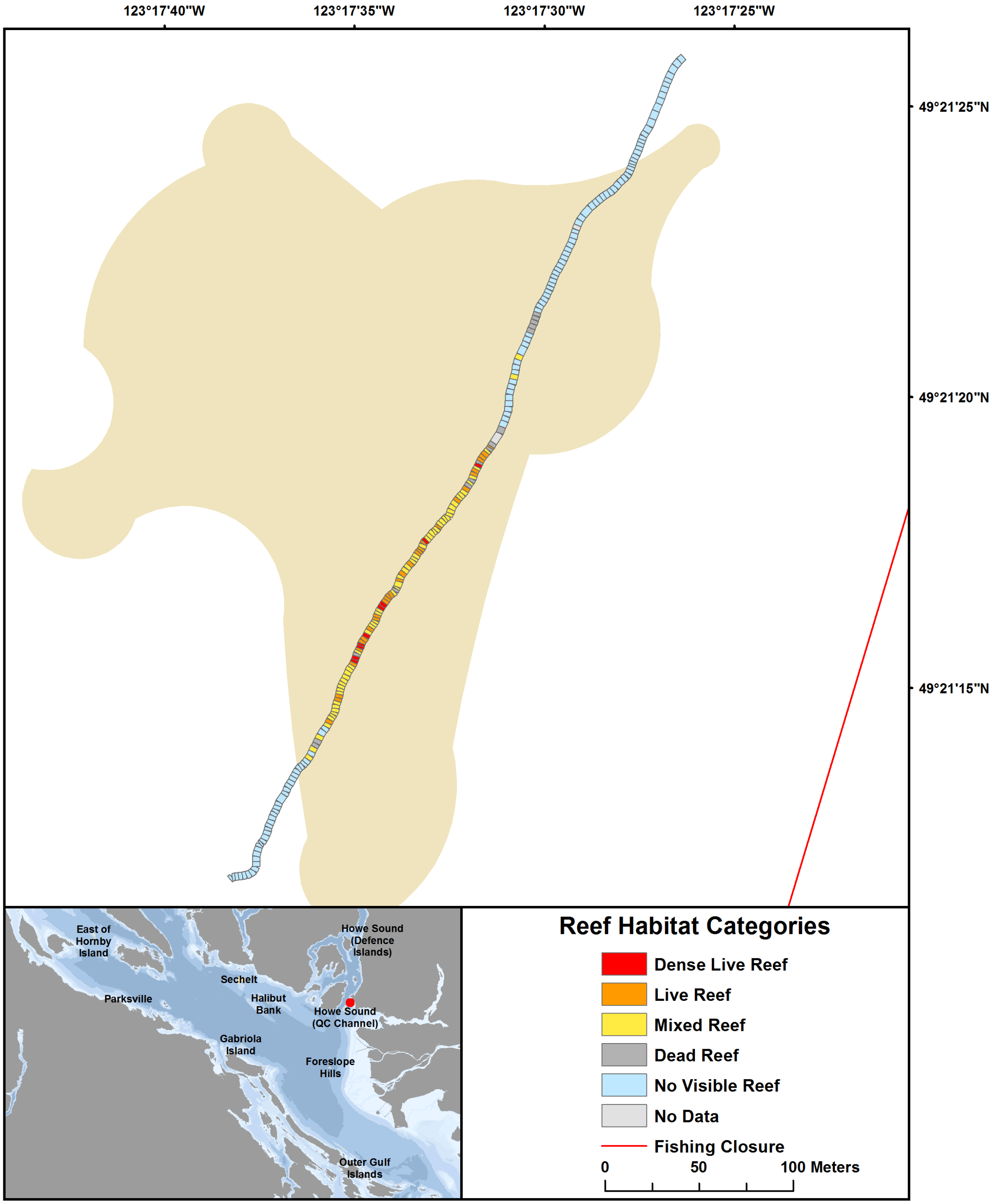


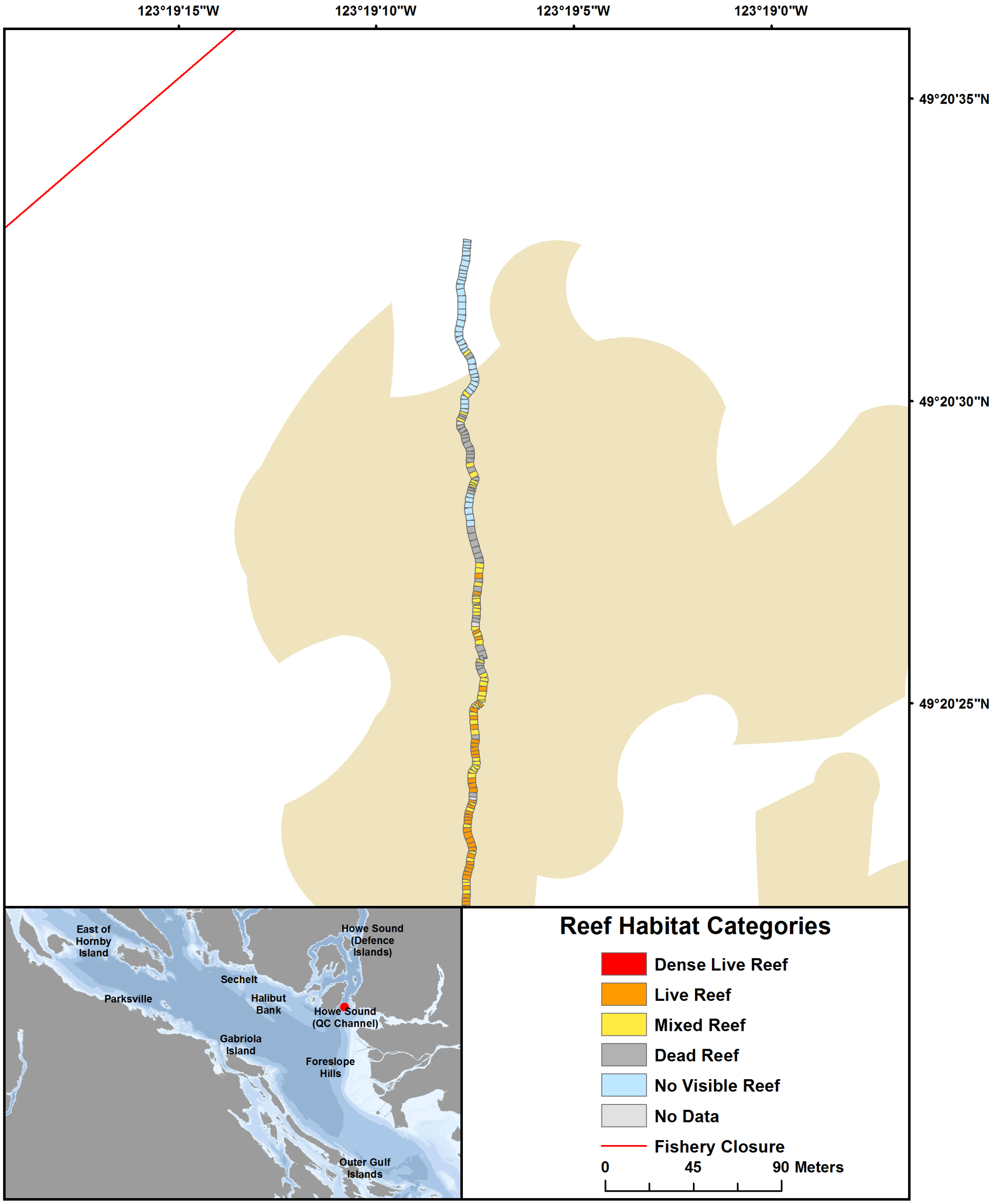
Howe Sound (QC Channel) - Pac2013-070 Transect 33



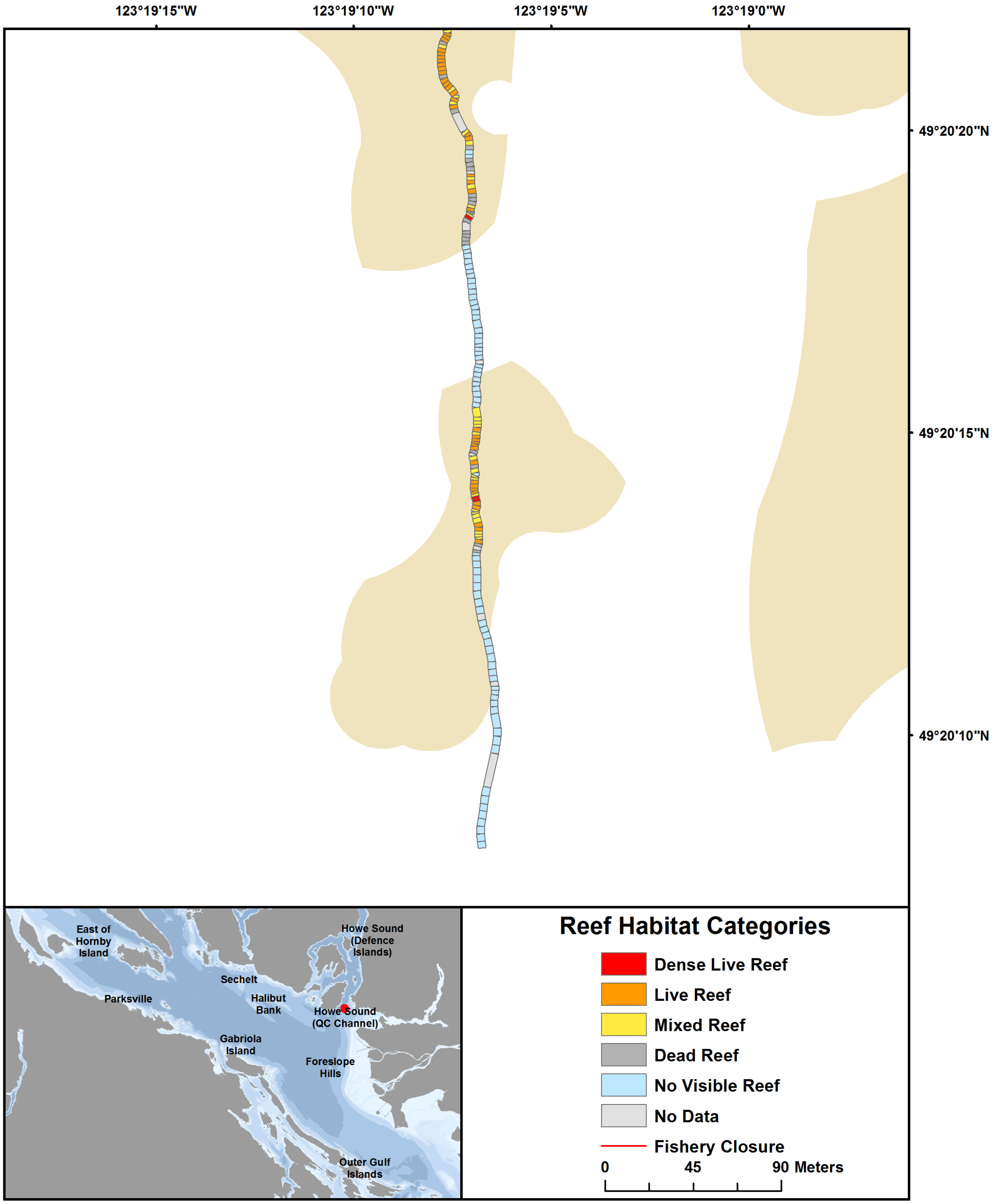
Howe Sound (QC Channel) - Pac2013-070 Transect 34



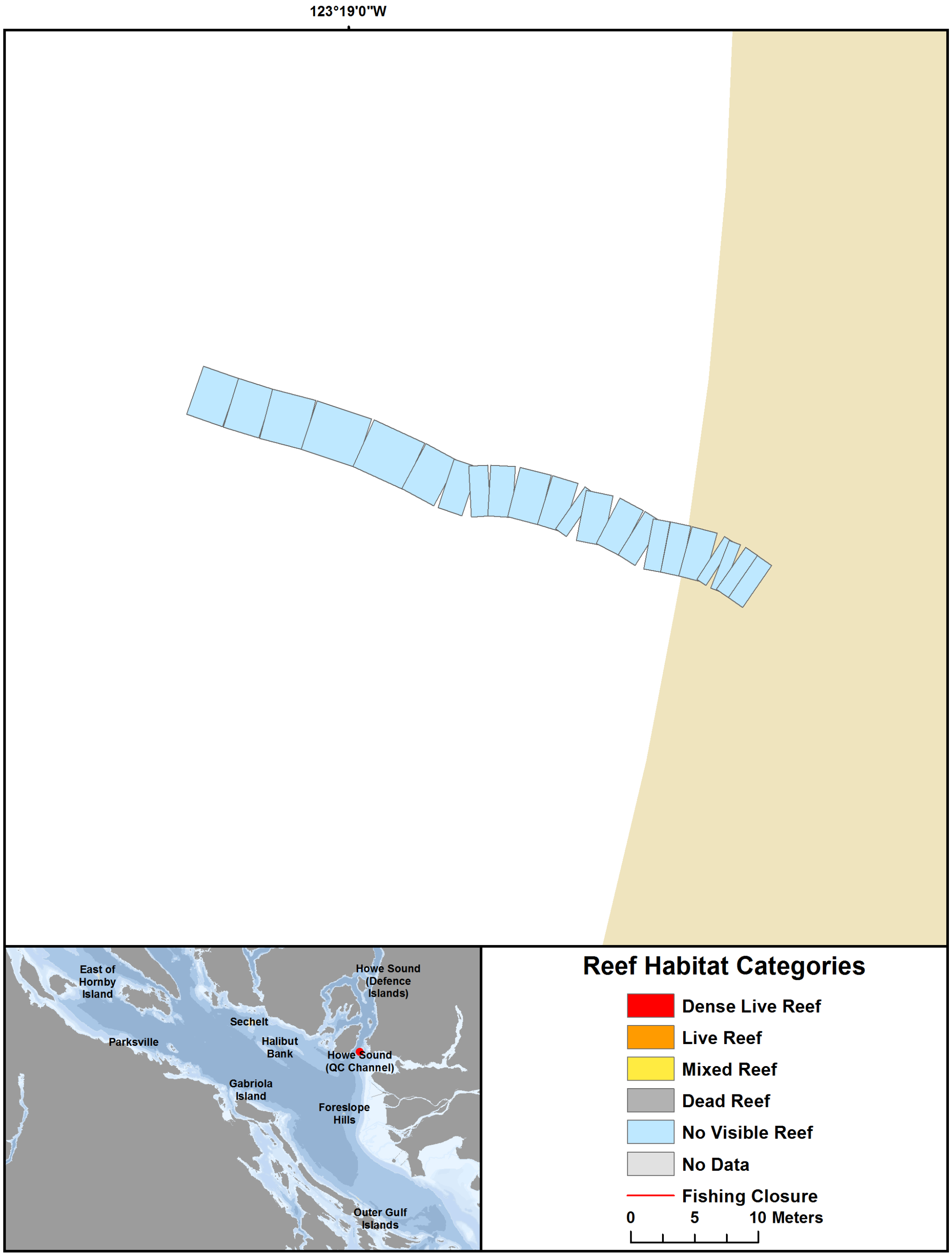
Howe Sound (QC Channel) - Pac2013-070 Transect 35 Part 1



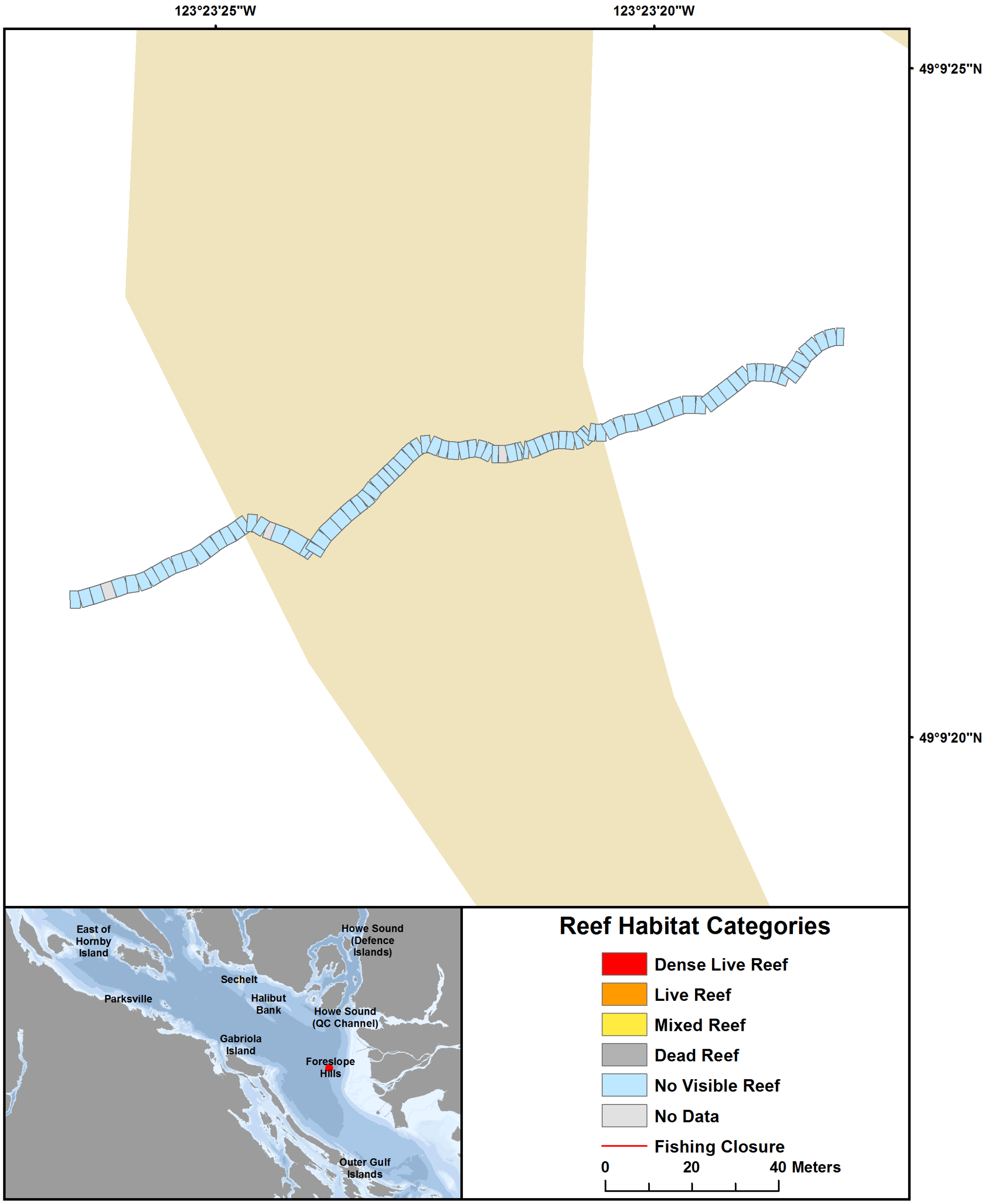
Howe Sound (QC Channel) - Pac2013-070 Transect 35 Part 2



