QUANTIFYING COASTAL BLUE CARBON: A LITERATURE REVIEW OF BLUE CARBON METHODS IN CANADIAN ECOSYSTEMS

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INTRODUCTION

This report provides a summary of published blue carbon methods applied in Canadian ecosystems. The report focuses on four main areas of scientific interest: carbon storage, carbon sequestration, greenhouse gas (GHG) fluxes, and carbon transport. The carbon storage section of the report summarizes methods that quantify the organic and inorganic carbon in coastal ecosystem sediment and biomass carbon pools. The carbon sequestration section summarizes methods used to quantify sediment and carbon accumulation rates as well as methods for dating sediments. The GHG flux section summarizes methods to determine changes in carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) concentrations at the surface of coastal vegetated ecosystems. And the carbon transport section summarizes methods to track the movement of carbon between ecosystems and determine blue carbon provenance.

All information in this report is based on a literature review conducted over the summer of 2022. Potential sources were identified using Google Scholar. Search terms included, but were not limited to: "Blue Carbon" "Canadian" "Salt Marsh" "Seagrass Meadow" "Carbon Storage" "Gas Flux" etc. This report is a synthesis of published methods applied in Canadian blue carbon ecosystems and is meant to be used as a reference for those interested in measuring carbon in Canadian nearshore marine and coastal ecosystems. It provides a basic overview of the procedures and equipment needed to quantify carbon stocks, carbon sequestration, GHG fluxes, and carbon transport. However, those looking to perform these methods should directly review the sources that are cited for further detail concerning the method of interest. As such, this report is meant to act as a guide to assist in finding the available and applicable resources for blue carbon measurement in Canadian ecosystems.

The geographic scope of this review was limited to Canada and Canadian-applicable ecosystems (e.g., northern United States) due to time constraints associated with a summer internship. Exceptions were made where publications focused on Canadian ecosystems referenced international sources as a basis for their methods, and where few publications on a subject were found. For example, much of the information regarding emerging blue carbon transport methods were not found in Canadian-based studies, thus international methods were included here in hopes that they may be used to assist in developing Canadian protocols.

BLUE CARBON METHODS

CARBON STORAGE

Carbon storage is a valuable ecosystem service that is gaining increased attention as governments and communities look for ways to mitigate climate change. Most carbon in nearshore marine and coastal ecosystems is stored in sediments or soil and is quantified by analyzing sediment or soil cores. Above- and below-ground biomass carbon can also be quantified to provide a complete estimate of carbon stocks. The following summaries of field sampling and laboratory analysis methods are based on literature relevant to sampling along Canada's coastlines and focus on salt marsh and seagrass systems.

FIELD SAMPLING

Sediment and soil (used interchangeably here) are the dominant carbon pools in marine ecosystems (Howard et al. 2014) and therefore recent literature has focused on the determination of sediment and soil carbon as the main approach to quantifying carbon stocks. Techniques for sampling sediment and soil carbon are similar across seagrass and salt marsh ecosystems (Howard et al. 2014), but often include modifications to account for ecosystem-specific challenges, such as tidal inundation. Biomass is often, but not always, sampled and is analysed separately. Biomass sampling techniques vary depending on ecosystem and vegetation type. Biomass and sediment carbon can be summed to provide a representative value of total-ecosystem carbon stocks.

Site Selection

Salt Marshes

When sampling for carbon data, sampling locations should be chosen to represent the entire habitat range for which carbon stocks are quantified. Some considerations when choosing sampling sites include the type of plant species present, tidal inundation, salinity gradients and anthropogenic disturbance (Chastain and Kohfeld 2016). Ditches and channels should be avoided when choosing sampling sites, as they likely yield slightly higher carbon content estimates (by approximately 5 per cent) (Chastain 2017).

The most common approach to sampling salt marsh is to divide the habitat into low and high marsh based on vegetation type (Chastain et al. 2021). Carbon is measured separately for low and high marsh, but measurements can be combined for an estimate of marsh-wide carbon stocks (Chastain 2017; Chastain et al. 2021; Gonneea et al. 2019; van Ardenne et al. 2018). Vegetation type can be used to differentiate between low and high marsh remotely through satellite imagery or colour orthophoto analysis, however, this type of remote sensing requires validation through field work. For example, a study by Chastain et al. (2021) used ArcMap 10.3 and 50 cm x 50 cm aerial orthophotos to delineate marsh zones for their study sites. Colour delineation in GIS verified with vegetation survey data resulted in an accuracy of 94 per cent. Gailis et al. 2020 used a 50 m x 50 m resolution Google satellite base map to delineate marsh zones using QGIS 3.0 and ground-truthed the analysis with vegetation surveys at 176 sampling points.