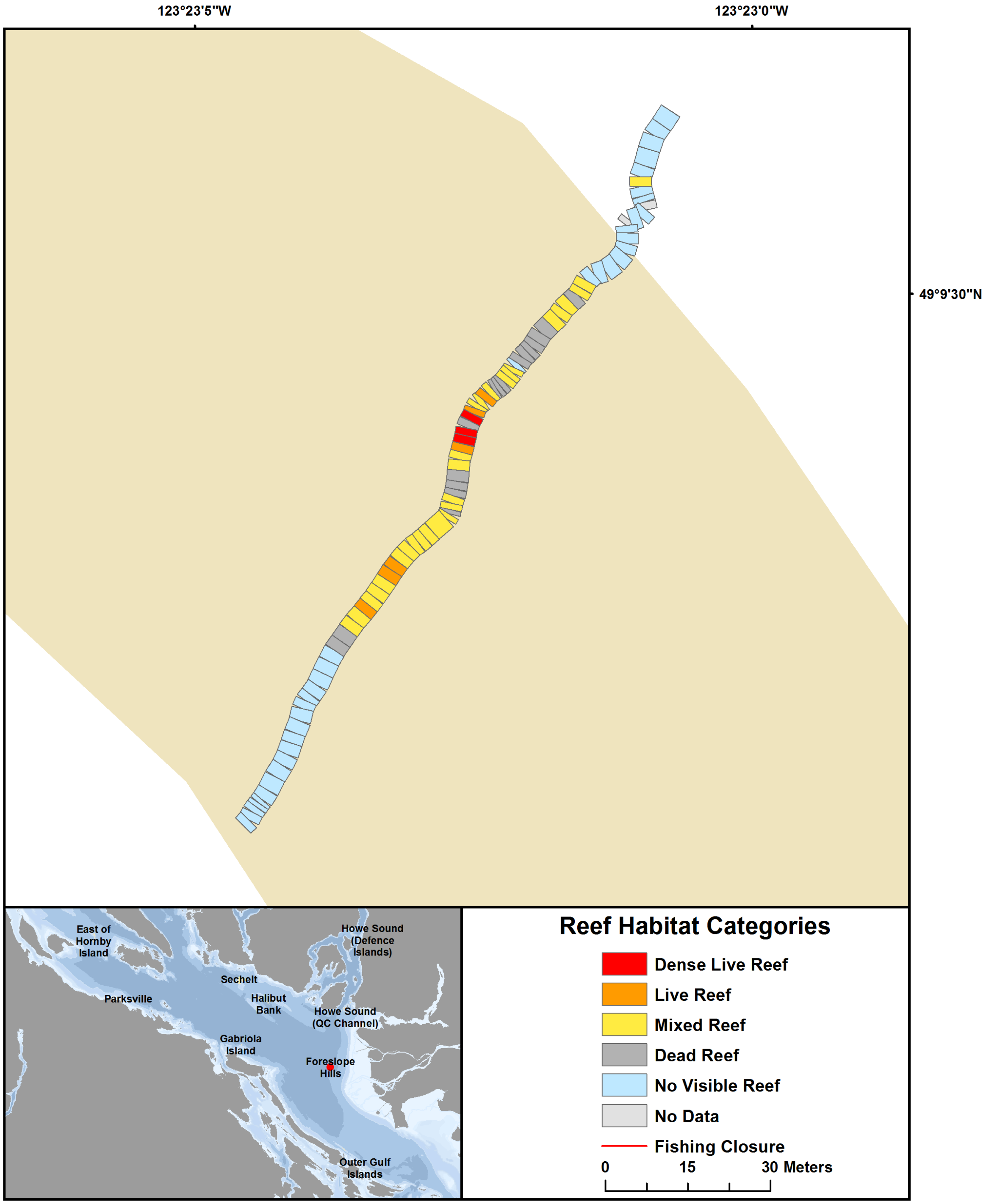
Howe Sound (QC Channel) - Pac2013-070 Transect 36



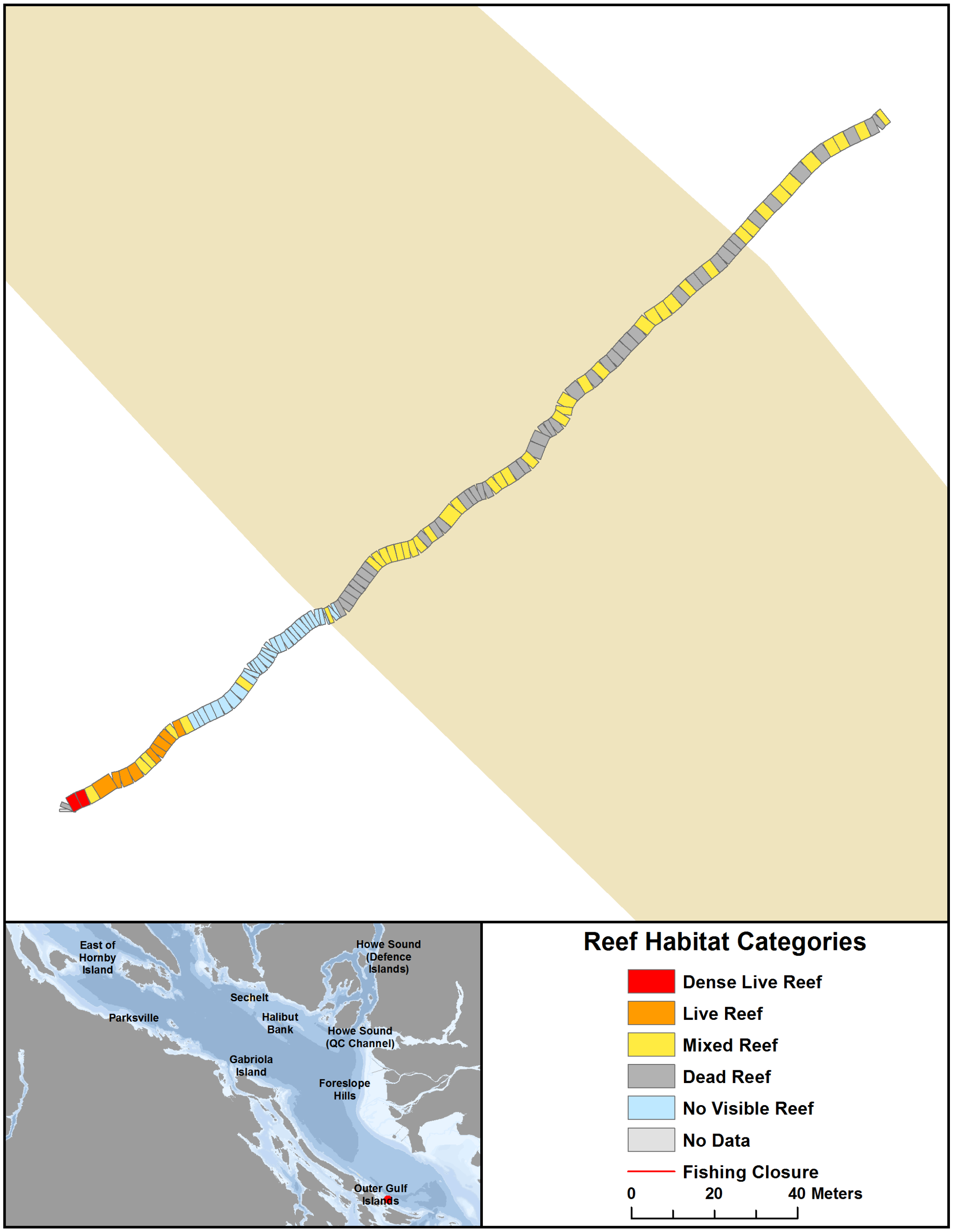
Foreslope Hills - Pac2013-070 Transect 38



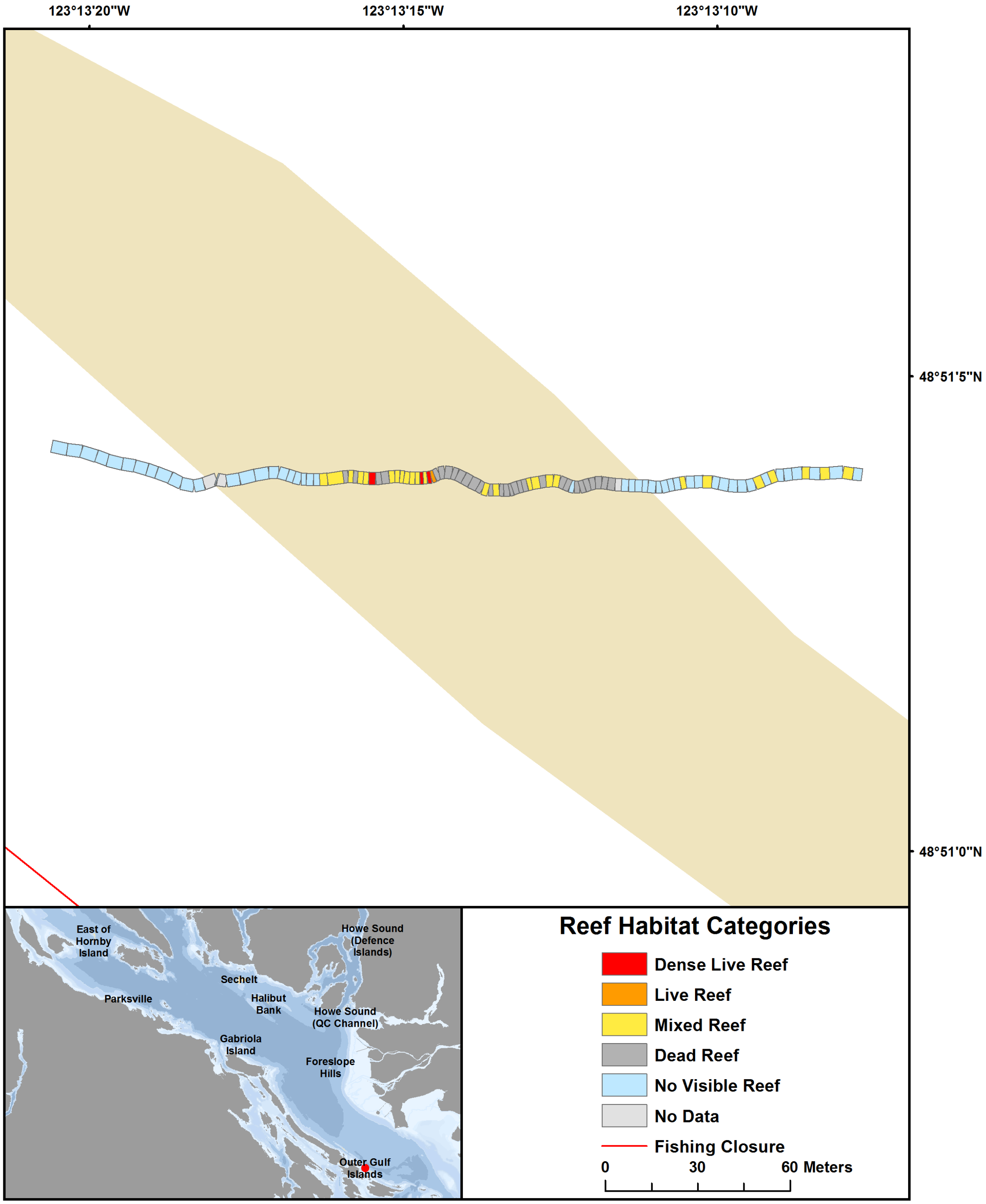
Foreslope Hills - Pac2013-070 Transect 39



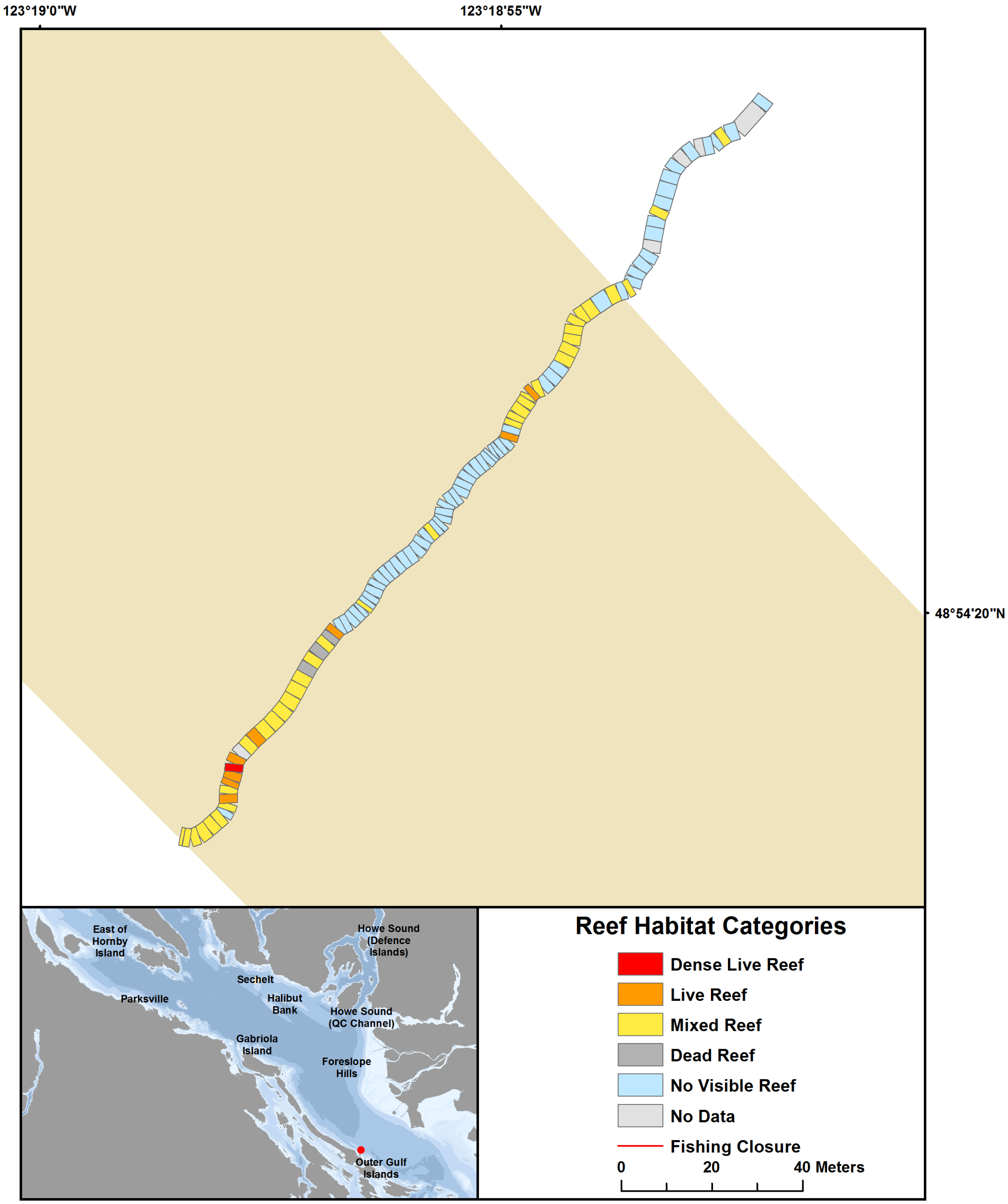
Outer Gulf Islands - Pac2013-070 Transect 40



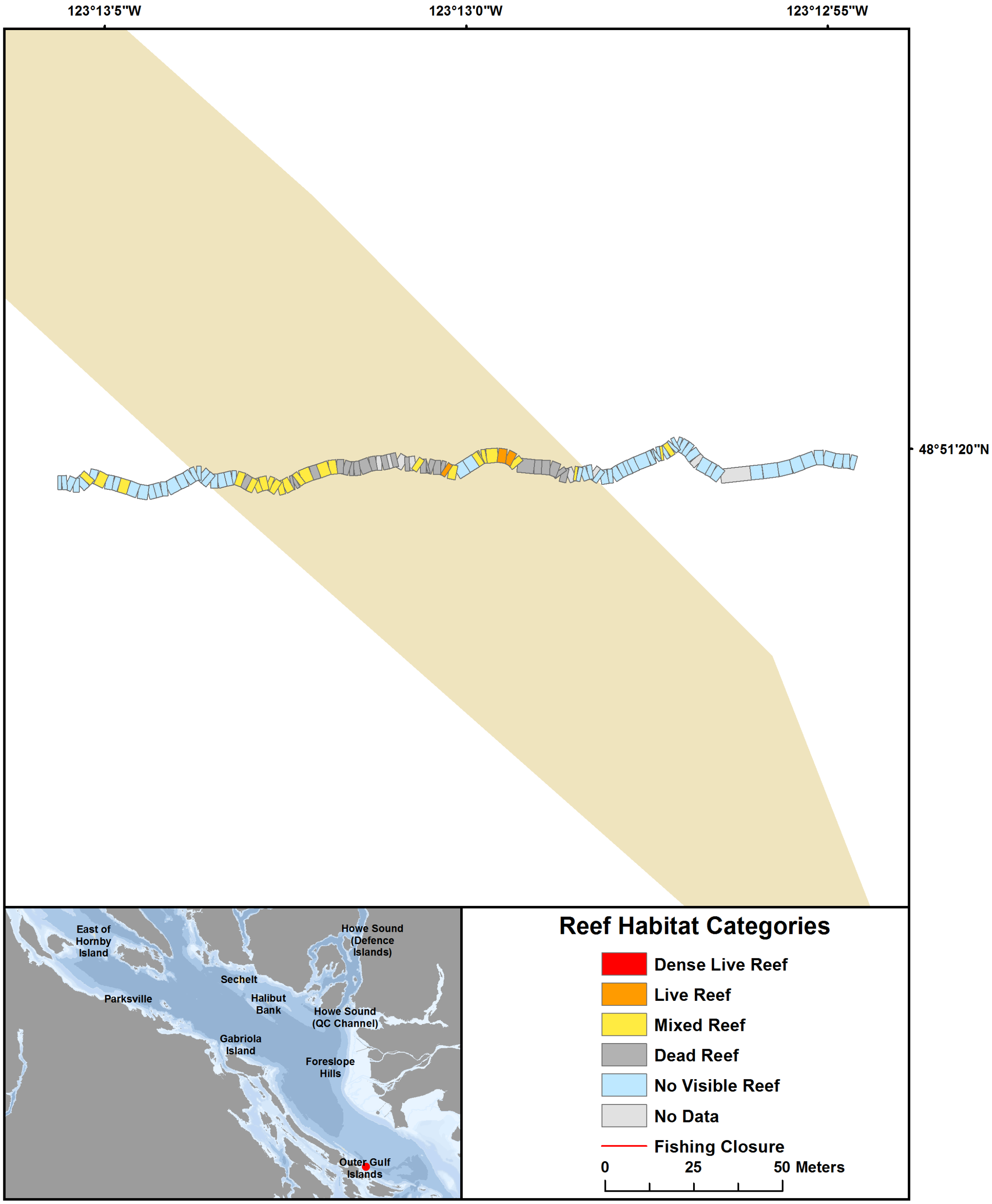
Outer Gulf Islands - Pac2013-070 Transect 41



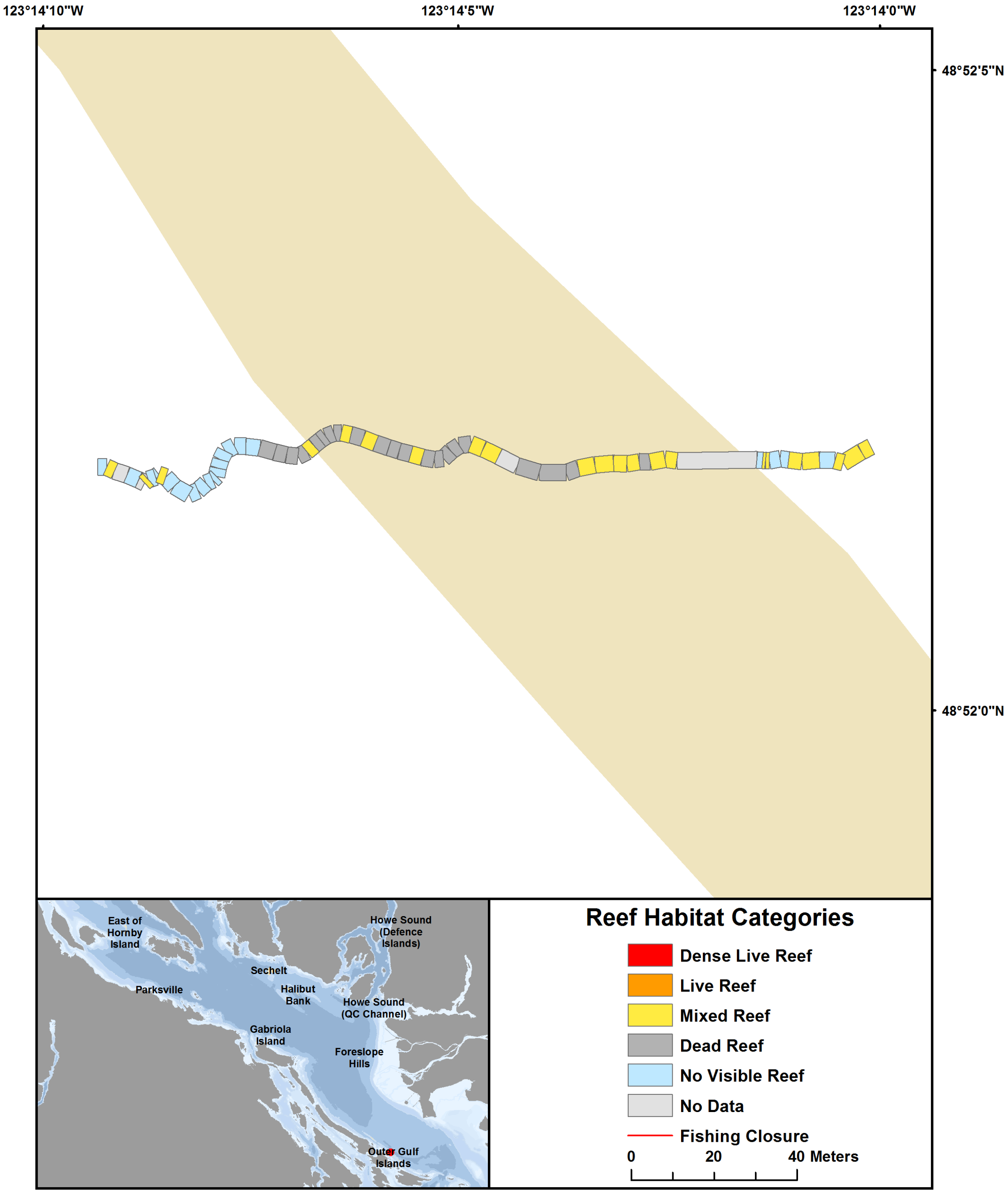
Outer Gulf Islands - Pac2013-070 Transect 42



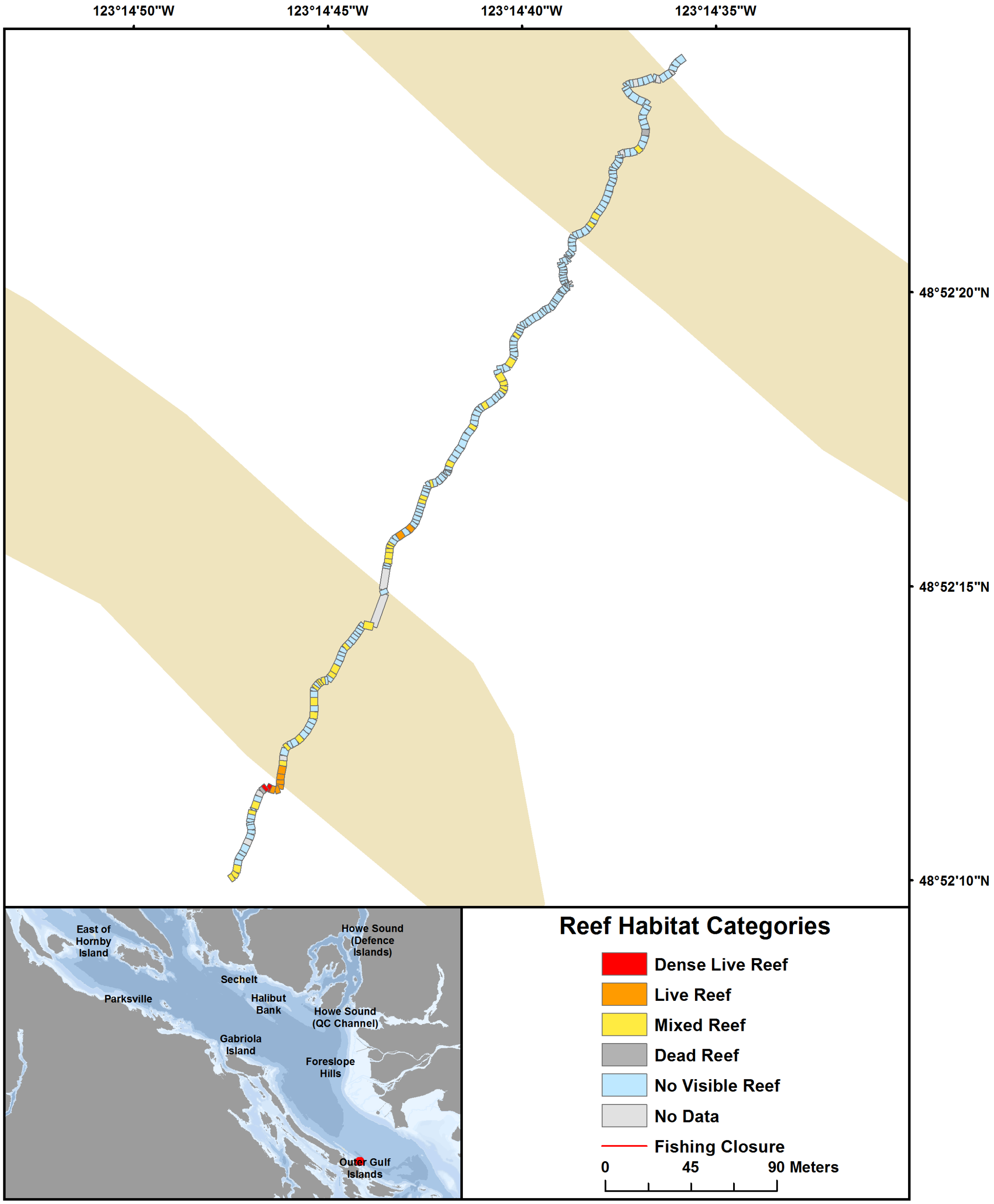
Outer Gulf Islands - Pac2013-070 Transect 44



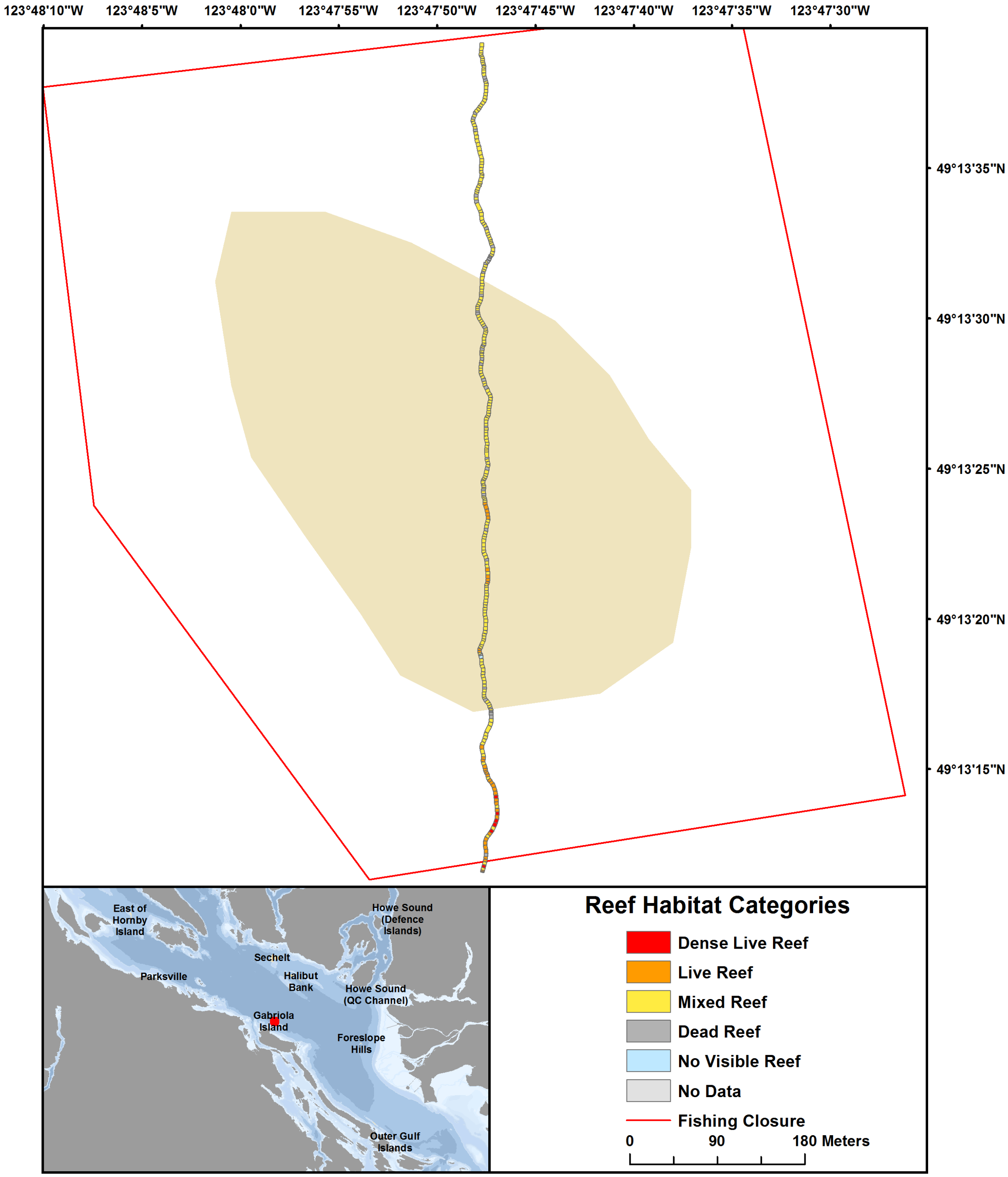
Outer Gulf Islands - Pac2013-070 Transect 45



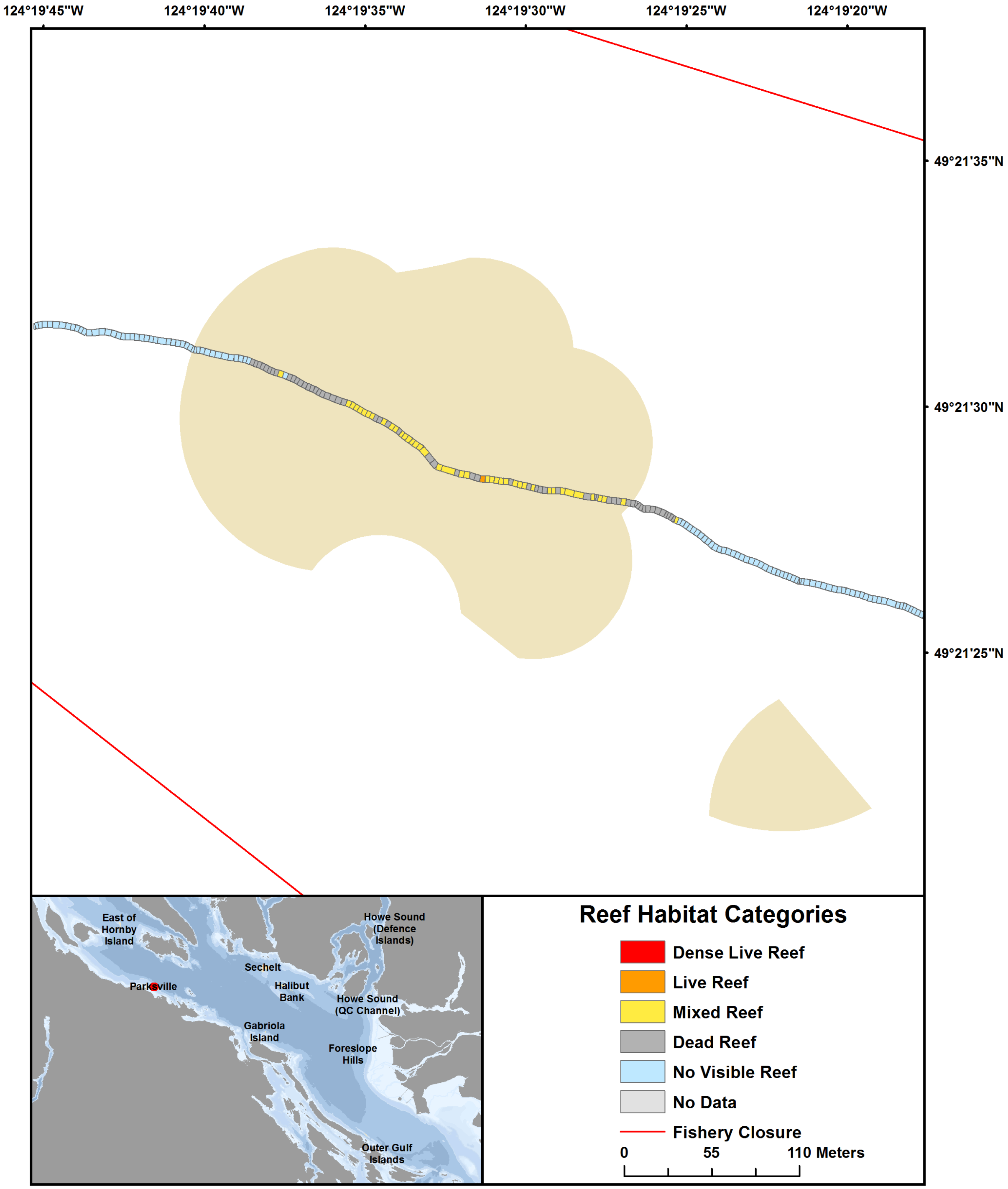
Outer Gulf Islands - Pac2013-070 Transect 46



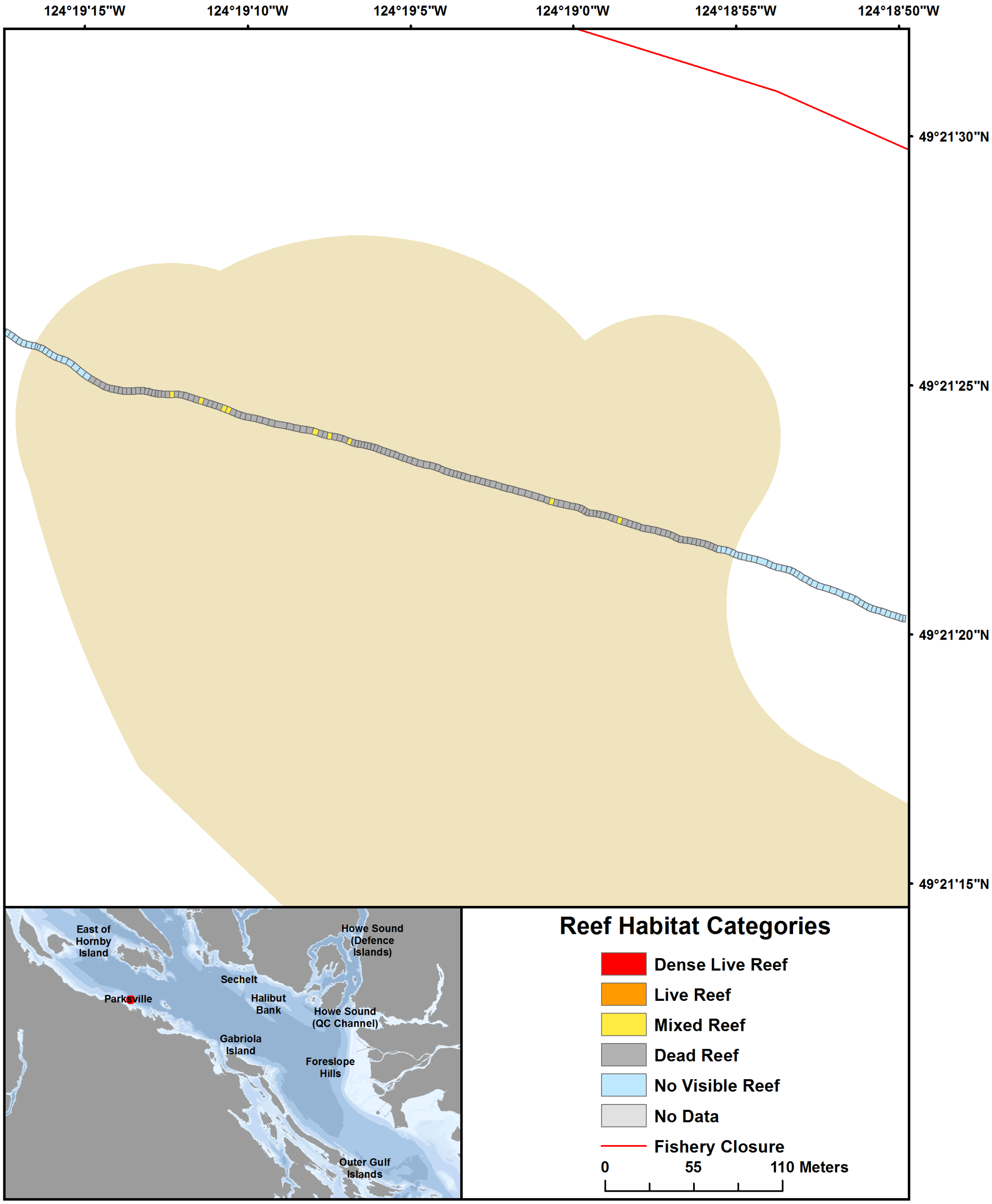
Gabriola Island - Pac2013-070 Transect 24