Transects for salt marsh sampling sites typically run from the upland edge to the seaward edge of marshes, perpendicular to the shoreline (Chastain 2017; Chastain and Kohfeld 2016; Howard et al. 2014; Gonneea et al. 2019; van Ardenne et al. 2021) and multiple parallel transects should be sampled (Chastain et al. 2021). Gridded sampling or standardised distances between samples is ideal for GIS analysis but not always cost effective. Alternatively, sampling intervals may be adjusted depending on the size and geomorphology of each marsh (van Ardenne et al. 2018) to acquire a representative sample of ecosystem variability across a range of elevations, vegetation types and tidal inundation (Chastain and Kohfeld 2016). van Ardenne et al. (2018) suggest that the carbon stock of an ecosystem can be reasonably estimated using a limited number of samples if sample locations are chosen to represent the major axes of variation in soil depth within a study site.

Seagrass Meadows

Tidal inundation is the most prevalent concern when sampling seagrass ecosystems. Unlike in salt marsh, sampling in seagrass meadows typically occurs along tidal transects that are parallel to the shoreline (Howard et al. 2014; Postlethwaite 2018) or parallel to the current (Greiner et al. 2013) and should align with depth (Howard et al. 2014). The method for selecting specific sampling sites varies, however sites should be chosen at random along a transect and samples can be taken in duplicate (Stephens and Eckert 2018) or at paired plots with similar elevation, aspect, slope, and stratum type (Spooner 2015).

Samples are taken in the intertidal zone at low tide for ease of access and to minimize sample disturbance by water (Douglas et al. 2022; Howard et al. 2014; Murray 2017; Postlethwaite 2018). For sampling in the subtidal zone, snorkelling or SCUBA techniques may be required (Howard et al. 2014). Seasonality can affect carbon stock estimates; therefore, sampling should be conducted when standing stocks are greatest, with repetition of measurements at the same time of year in subsequent years (Howard et al. 2014).

Biomass Sampling

Samples of living biomass can be taken to quantify the amount of carbon stored in living material within a habitat, though it is not expected to be as significant as the sediment or soil carbon pool.

Salt Marshes

Few available published studies on Canadian sites include biomass sampling in salt marsh ecosystems, generally because the amount of carbon stored in biomass would be small in comparison to the soil carbon pool, especially where soils extend beyond a meter in depth. Marsh ecosystems may also include a diverse range of vegetation, including grasses, shrubs, and trees, which can complicate quantifying the carbon stored in living biomass.

To quantify salt marsh biomass, aboveground and belowground biomass is sampled within a 30 cm x 30 cm quadrant (Howard et al. 2014; Kauffman e al. 2020). Biomass estimates are completed in one of two ways: by directly harvesting and measuring plant materials within each plot, or by developing species-specific allometric equations to estimate plant biomass.

The direct harvest method generally involves destructive sampling, whereby all plant material within a quadrat is collected. Belowground biomass is sampled by separating root and rhizome material from soil cores while aboveground biomass is cut at the soil surface. Plant material is rinsed with freshwater, and oven dried to produce a biomass estimate, which may then be ground and analyzed for carbon content (Diefenderfer et al. 2018; Kauffman et al. 2020).

Alternatively, allometric equations can be developed for each species present within the study area. This approach is considered the most accurate way to estimate the aboveground carbon content within a quadrat. To determine an allometric equation, a minimum of 50 stems are cut over a range of observable heights for each species and plant biomass is determined by oven-drying to constant weight. Dried specimens can then be analyzed for carbon content and the data can be used to develop the species-specific equations relating biomass to carbon content (Howard et al. 2014). Howard et al. (2014) includes detailed descriptions for how to quantify biomass of larger types of vegetation that may be encountered within a marsh setting, such as shrubs and dead or downed wood, however these are rarely significant contributors to the ecosystem carbon pool.

Seagrass Meadows

Biomass sampling is more common in seagrass ecosystems relative to salt marshes. Aboveground sources of biomass include epiphytes (benthic diatoms and seston), and macroalgae (commonly present as drift algae) (Greiner et al. 2016; Rohr et al. 2018) in addition to seagrass. Several different methods have been used to quantify seagrass biomass. Seagrass densities can be measured by counting shoots within a specified area (Greiner et al. 2016; Rohr et al. 2018) or by harvesting plant material within quadrats (Douglas et al. 2022). Quadrats used to sample biomass in seagrass meadows range from 0.25 – 1 m^2 in size. Biomass can also be harvested by coring, pushing cores through aboveground plant material without damaging leaves, into upper root-dominated soil, which typically extends 40 cm from the sediment surface. Biomass is then separated from sediments, and dead biomass is separated from living biomass, and not counted as part of the carbon pool

(Howard et al. 2014). Determining a relationship between aboveground and belowground biomass can be useful for estimating carbon stocks; to determine the relationship, several fully intact seagrass plants may be harvested by hand or extracted by coring (Douglas et al. 2022; Howard et al. 2014), the root and shoot material is separated out and dried, after which the dry weight of root and shoot, material is fitted to a linear regression (Douglas et al. 2022).

When measuring the carbon content in aboveground biomass in seagrass meadows, a key consideration is the number of epiphytes present on seagrass blades. Epiphytes on leaf blades should be removed prior to carbon analysis as they can affect the quantity of inorganic carbon present in the samples. Epiphytes can be analysed as a separate carbon pool but are often excluded due to reports of their small overall impact, yet there is debate around their significance (Rohr et al. 2018). Epiphyte removal techniques include scraping (Kennedy et al. 2010; Poppe and Rybczyk 2018), acid washing, and vertical migration. Manual scraping is recommended when possible (Poppe and Rybczyk 2018) but can result in incomplete removal or damage to the seagrass leaf surface. Scraping can also contaminate the epiphyte sample with carbon from seagrass blades, which is an issue if planning to analyze epiphyte carbon. Acid washing can be used to remove carbonate epiphytes but can also cause organic compounds to leach from the seagrass samples (Poppe and Rybczyk 2018). A vertical migration technique can be used to collect benthic diatoms (Greiner et al. 2013). Seston samples can be collected by filtering water from seagrass sites through GF/F glass microfiber filters. Benthic diatoms and seston are considered a single carbon source, as they are functionally and isotopically similar (Greiner et al. 2013).

Sediment Sampling

Sediment carbon pools are expected to store the largest quantity of blue carbon in salt marsh and seagrass ecosystems. Sediment coring is the predominant sediment sampling technique for sediments in both coastal and terrestrial sampling sites. Sampling to depths of at least 1 m is recommended where possible and further if able (Howard et al. 2014). The general coring method for sediments is similar across Canadian blue carbon publications, however salt marsh and seagrass ecosystems each present their own unique challenges due to the physical differences between these habitats.

In both ecosystems, the choice of coring device and method affects the degree of compaction of the sample and may also influence the detected depth of refusal[1] (Chastain and Kohfeld 2016). Therefore, coring equipment should be chosen to minimize disturbance (Howard et al. 2014), especially if planning to date the sediment core. This section of the report includes details on coring devices and methods used in publications on salt marsh ecosystems in Canada.

Salt Marsh

When coring in salt marshes, there are multiple considerations to ensure accurate carbon data. Salt marsh terrain is often uneven and therefore the maximum depth of the marsh can be difficult to determine. Therefore, soil depth should be measured in multiple locations, and different coring devices may be necessary at different locations within a site to penetrate the full depth profile. As well, corers may be unsuccessful at salt marsh sites if unable to cut through fibrous root systems (Howard et al. 2014). Table 1 provides a list of different coring devices, along with device description and the advantages and disadvantages of the device.

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1. The depth of refusal is the deepest point to which a corer or pole may be effectively forced into the sediment and typically aligns with a transition to rock or sand.

Table 1. List of coring devices used in salt marsh publications reviewed for this report.