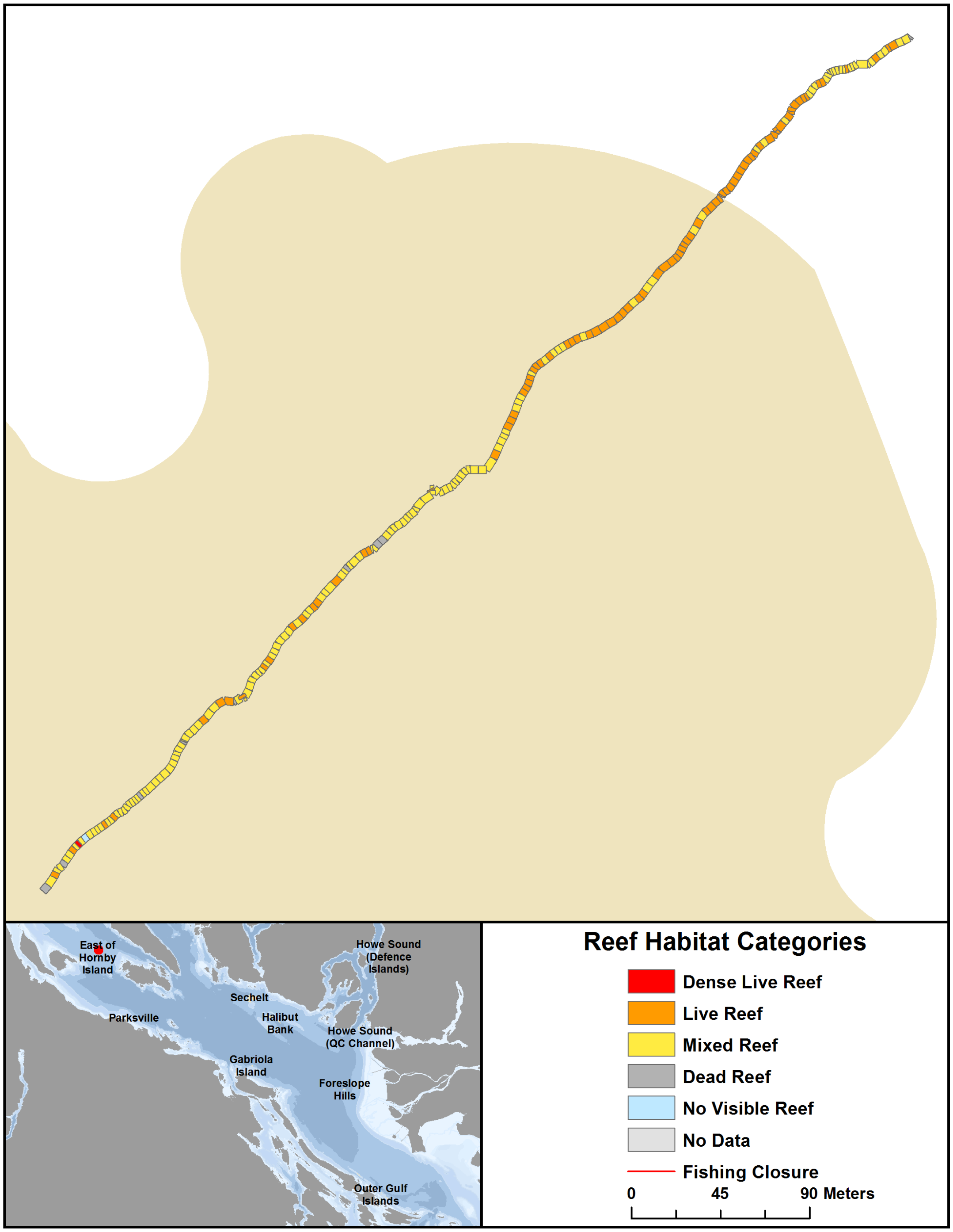
Parksville - Pac2013-070 Transect 2 Part 1



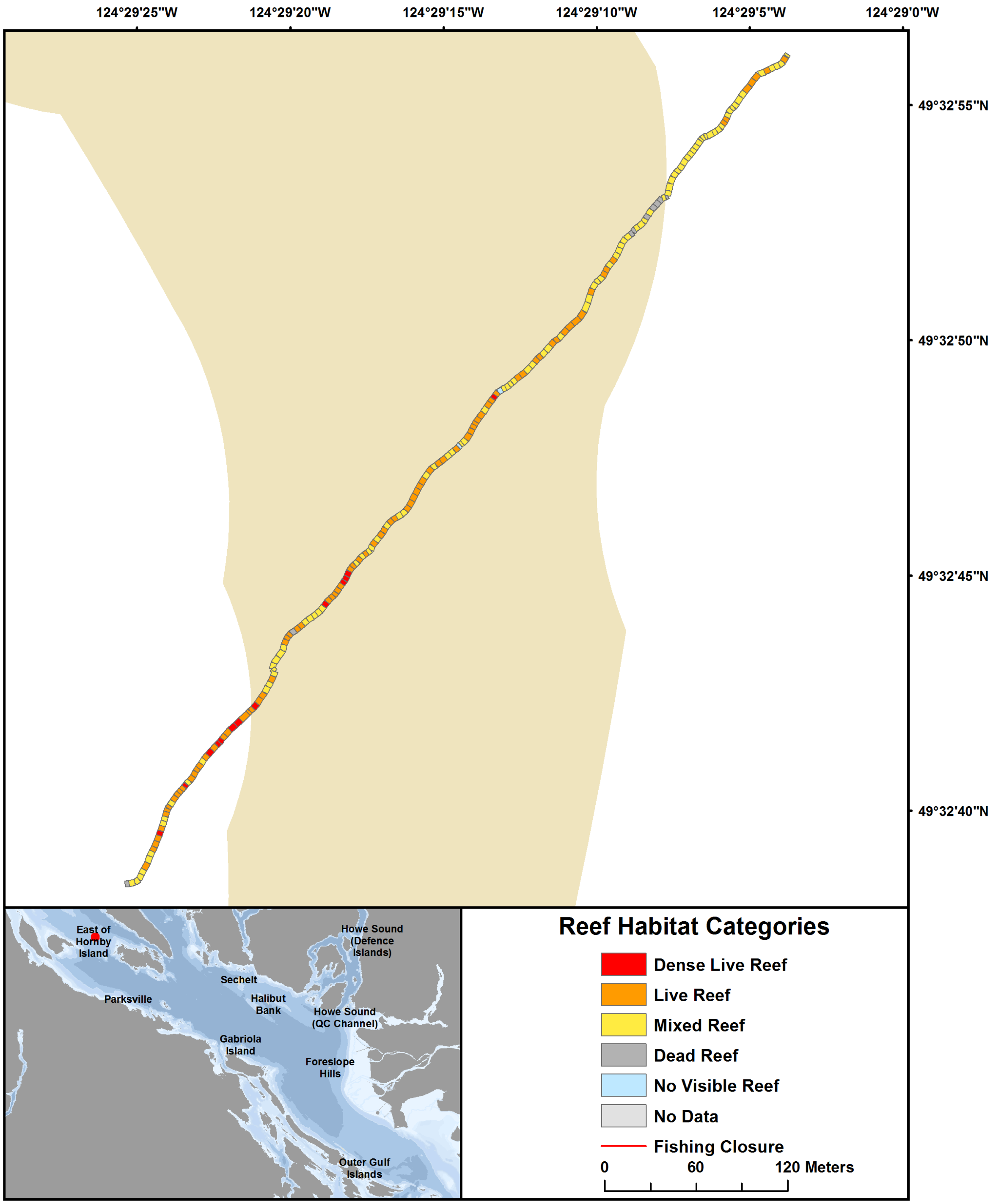
Parksville - Pac2013-070 Transect 2 Part 2



East of Hornby Island - Pac2013-070 Transect 3



East of Hornby Island - Pac2013-070 Transect 4



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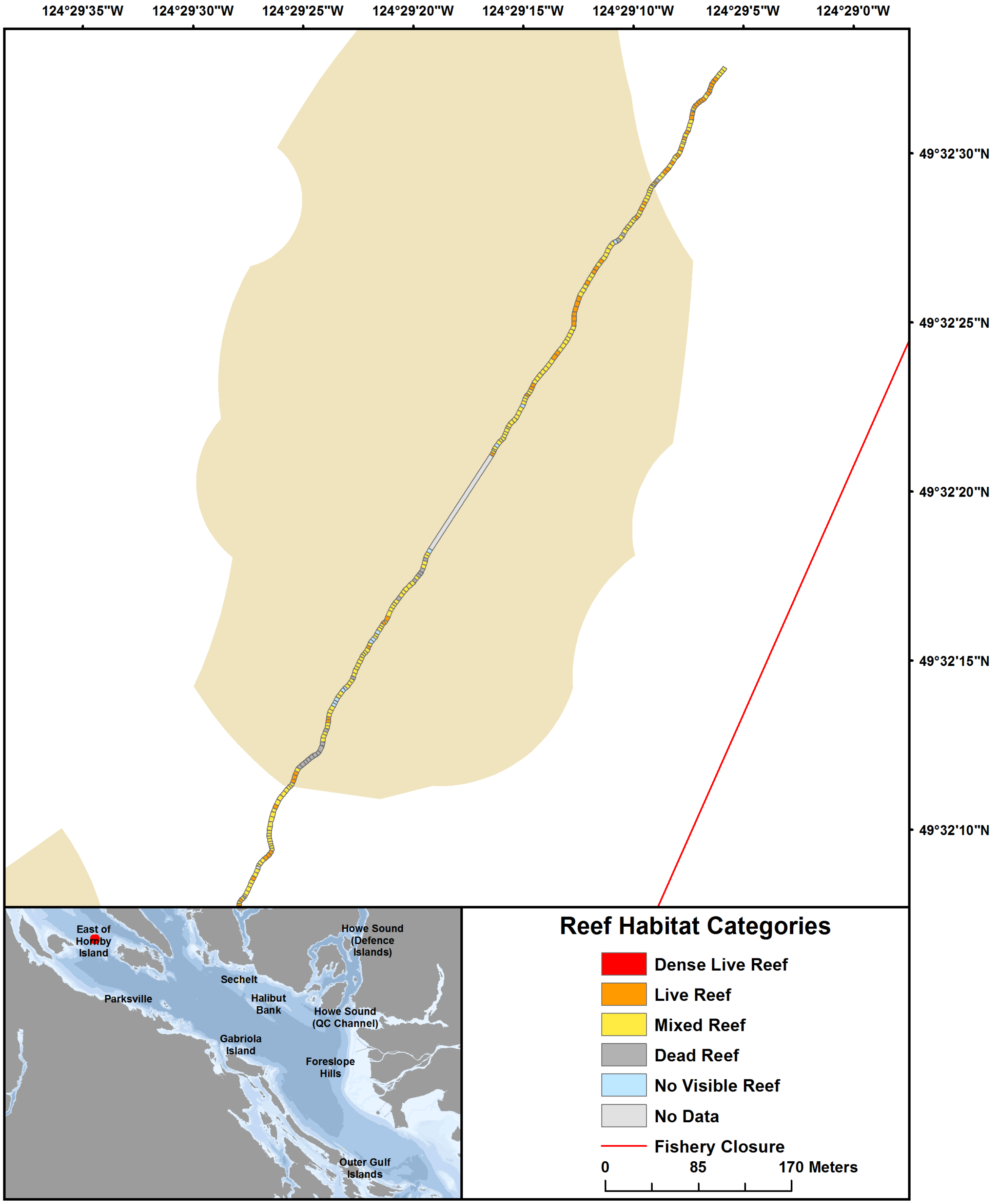

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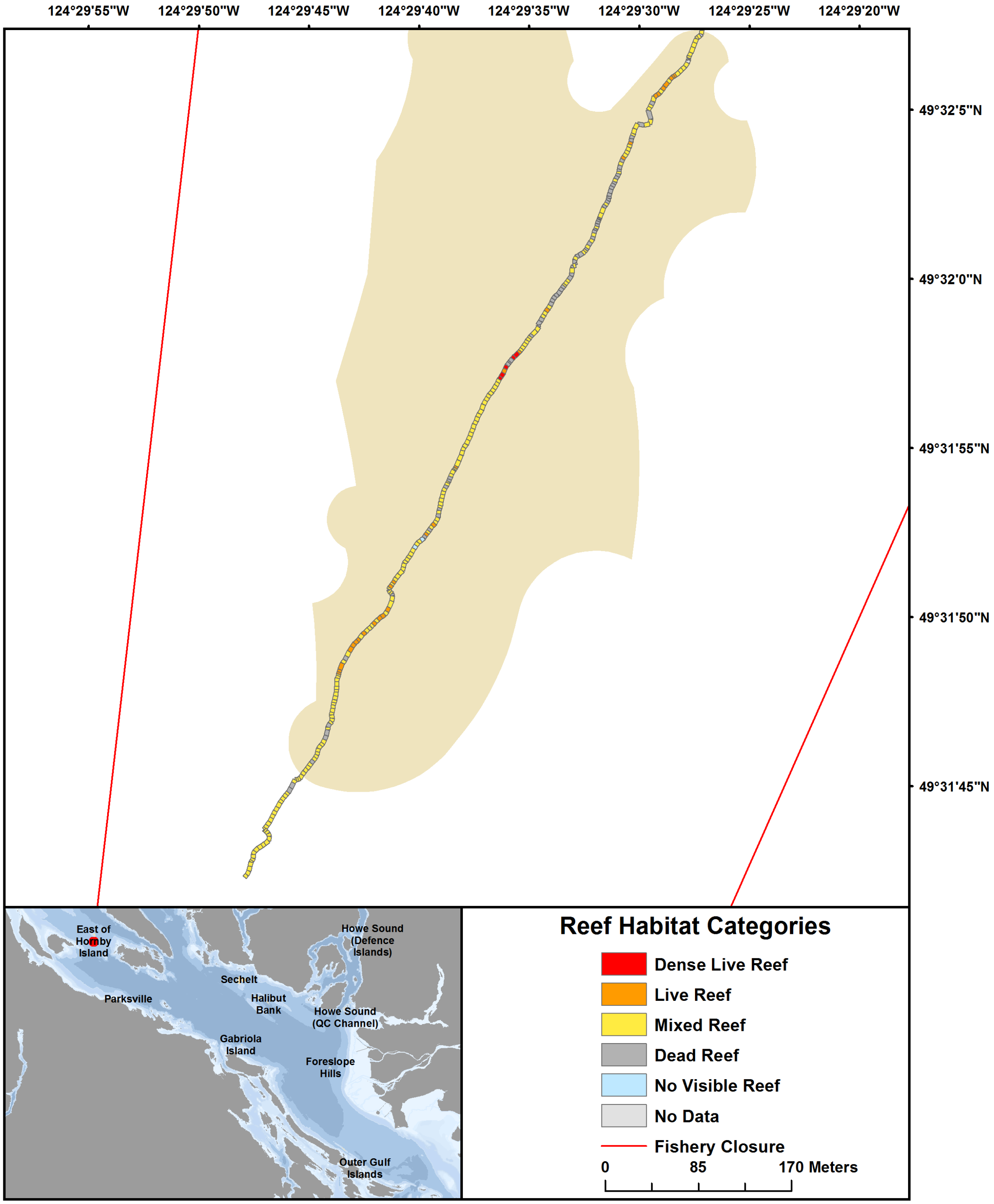
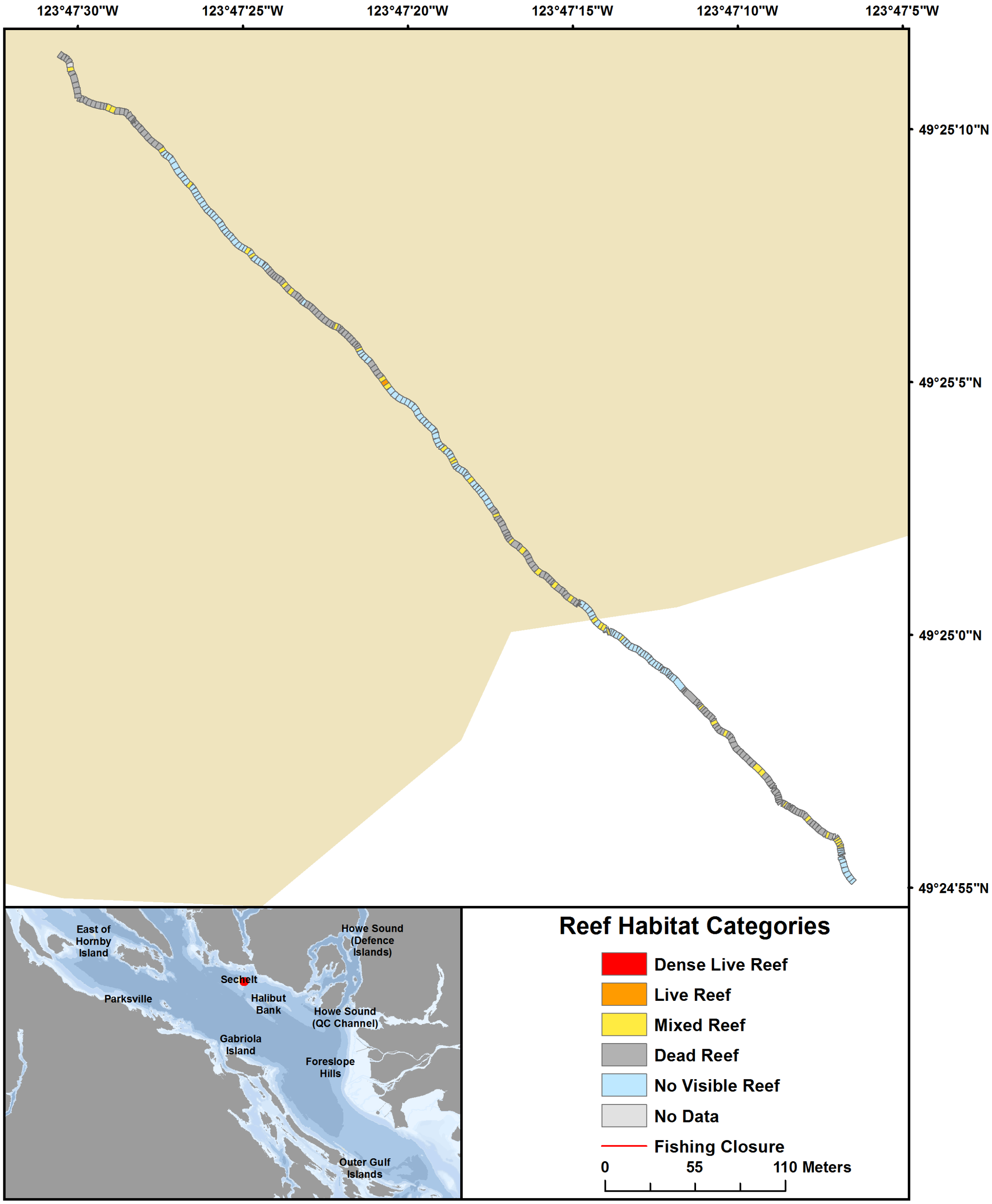


Sechelt - Pac2013-070 Transect 6



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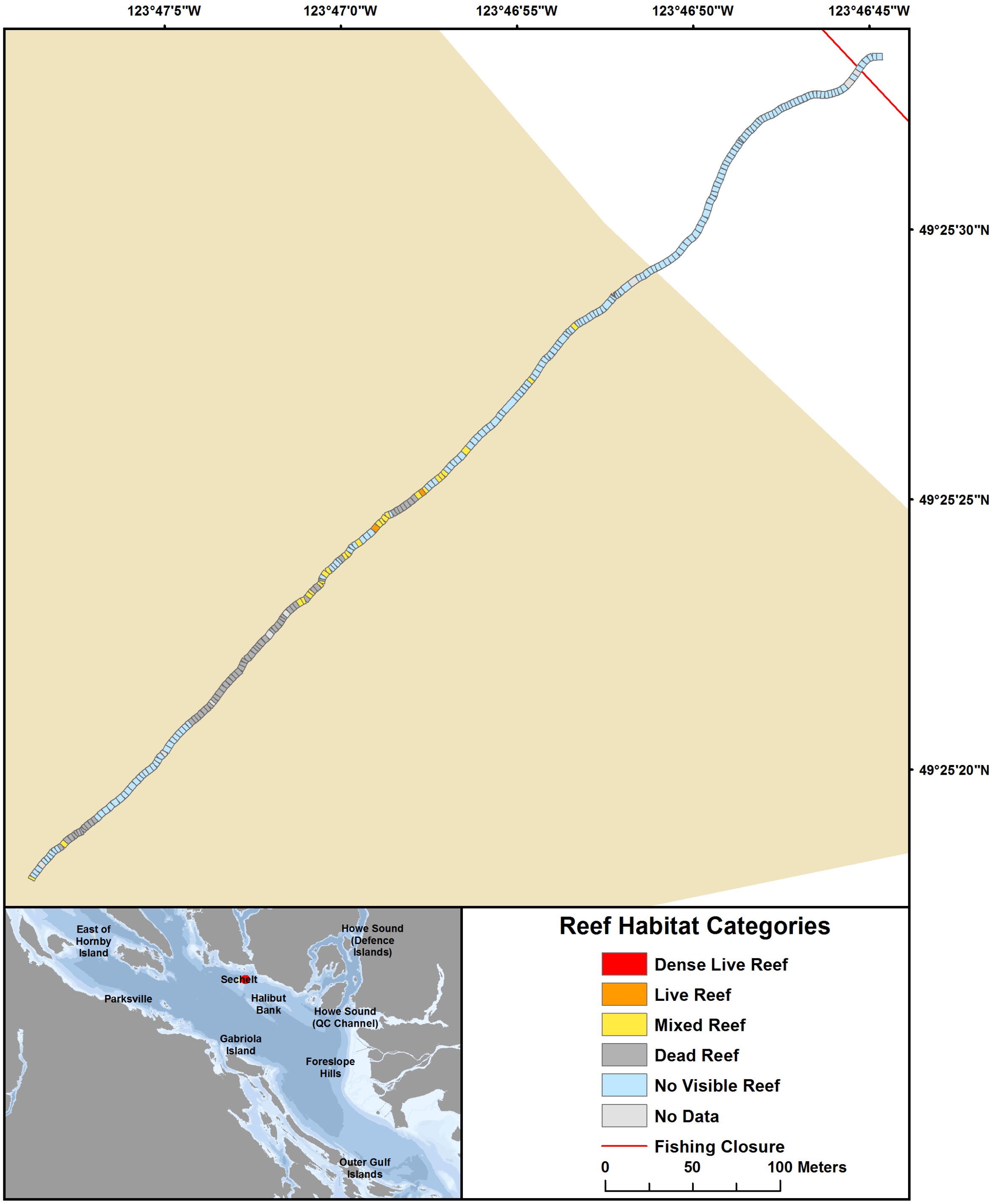

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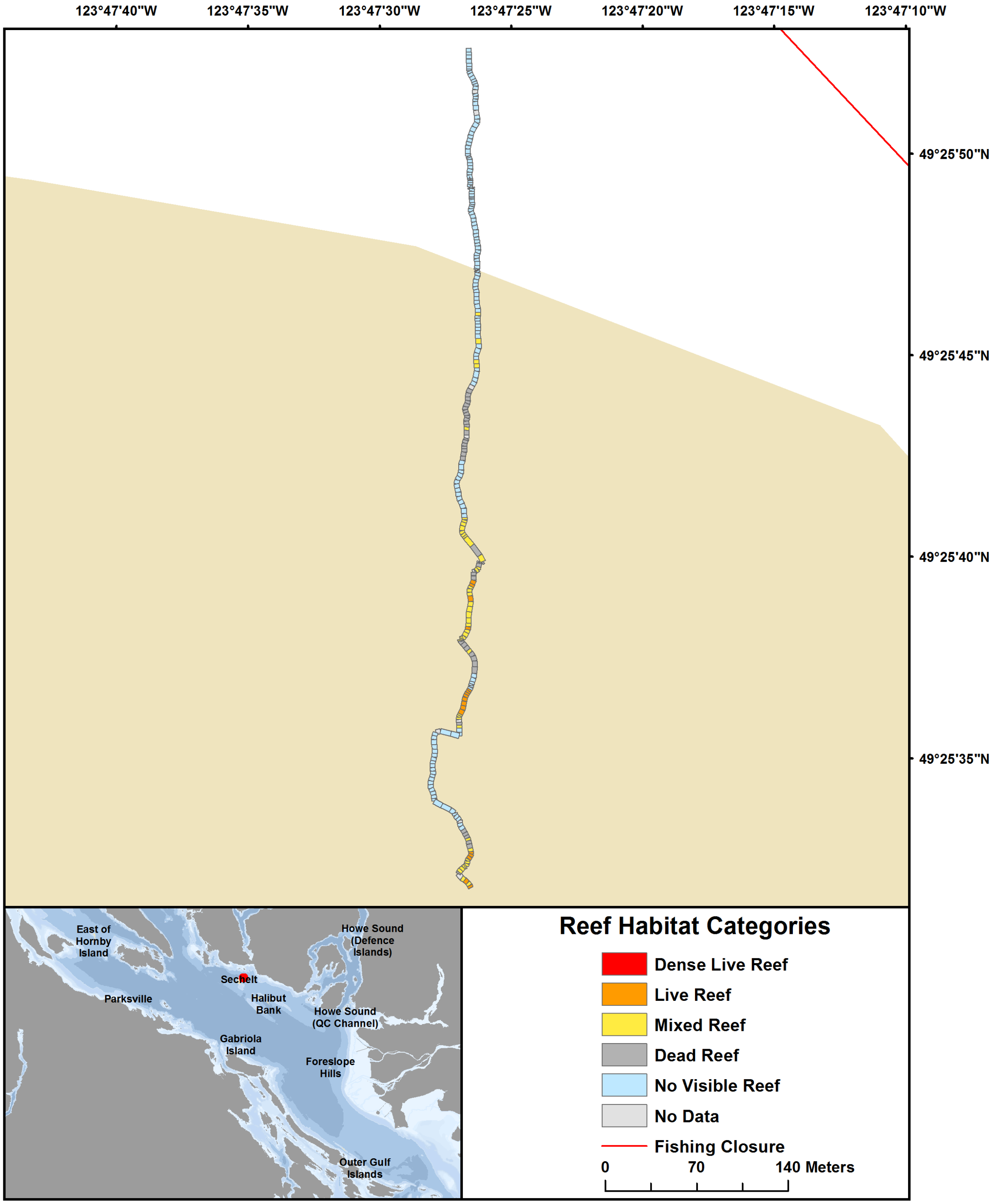

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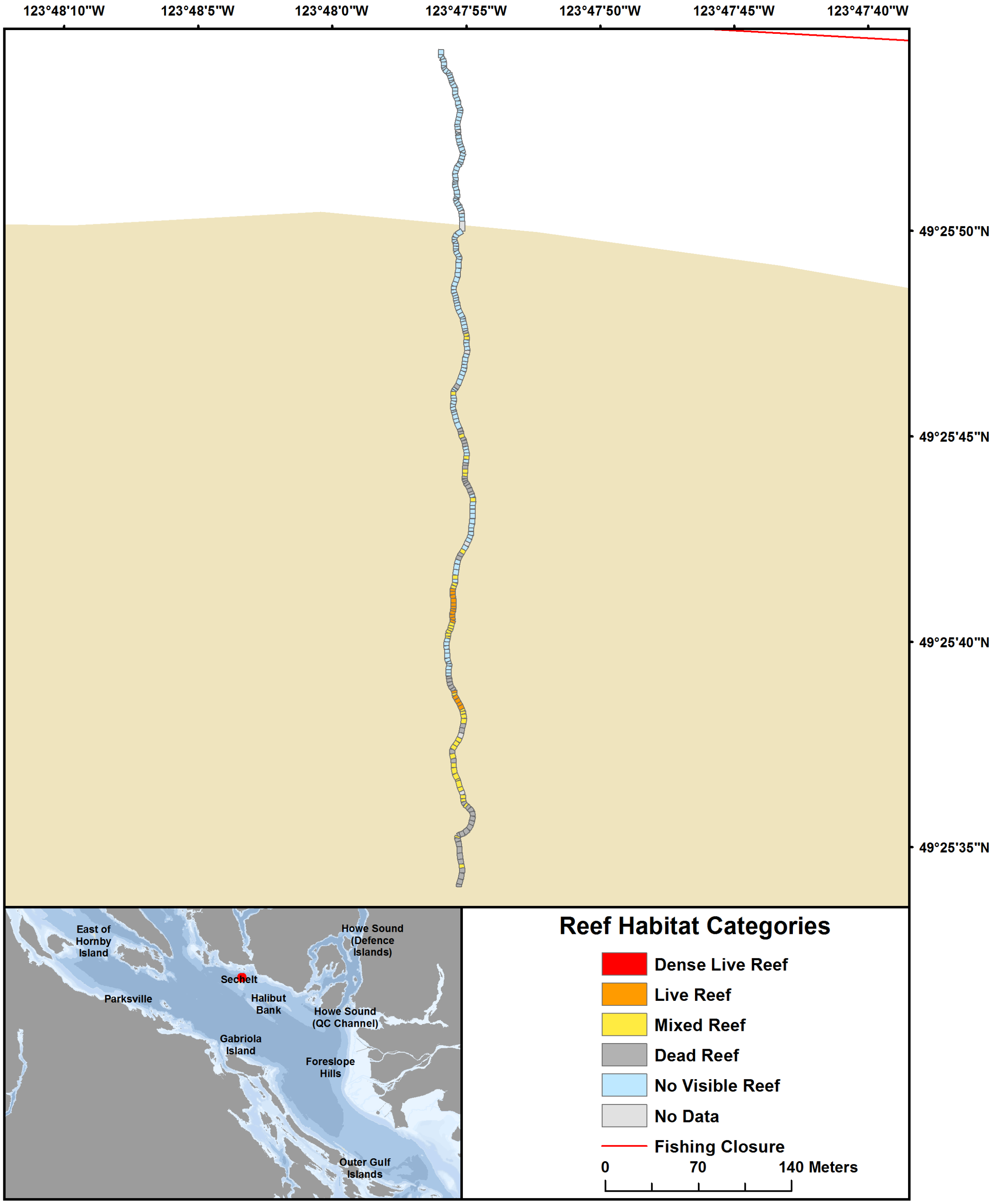

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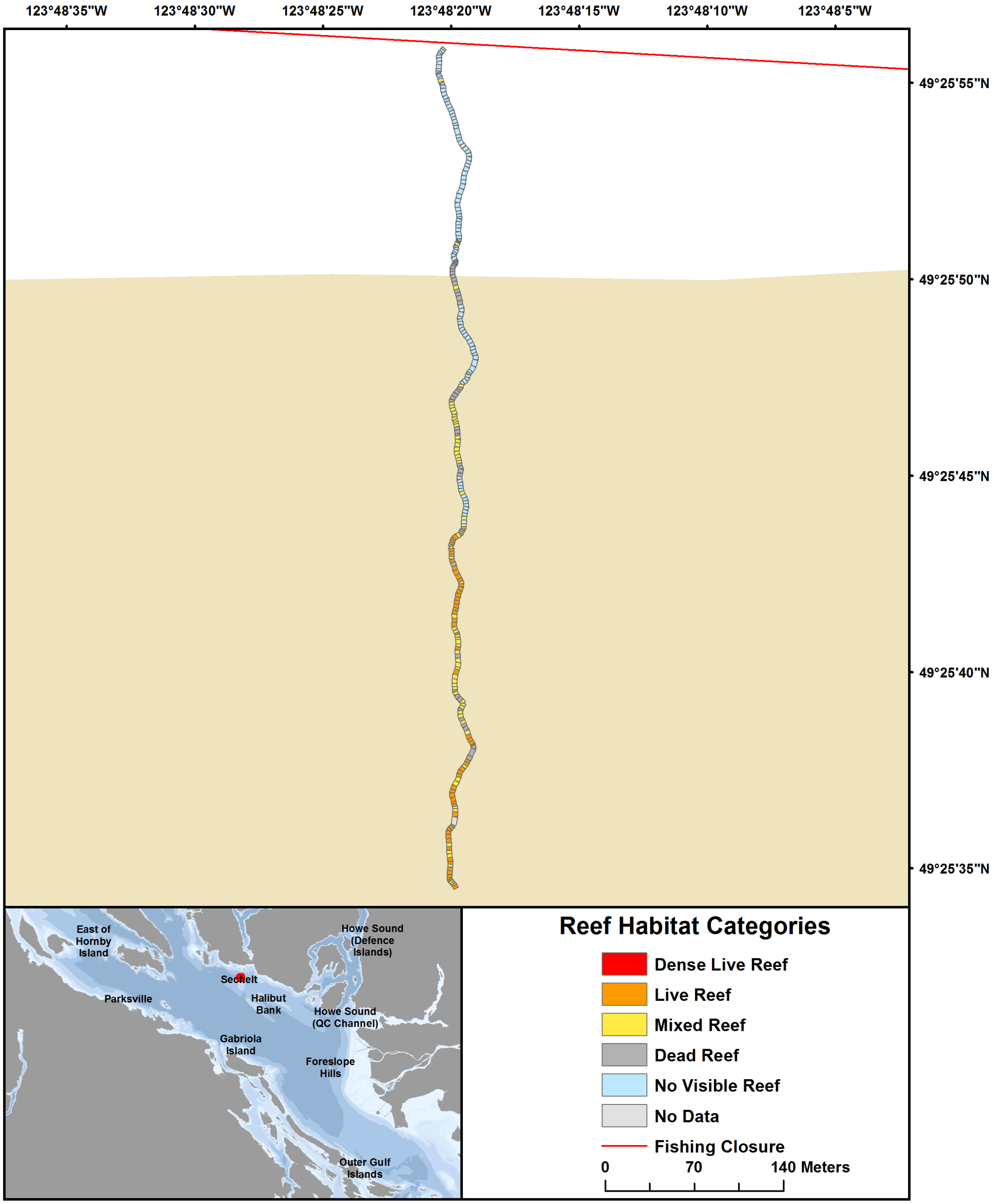