CORER TYPE	DESCRIPTION	ADVANTAGES AND DISADVANTAGES	COST (USD)	PUBLICATIONS
RUSSIAN PEAT CORER	Designed to fill sediment chamber from the side, samples are fully exposed once corer is removed and opened. Steel construction. Manual forced sediment entry. Proven to be reliable for quantitative sampling in peat, low marsh, and high marsh areas. Cores are transferred from corer into PVC pipe for travel to lab or sectioned in-field. Corer disassembles into 3 parts for portability.	Advantages: - minimal compaction and sample disturbance. - extensions often available up to 5m in length. - immediate detection and discard of defective samples. - minimal to no sediment loss out of the bottom, no core catcher required. Disadvantages: - smaller sampling barrel size. - sampling depth dependent on strength of corer. - possible mechanism jamming when attempting to close.	Complete Kit: $1.8-3,000 Replacement Parts & Extensions: $200-$300 each	Gonneea et al. 2019 van Ardenne et al. 2018 Artigas et al. 2015 Orson et al. 1998 Description and Assessment of the Peat Corer: Pitkanen et al. 2011 Jowsey 1966 Howard et al. 2014

| PISTON CORER | Plastic sharpened core liner fitted with a gasketed piston.

Semi-cylindrical chamber with open end. | Advantages:
- extensions available for several meters
- used in saturated soils
- easy use

Disadvantages:
- possible compression and disturbance of sediments, cores generally compacted due to extraction method
- slow sampling process
- produces slight underestimates compared to Russian and Box type corers, and where peat is rich in sedge, cottongrass or wood remains. Bias is non-significant in single-site inventories but will produce uncertainty in nationwide assessments. | Models Used: Livingston Piston Corer (2020) | Gailis et al. 2020

Gonneea et al. 2019

Description and Assessment

Pitkanen et al. 2011

Connor et al. 2001

Buckley et al. 1994

Howard et al. 2014 |

SIMPLE PERCUSSION OR SIMPLE PUSH	PVC, Acrylic, tubing or piping and plastic core catcher. Corer hammered into ground Simple Push: Sharpened pipe pushed into ground, plumbers' valve to seal and remove	Advantages - Simple design - Inexpensive Disadvantages - not suitable for deep depths - usually results in compaction of soils - simple push results in cores typically 30 cm or less	Made in-lab AMS core catcher $2-$10	Chastain et al. 2021 Chastain 2017 Douglas et al. 2022 Yu and Chmura 2009
MODIFIED PISTON / SIMPLE PERCUSSION CORER	Aluminum cutting head, acrylic or aluminum cylinder, piston, rubber caps Uses combination of piston and sharp cutting edges to overcome compaction when forcing cylinder through sediments	Advantages: - cutting head can be removed and attached to coring tubes, allowing repeated sampling of intact cores - clear acrylic tubing allows for visual inspection Disadvantages - compaction possible - Callaway et al. (2012) report average compaction of 0.3 cm - modified design requires in-lab construction to create	Made in-lab	Callaway et al. 2012 Original Design and required parts: Hargis and Twilley 1994

| VIBRACORER | Large pipe with vibrating motor, pipe is vibrated into soil, forced to the bottom. | Advantages:
- extremely long cores possible
- 1-step core recovery
- coring process user-friendly and not physically intensive

Disadvantages:
- high degree of compaction likely with long cores, compaction must be measured and accounted for in further calculations
- continuous or intermittent compaction measurements are impractical
- tripod or lifting equipment needed for extraction
- not portable | Expensive

For cost estimate: info@ vibracorer .com | Chmura et al. 2001

Connor et al. 2001

Howard et al. 2014

Described by:

Daoust et al. 1996 |

| SOIL AUGERS | Cylindrical or semi-cylindrical chamber to hold soil. Soil is forced into barrel by cutting lips as device is pushed downwards.

Multiple specialty augers available to adapt to physical constraints of sampling site. | Advantages
Dutch model:
- cutting edge for heavily rooted soils
- well designed for wetland use

Open-Faced model:
- undisturbed cores with minimal compaction

Eljkelkamp model:
- long extensions for several metres depth

Bucket model:
- universal approach for diverse settings

Disadvantages:
Ejkelkamp model:
- possible bottom loss
- not ideal for wet or semi-liquid sediments

Bucket model:
- affects soil profile, samples semi-undisturbed | Complete Soil Coring Kit

$1,100 - $2,200

Auger component

$100-$400

Easy to purchase | Dutch model:
Van Ardenne et al. 2018
Van Ardenne et al. 2021

Open-Faced model:
Kauffman et al. 2020
Donato et al. 2012
Kauffman and Donato 2012

Ejkelkamp model:
Howard et al. 2014

Bucket model:
Howard et al. 2014 |

Coring Depth

Depth of refusal is the most common coring depth used in the literature reviewed for this report (Chastain 2017; Chastain et al 2021; Gailis et al. 2020; Howard et al 2014; van Ardenne et al. 2018). Depth of refusal is used as a proxy for maximum depth of organic accumulation (Chastain 2017), and it assumes organic soil is easier to penetrate than underlying bedrock or sand. Depth of refusal should be verified at multiple locations to firmly establish organic layer depth, and to ensure the chosen coring location is not obstructed by rock or difficult mineral-rich soil (Howard et al. 2014). Alternatively, van Ardenne et al. (2018) assume depth of salt marsh ceases at the depth where the presence of rhizomes and roots of marsh vegetation vanish, signifying the end of organic matter accumulation. To determine depth of refusal, a metal meterstick or aluminum rod is pushed downwards until it hit a sand layer, or sediment cores may be taken to identify the depth of a transition layer from organic matter to sand.

To compare between studies, Howard et al. (2014) suggests a minimum coring depth of 1 meter should be standard. Several studies core to a lesser depth and extrapolate results to 1 meter, which allows for comparison of results between studies and across study sites. However, this could lead to over- or under-estimation of soil carbon stocks. A case study provided by van Ardenne et al. (2021) demonstrates this risk: organic matter depth at Kilbella Marsh in British Columbia extends beyond 1 meter, and therefore restricting carbon stock calculations to the 1 m standard underestimates carbon stocks (only 40 per cent of the marsh's total carbon is stored in the top surface meter). In contrast, Wanock Marsh has an organic matter depth of only 74 cm, and therefore extrapolating to 1 meter overestimates carbon stock. In the published literature reviewed here, depth of refusal is the most common core collection depth (Chastain 2017; Chastain et al. 2021; Gailis et al 2020; Howard et al. 2014; van Ardenne et al. 2018), however several studies quantified carbon content for the top 20 - 50 cm without specifying that depth of refusal had been reached (Callaway et al. 2012; Craft 2007; Douglas et al. 2022; Emery and Fulweiler 2014; Magenheimer et al 1996; Yu and Chmura 2009). In contrast, a study by Artigas et al. (2015) cored to a depth of 5.5 m in 50 cm increments, using only the upper 2.5 m of least disturbed core for sediment analysis.

Coring Diameter

The diameter of coring tubing can affect the compaction of soil cores, with large-diameter tubes causing less core compaction than small diameter tubes (Morton and White 1997). Despite this, coring diameters cited in the published literature and reviewed here range in diameter from 2.5 cm to 11 cm across 10 publications. No particular diameter size emerged as the most popular.

Core and Sample Storage

Cores collected from salt marsh are either transported intact and upright (Chastain 2017; Chastain et al. 2021) immediately to a laboratory for storage or sub-sectioned in the field before transport. Depending on the coring method, cores may be transferred from the corer chamber to a PVC pipe or tube in the field (Artigas et al. 2015; Gailis et al. 2020; Gonneea et al. 2019; van Ardenne et al. 2018) or wrapped in plastic wrap before transport to the laboratory (Murray 2017). If cores are sub-sectioned in the field, sectioned intervals are ziplocked and kept cool or on ice until transported to the laboratory (Callaway et al. 2012; Craft 2007). In the lab, core or core subsections are refrigerated at 4 °C (Artigas et al. 2015; Chastain 2017; Chastain et al. 2021; Gailis et al. 2020; Gonneea et al. 2019; van Ardenne et al. 2018), or subsamples are frozen (Douglas et al. 2022; Johannessen et al. 2003) or freeze dried (Gonneea et al. 2019), ideally within 24 hours of sampling (Howard et al. 2014).

Core Sub-Sectioning

Where there is significant variation in carbon content, usually in the top 20 - 50 cm, a detailed depth profile should be taken (Howard et al. 2014). Howard et al. (2014) suggests 5 cm intervals throughout the upper 50 cm of the sediment core, shifting to larger intervals with increasing depth if appropriate. Subsections are homogenized before analysis for carbon content. Note that the original volume of sections needs to be recorded for future dry bulk density calculations. The sampling intervals for salt marsh cores reported in studies are provide in Table 2.