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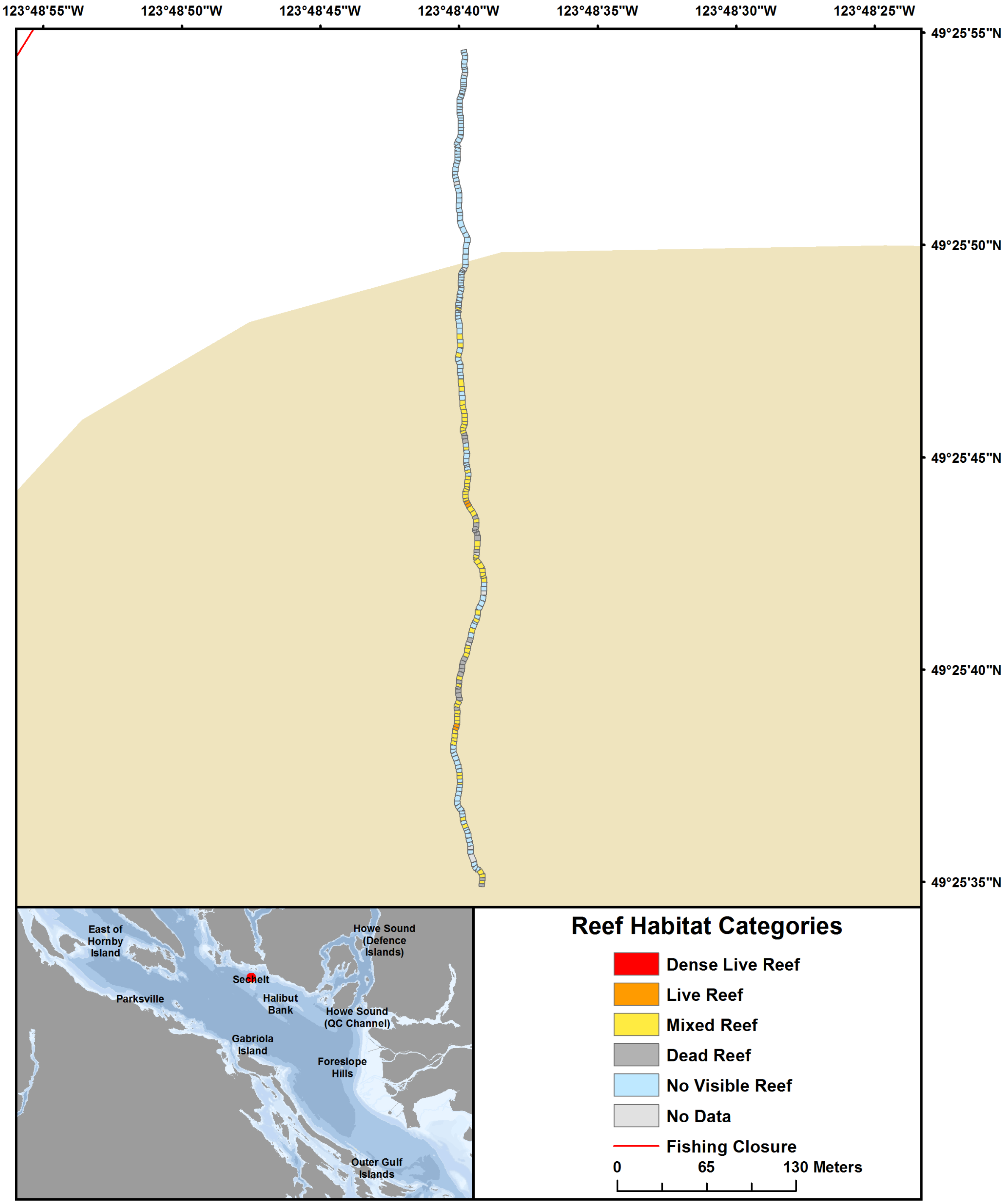

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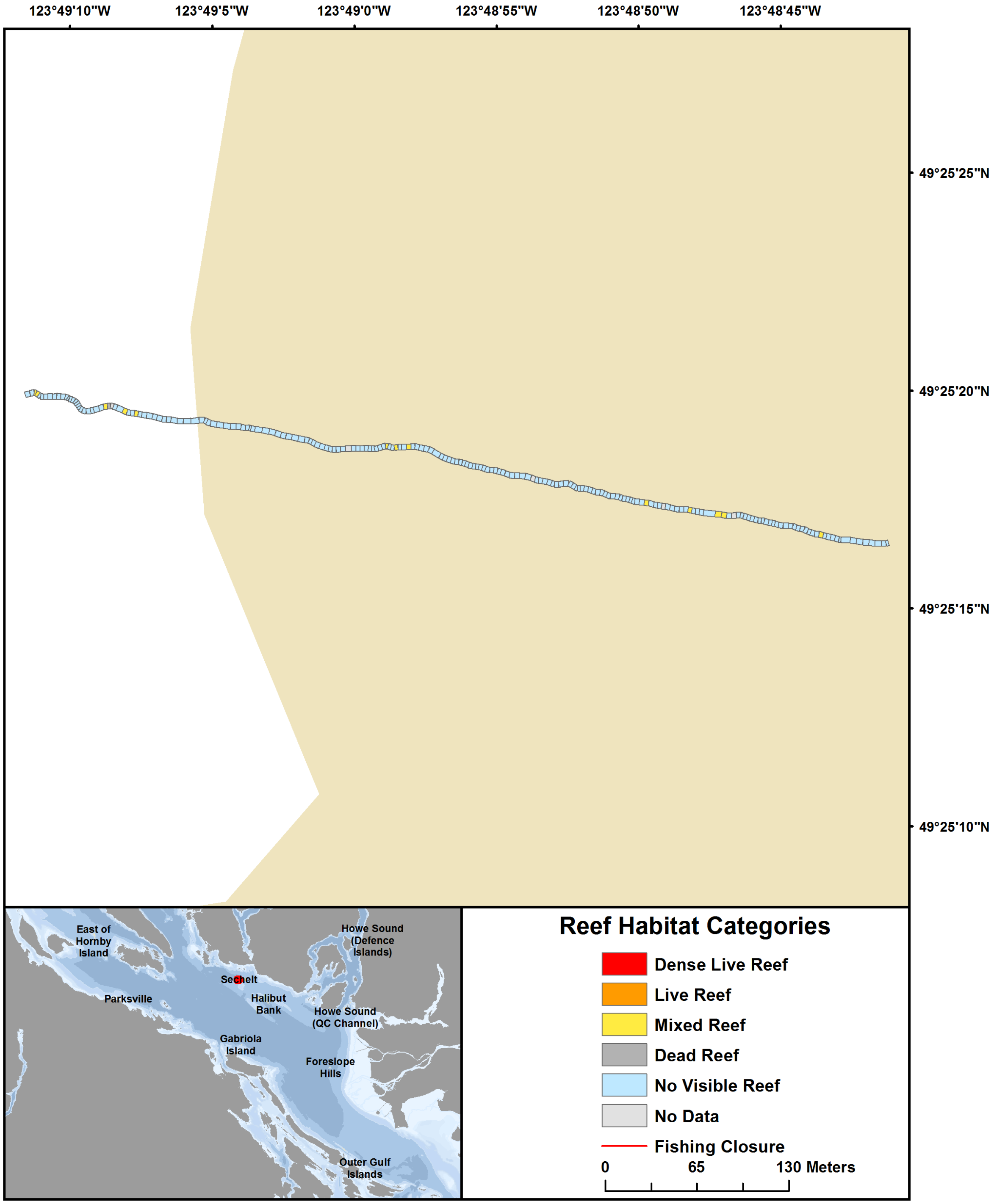

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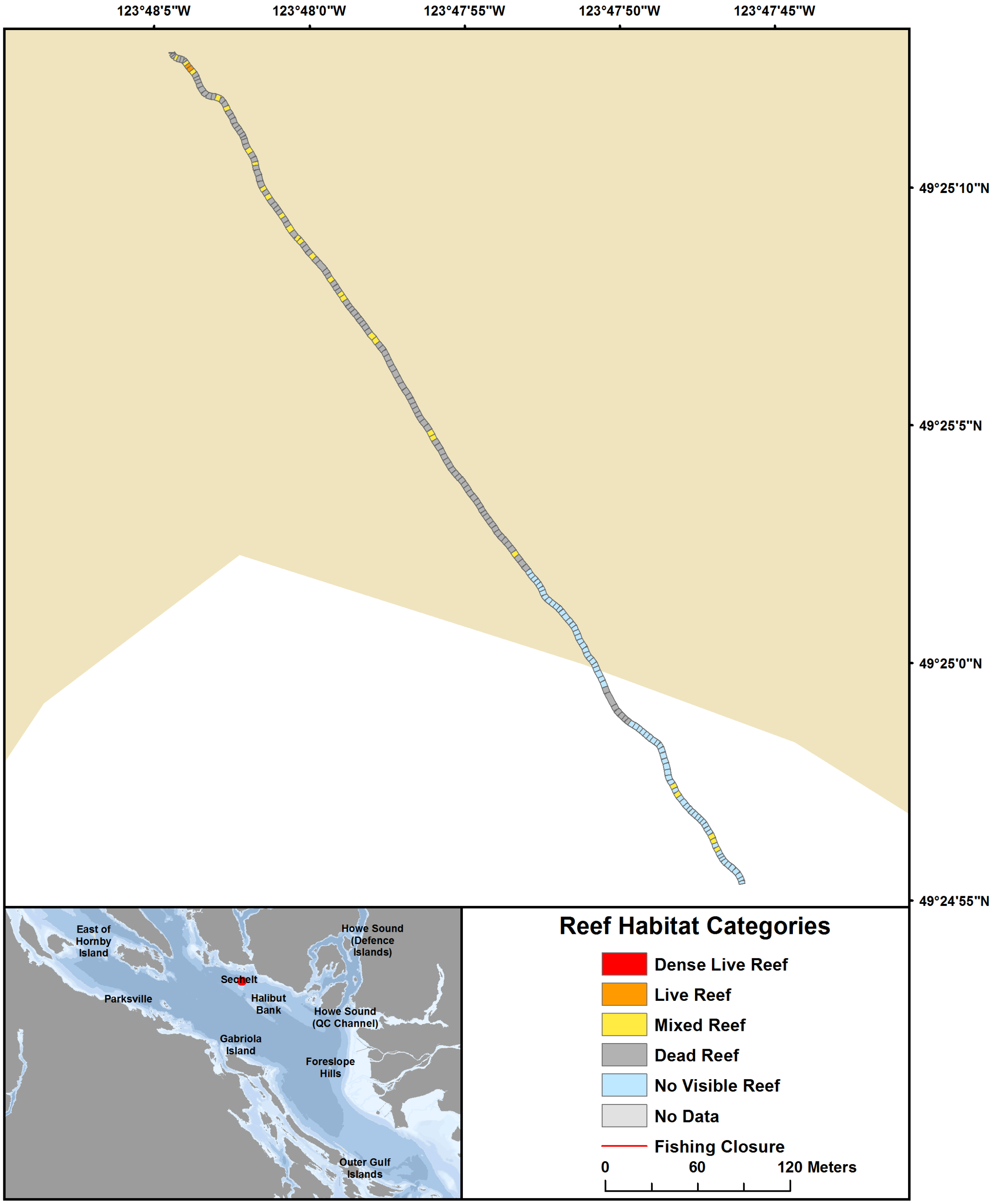

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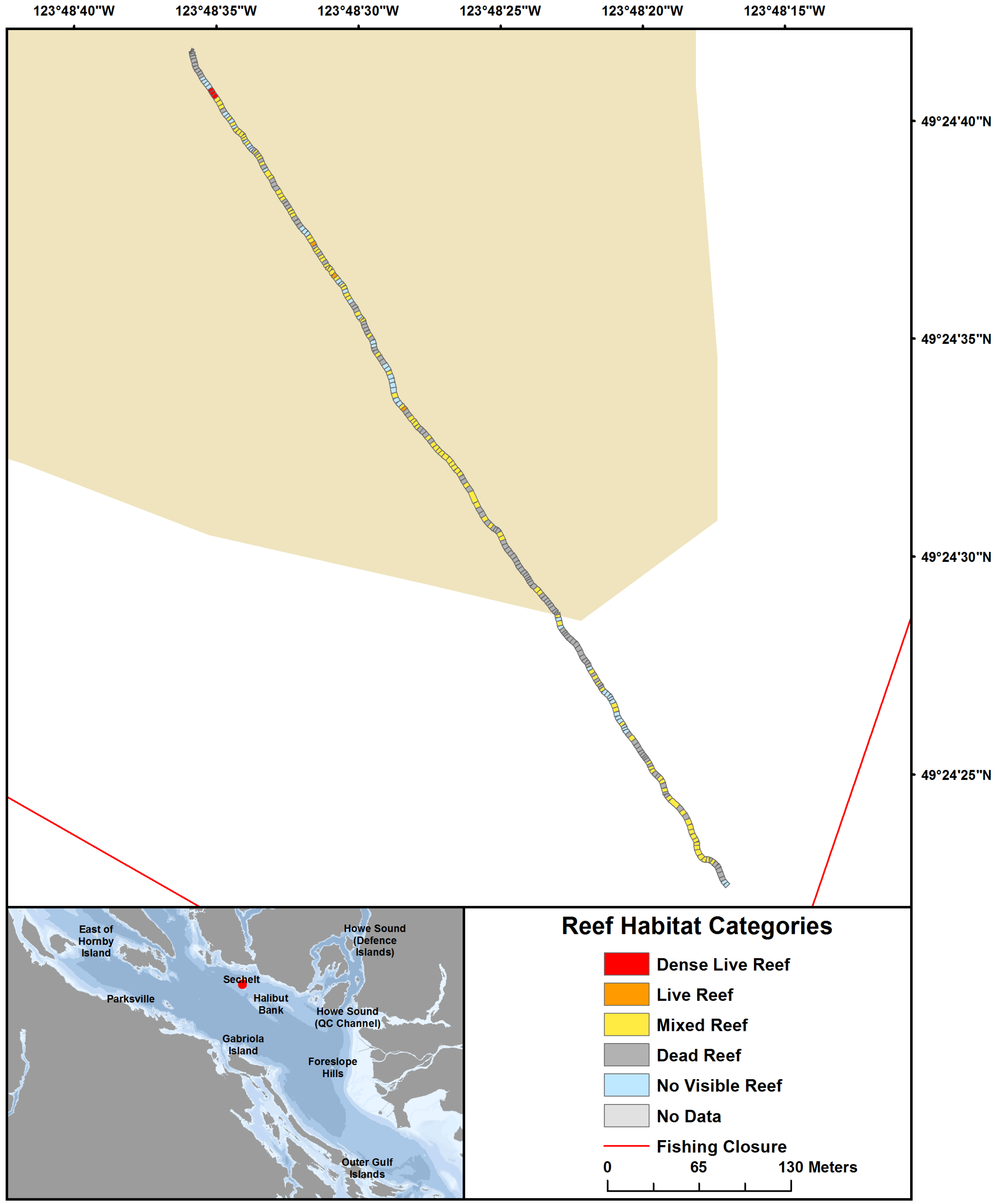

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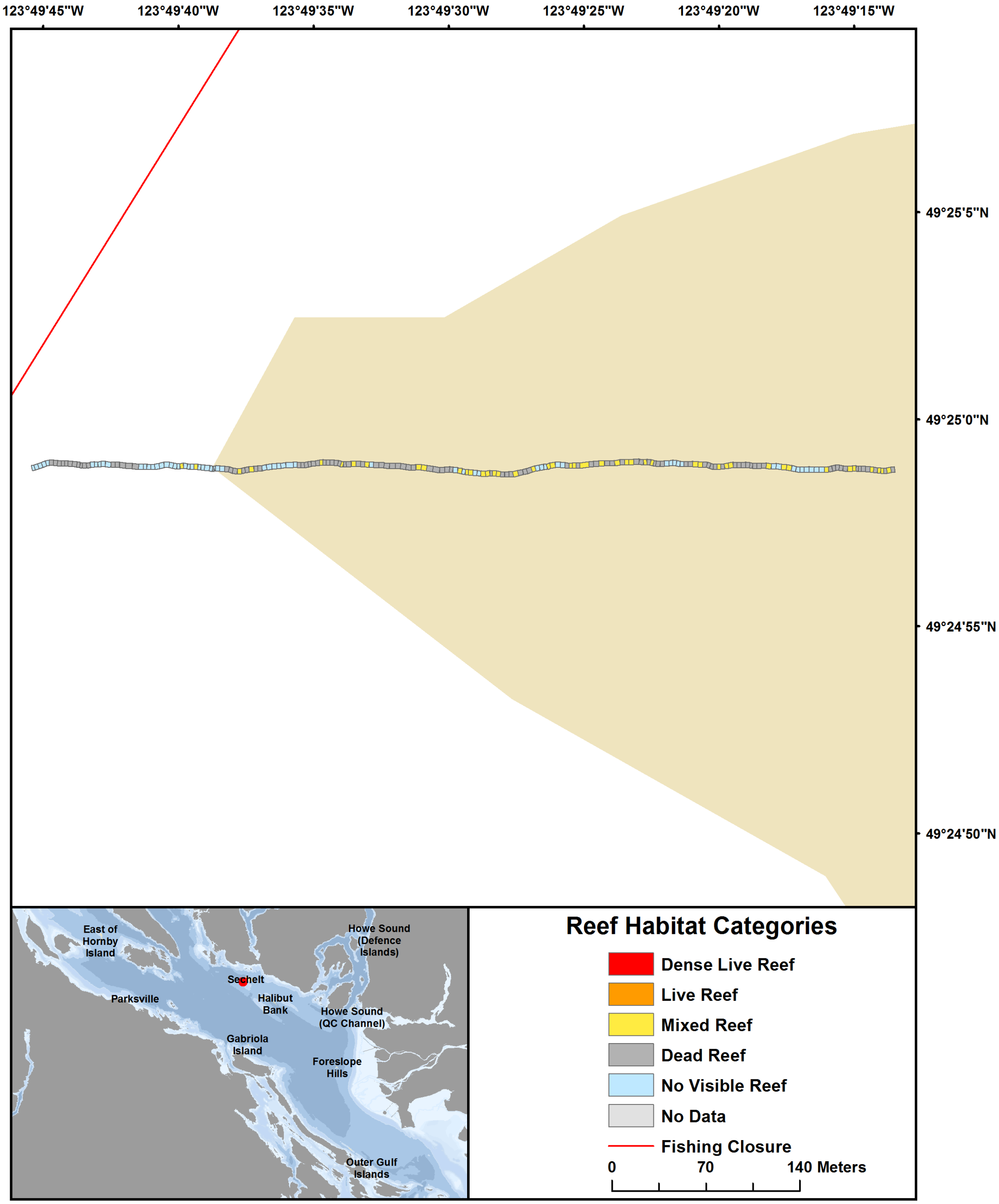

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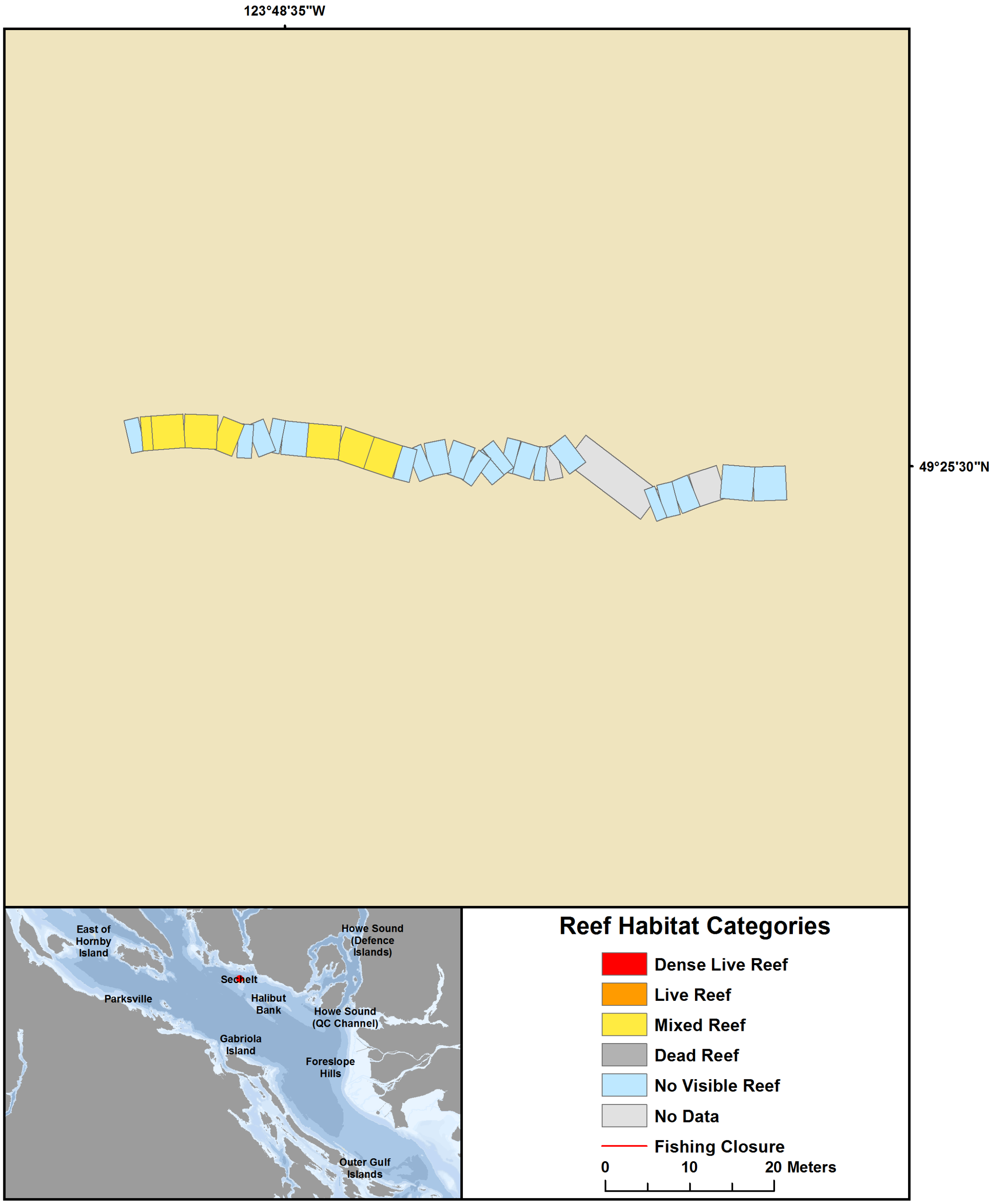
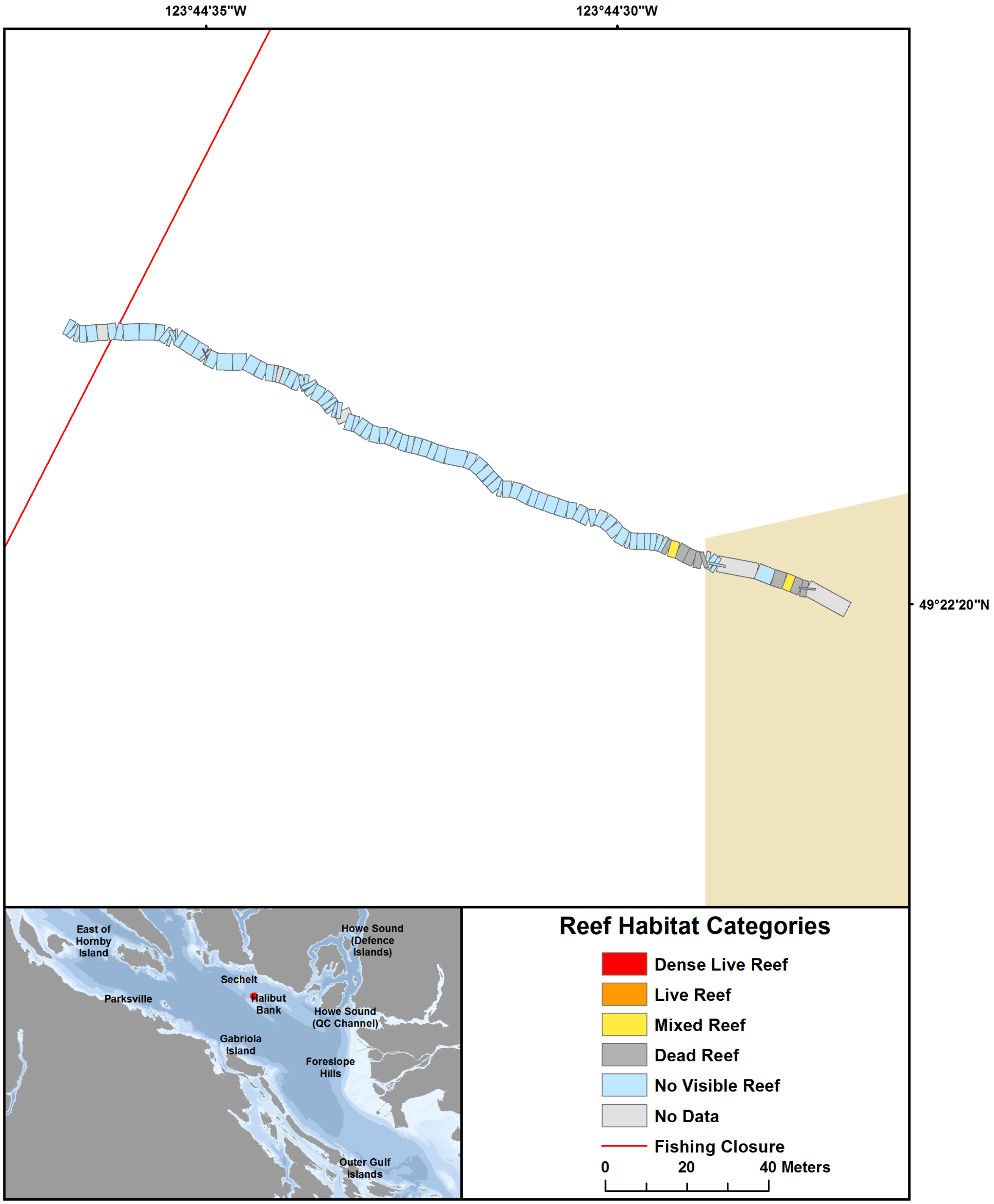
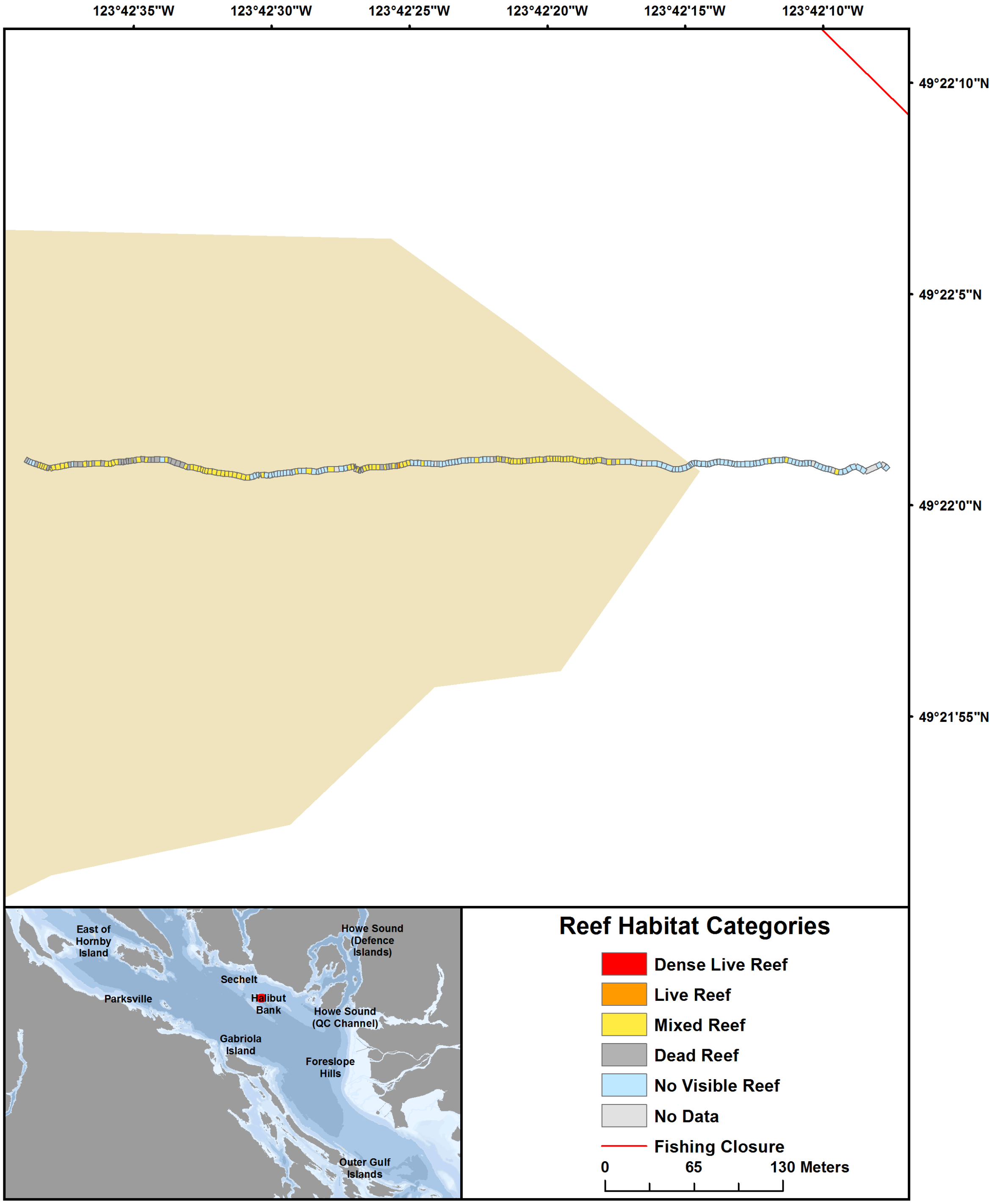


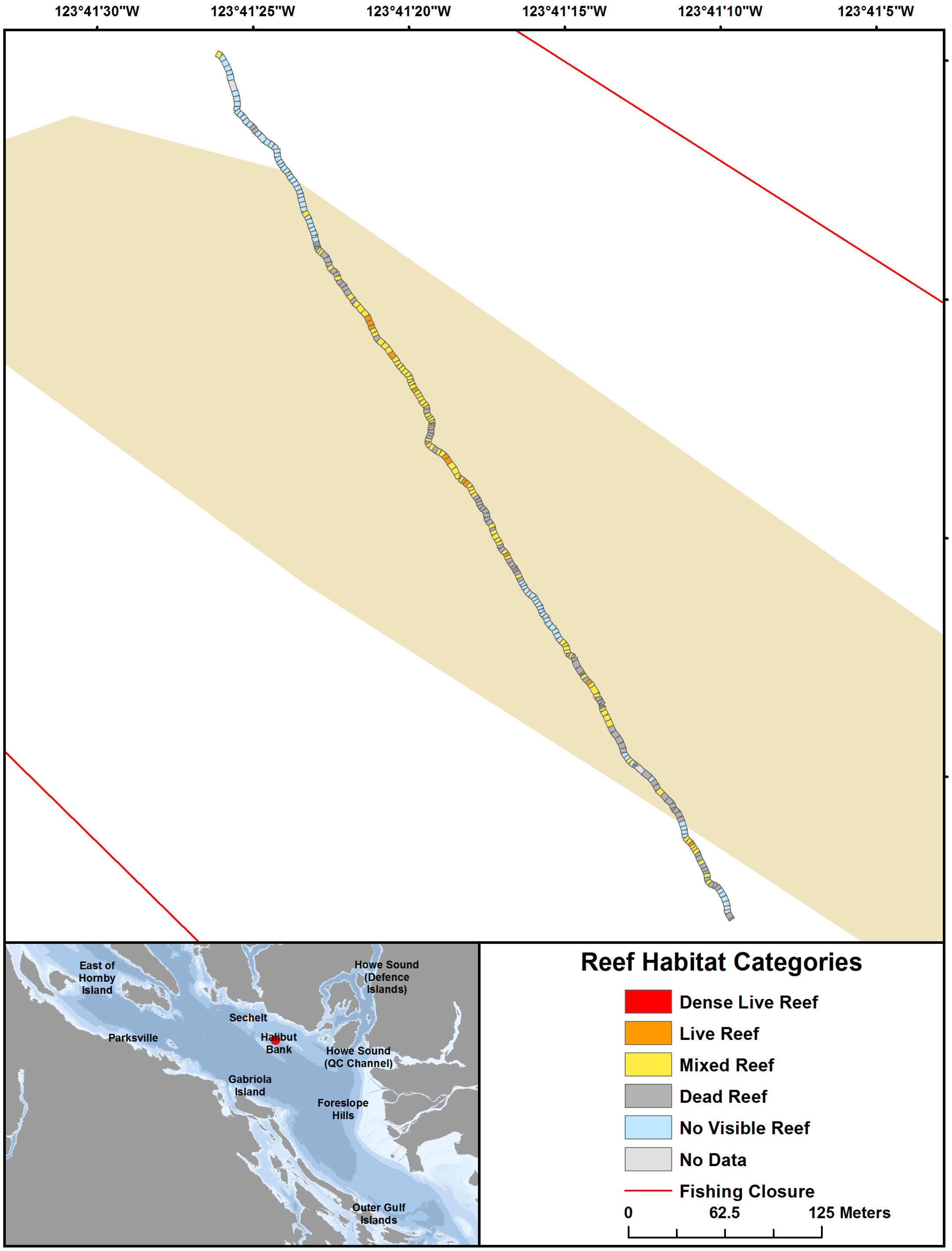
Halibut Bank - Pac2013-070 Transect 13



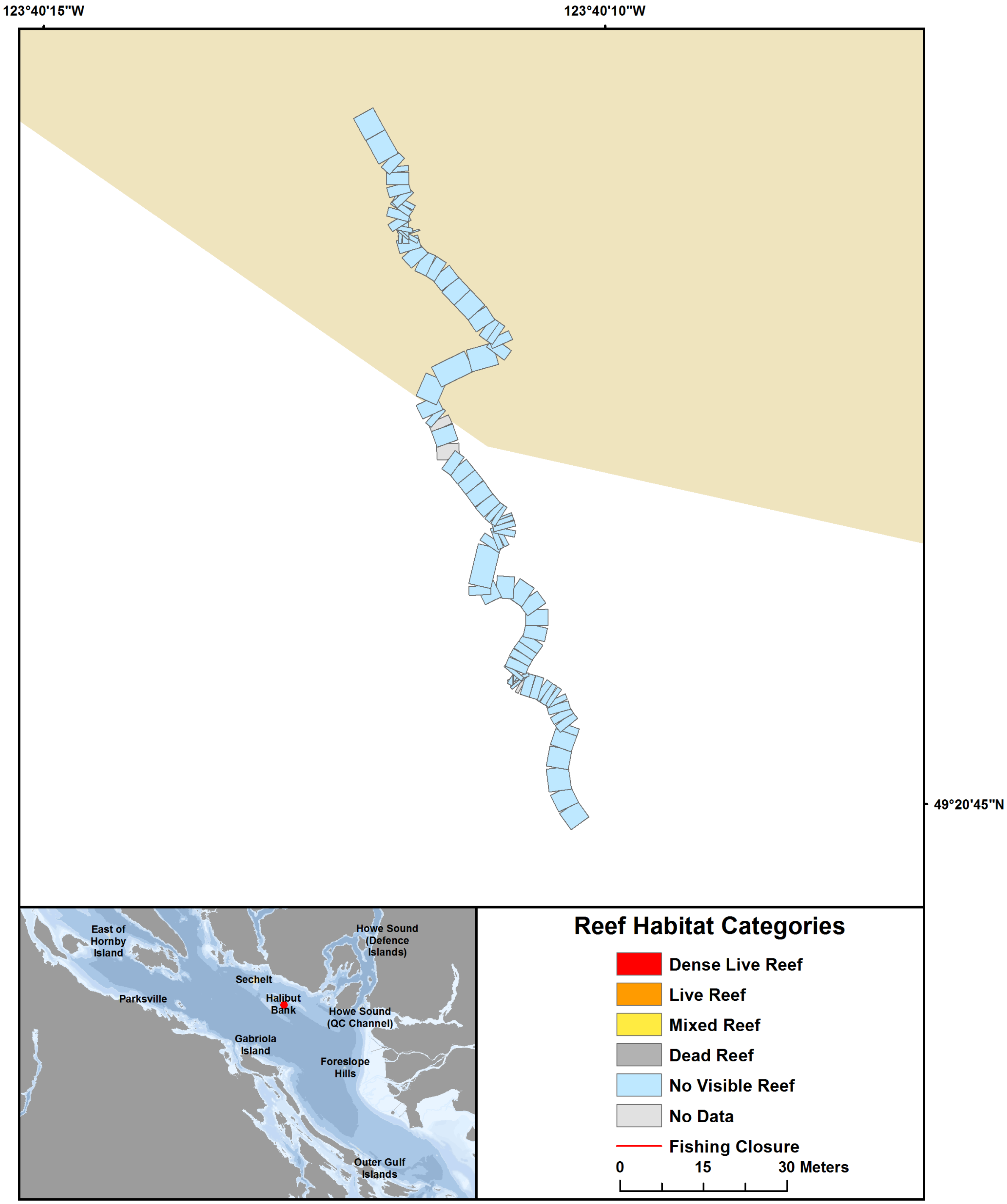
Halibut Bank - Pac2013-070 Transect 14



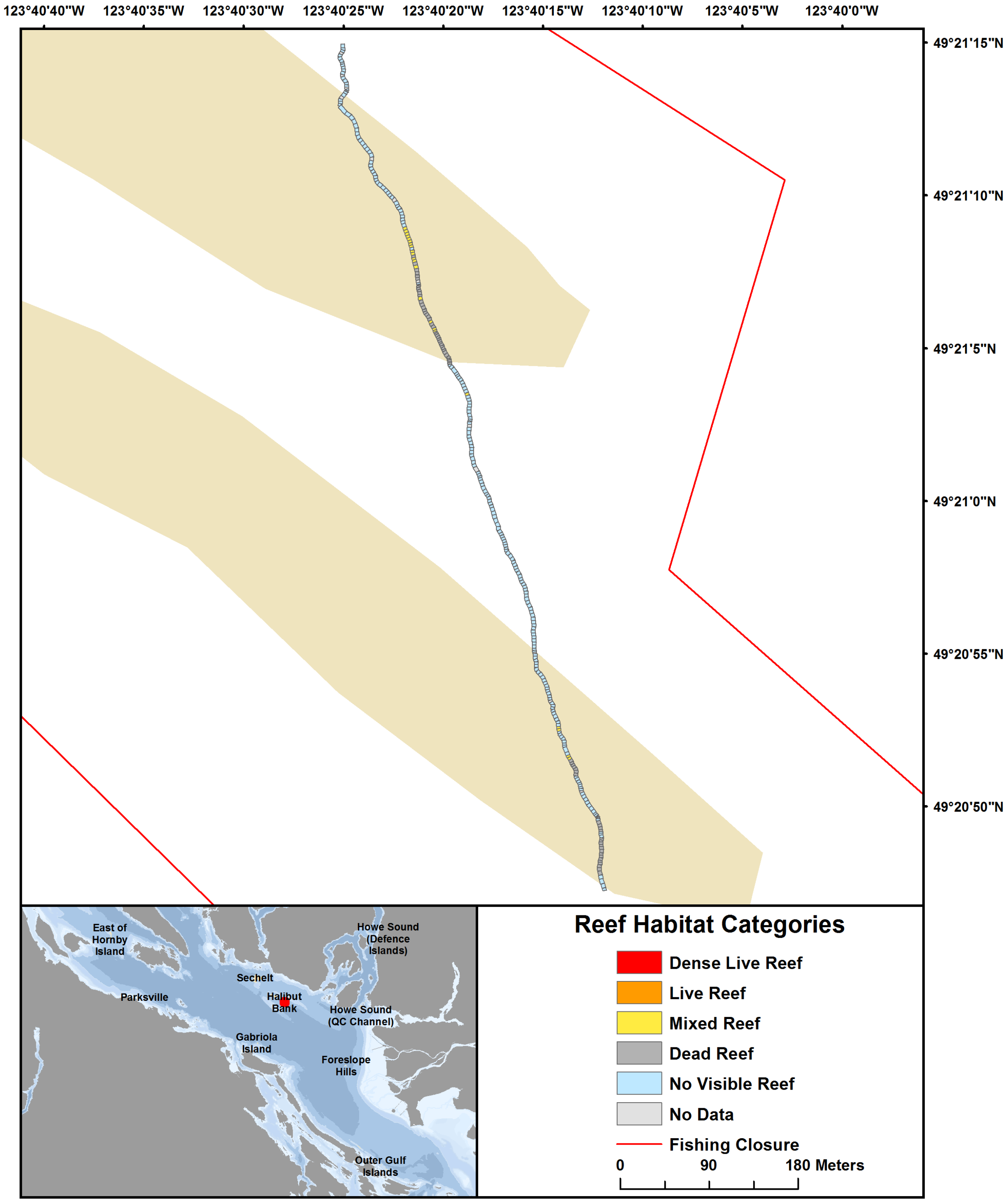
Halibut Bank - Pac2013-070 Transect 15



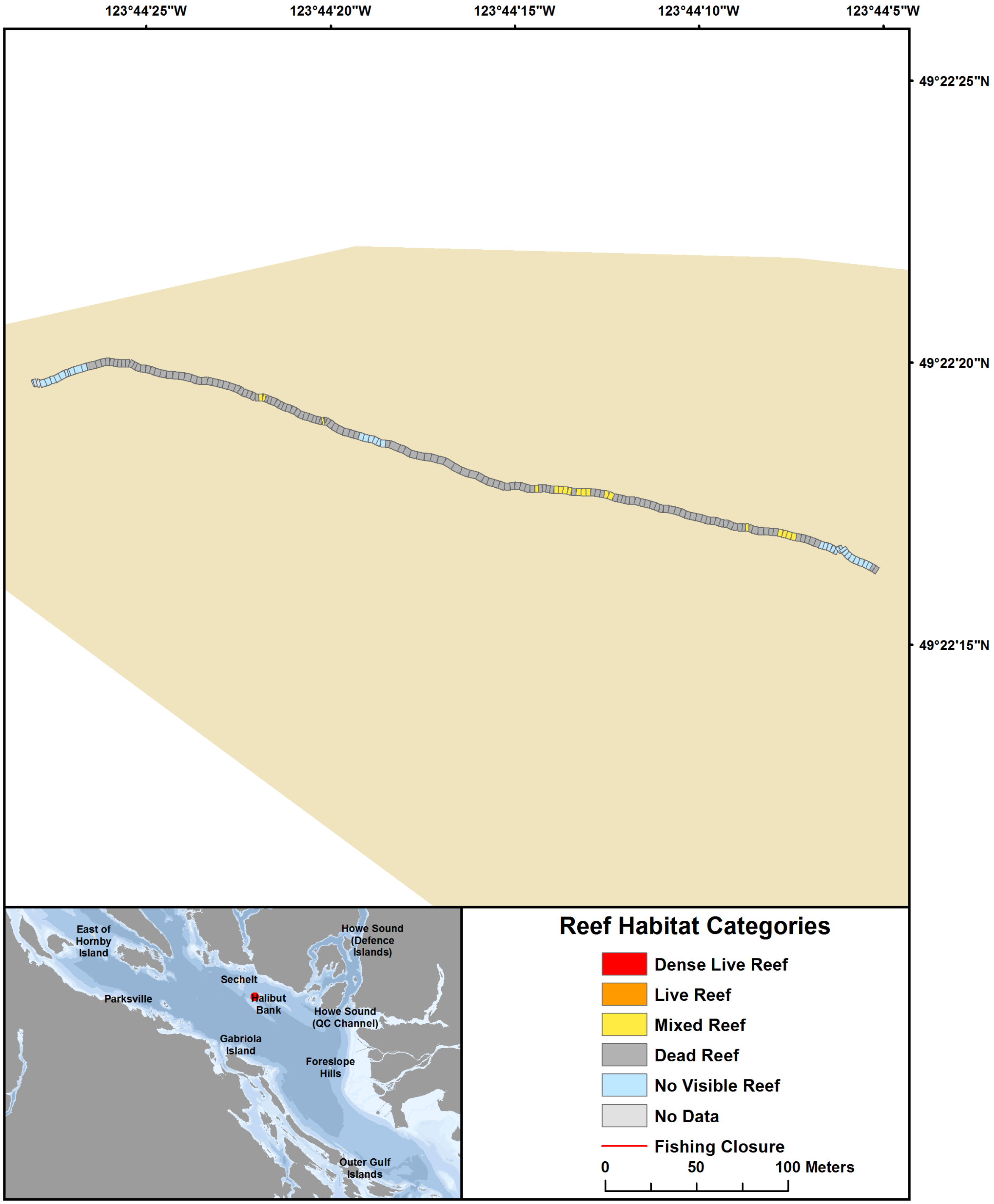
Halibut Bank - Pac2013-070 Transect 16



Halibut Bank - Pac2013-070 Transect 17



Halibut Bank - Pac2013-070 Transect 18



APPENDIX 8. PAIRWISE COMPARISONS OF COMMUNITY STRUCTURE BETWEEN REEF COMPLEXES.

Table A7-1: Pairwise comparisons of community structure between reef complexes (PerMANOVA post hoc tests, α = 0.05, P≤0.0375 after Benjamini and Hochberg (1995) correction)

Reef A	**Reef B**	**F**	**R2**	**BH adjusted p-value**
East of Hornby Island	Halibut Bank	6.25	0.32	0.003
East of Hornby Island	Howe Sound (QC Channel)	8.25	0.26	0.003
East of Hornby Island	Outer Gulf Islands	10.49	0.40	0.003
Foreslope Hills	Howe Sound (QC Channel)	4.52	0.18	0.003
Foreslope Hills	Sechelt	5.89	0.20	0.003
Halibut Bank	Howe Sound (QC Channel)	9.34	0.26	0.003
Halibut Bank	Outer Gulf Islands	5.89	0.24	0.003
Howe Sound (QC Channel)	Outer Gulf Islands	11.28	0.28	0.003
Howe Sound (QC Channel)	Parksville	8.90	0.31	0.003
Howe Sound (QC Channel)	Sechelt	12.36	0.25	0.003
Outer Gulf Islands	Parksville	8.56	0.40	0.003
Outer Gulf Islands	Sechelt	12.37	0.29	0.003
Howe Sound (Defence Islands)	Sechelt	4.08	0.16	0.005142857
Parksville	Sechelt	6.88	0.24	0.005142857
Halibut Bank	Sechelt	4.25	0.13	0.0072
Halibut Bank	Parksville	6.99	0.41	0.009
East of Hornby Island	Foreslope Hills	8.54	0.52	0.012
Howe Sound (Defence Islands)	Outer Gulf Islands	3.71	0.22	0.012
East of Hornby Island	Parksville	5.77	0.45	0.013263158
East of Hornby Island	Howe Sound (Defence Islands)	6.46	0.48	0.018857143
Gabriola Island	Outer Gulf Islands	3.54	0.23	0.018857143
Howe Sound (Defence Islands)	Howe Sound (QC Channel)	2.58	0.11	0.024545455
Gabriola Island	Howe Sound (QC Channel)	2.81	0.13	0.031304348
Foreslope Hills	Halibut Bank	2.64	0.19	0.0345
East of Hornby Island	Sechelt	2.65	0.10	0.03456

Reef A	Reef B	F	R2	BH adjusted p-value
Foreslope Hills	Howe Sound (Defence Islands)	2.42	0.33	0.041538462
Foreslope Hills	Parksville	10.53	0.68	0.046666667
Gabriola Island	Halibut Bank	2.12	0.19	0.048413793
East of Hornby Island	Gabriola Island	2.23	0.27	0.048413793
Foreslope Hills	Gabriola Island	4.14	0.51	0.08
Foreslope Hills	Outer Gulf Islands	1.73	0.11	0.080129032
Gabriola Island	Howe Sound (Defence Islands)	2.72	0.48	0.105882353
Gabriola Island	Parksville	13.22	0.82	0.105882353
Howe Sound (Defence Islands)	Parksville	9.15	0.70	0.105882353
Halibut Bank	Howe Sound (Defence Islands)	1.64	0.14	0.118285714
Gabriola Island	Sechelt	1.56	0.07	0.16

APPENDIX 9. RESULTS OF INDICATOR SPECIES ANALYSES FOR INDIVIDUAL REEF COMPLEXES.

Indicator species	**Howe Sound - Defence Islands**			**Howe Sound - QC Channel**			**Foreslope Hills**			**Outer Gulf Islands**			**Gabriola Island**		
	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value
Sebastes maliger	🟨	0.117	0.885	🟥+🟧+🟨+◼	0.124	**0.005**	🟧+🟨	0.152	0.170	🟥+🟧	0.281	**0.035**	Not Observed		
Chorilia longipes	🟥	0.351	0.085	🟥+🟧+🟨	0.296	**0.005**	🟧+🟨	0.309	**0.005**	🟥	0.213	0.070	Not Observed		
Rhabdocalyptus dawsoni	🟧+🟨	0.279	0.190	🟧+🟨	0.255	**0.005**	Not Observed			🟨	0.140	0.145	No Association		
Pandalus platyceros	🟥+🟧	0.671	**0.010**	No Association			🟨+◼	0.180	0.100	🟥+🟧	0.152	0.230	🟧+🟦	0.276	0.360
Munida quadrispina	🟥+🟧+🟨+ ◼	0.497	**0.030**	🟥+🟧+🟨	0.482	**0.005**	🟧+🟨+◼+🟦	0.402	**0.015**	🟥	0.481	**0.005**	🟥+🟧+🟨+ ◼	0.715	**0.005**
Ophiuroidea	🟦	0.443	**0.040**	🟦	0.133	**0.005**	Not Observed			◼+🟦	0.140	0.155	Not Observed		
Pennatulacea	🟦	0.582	**0.005**	◼+🟦	0.385	**0.005**	◼+🟦	0.478	**0.005**	🟦	0.038	1.000	🟦	0.430	0.050

Indicator species	**Parksville**			**East of Hornby Island**			**Sechelt**			**Halibut Bank**		
	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value	Habitat category	Indicator value	*P* value
Sebastes maliger	🟧	0.995	**0.005**	🟥+🟧	0.125	0.165	🟧+🟨	0.088	0.100	Not Observed		
Chorilia longipes	🟦	0.043	1.000	🟥	0.215	**0.015**	🟥	0.301	**0.005**	🟨+◼	0.077	0.185
Rhabdocalyptus dawsoni	🟧+🟨+◼	0.920	**0.005**	No Association			🟧+🟨+◼	0.531	**0.005**	🟧+🟨	0.356	**0.010**
Pandalus platyceros	🟧	0.672	0.155	No Association			No Association			🟨	0.047	0.815
Munida quadrispina	🟨+◼	0.846	**0.010**	No Association			🟥+🟧+🟨+ ◼	0.685	**0.005**	🟧+🟨+ ◼	0.572	**0.005**
Ophiuroidea	🟦	0.086	0.715	Not Observed			Not Observed			◼	0.036	1.000
Pennatulacea	🟦	0.470	0.170	Not Observed			🟦	0.189	**0.025**	🟧	0.469	**0.005**

Figure A9-1. Taxa identified as indicator species: habitat associations within reef complexes determined by the Dufrêne-Legendre Indicator Species Analysis using video dataset. Higher indicator values indicate stronger associations (maximum indicator value is 1). Statistically significant associations are bolded (P<0.05). Habitat categories are: 🟥 = dense live reef, 🟧 = live reef, 🟨 = mixed reef, ◼ = dead reef, 🟦 = no visible reef.

APPENDIX 10. MINIMUM NUMBER OF STILL IMAGES PER TRANSECT TO BE ANALYZED.

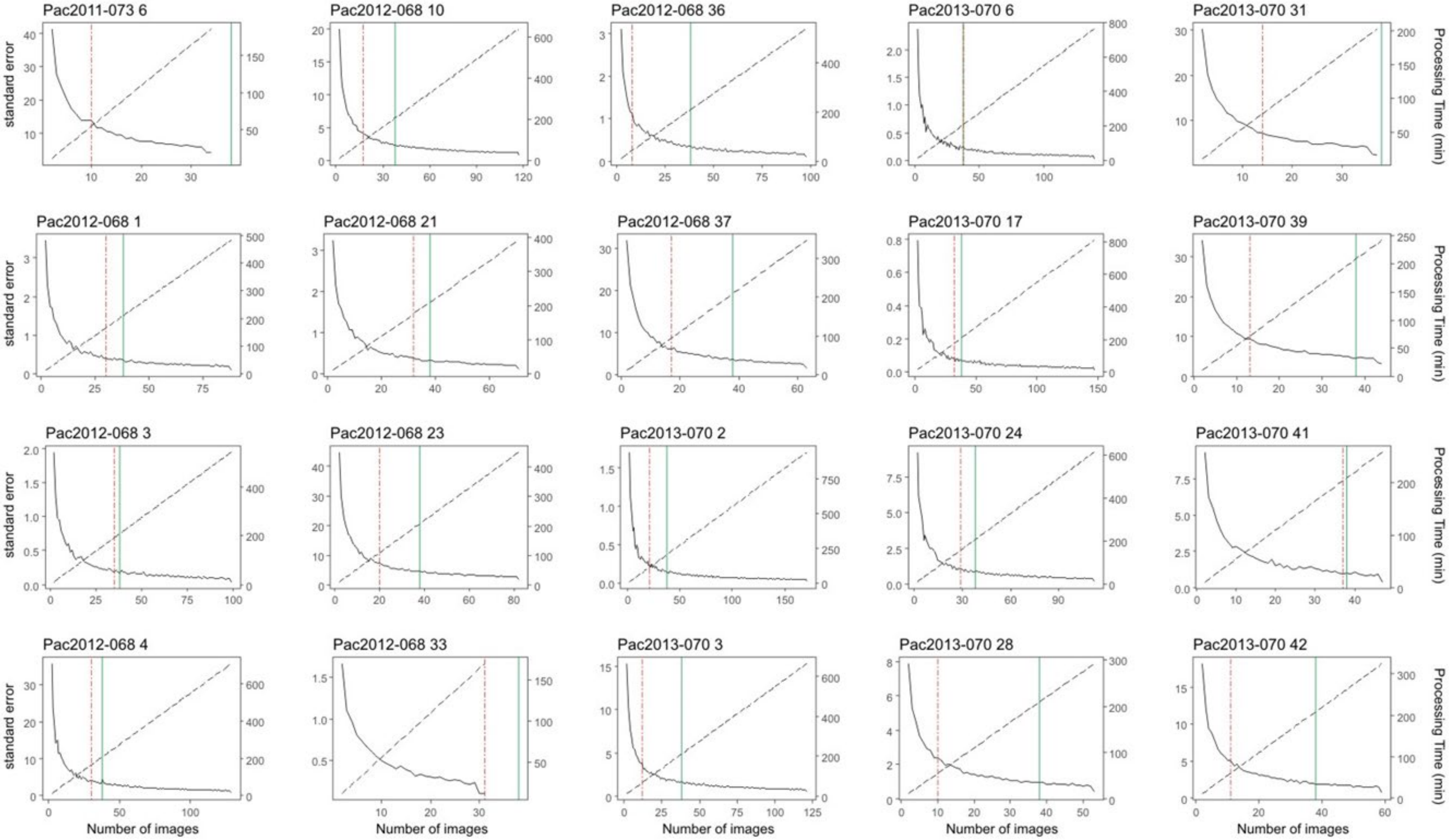


Figure A10-1. Standard error (95th percentile; solid black line) and image processing time(dotted black line) as a function of the number of still images analyzed, per transect, using the grid method, for 20 transects. The cut-offs (shown as red dotted lines) were determined by applying Hewitt et al. (1992) modification of the Bros and Cowell (1987) randomization technique for optimizing sample size; for each transect, the cut-off was set as the point at which the slope of the curve was ≤1% of the initial slope. Maximum observed cut-off value of 38 images per transect – recommended as minimum number of images per transect to be analyzed - is shown as a green line.