Table 2. Sampling intervals for salt marsh cores reported in studies reviewed for this report.

Core Depth (cm)	Interval (Section Thickness, cm)	Publication(s)
0-10	1	Douglas et al. 2022 Johannessen et al. 2003
0-30	1	Gonneea et al. 2019
0-50	5	Howard et al. 2014
10-20	2	Douglas et al. 2022
>10	Increasing 5 to 10 with depth	Johannessen et al. 2003
>30	2	Gonneea et al. 2019
Throughout	2	Callaway et al. 2012 Craft 2007 Orson et al. 1998
Throughout	6	Artigas et al. 2015
Throughout	10	Yu and Chmura 2009

Seagrass Meadow

Methods for coring in seagrass meadows follow many of the same steps as coring in salt marsh ecosystems, however coring devices are listed separately in Table 3 given the specialized seagrass considerations for working in areas that experience tidal inundation and sandy sediments. In seagrass systems, intertidal measurements are taken at low tide and subtidal measurements are taken using SCUBA. Water depth should be considered when choosing a corer for seagrass systems, prioritizing equipment with simple mechanisms to stand up to submersion. Cores extracted from seagrass meadows are more likely to include water or be less solid than those extracted from a salt marsh, therefore care must be taken to minimize vibrational disturbance when transporting intact cores from the field to the lab.

Table 3. List of coring devices used in published literature focused on Canadian seagrass ecosystems.

CORER TYPE	DESCRIPTION	ADVANTAGES AND DISADVANTAGES	COST (USD)	PUBLICATIONS
PVC PIPE PERCUSSION	Manual insertion of PVC pipe core Thin walled for fine grained sediments. Thick walled for coarse grained sediments. Sledgehammer percussion on metal or wood platform to prevent pipe cracking, top is capped at desired depth	Advantages - inexpensive - simple design - SCUBA compatible	Lab-made PVC Pipe: $10-$100 PVC Pipe (Clear): $250-$500	Stephens and Eckert 2018 Howard et al. 2014 Prentice et al. 2020 Murray 2017
PVC PIPE PERCUSSION WITH STEEL OR GALVALNIZED CORE CATCHER	Addition of core catcher, preferably steel or galvanized, equipped with metal teeth. Not necessary at all coring locations.	Advantages - minimizes sediment loss out of bottom Disadvantages: - usually ~15cm below core catcher is lost - compaction possible	Lab-made Steel core catcher cut from metal sheet, 0.254 mm suggested thickness. Equipment list is provided in each publication	Prentice et al. n.d. Short et al. n.d.
SIMPLE PUSH	3-inch polycarbonate tube, bevelled at one end, pushed into the ground.	Advantages: - cheap - durable - simple Disadvantages: - shallow depth - not feasible in difficult sediments - compaction possible	$10-$30	Postlethwaite 2018

ACRYLIC TUBE PERCUSSION	Manually forced into the ground and capped at both ends underwater (if applicable). Hammered with mallet with lumbar or high-density polyethylene plastic board placed over corer, dug out with shovel (Spooner 2015) 5lb cannonball weight (dropped repeatedly) onto high density polyethylene plastic board with 5mm holes drilled, useful when underwater Removal with shellfish harvesting stinger (Spooner 2015)	Advantages: - clear tube allows for compaction visibility	Lab-made Acrylic Tubing: $50-$500 (diameter dependent)	Douglas et al. 2022 Rohr et al. 2018 Spooner 2015
BOX CORER	Stainless steel sampling box Core sample size controlled by speed of lowering to ocean floor	Advantages - subtidal coring - immediate inspection and discard of defective samples - minimal disturbance Disadvantages - outermost 5 cm discarded to avoid sediment smear by core wall - not portable, heavy to transport and deploy - may require use of research vessel	$6,400 Contact for Quote: Ocean Instruments KC Denmark	Johannessen et al. 2003 Pitkanen et al. 2011

Coring Depth and Diameter

Howard et al. (2014) recommends the same 1 m standard of coring depth for seagrass meadows as recommended for salt marshes. Reviewed literature reports a range of sampling from 20 cm to 150 cm in depth. Coring to a predetermined depth (Branimir et al. 2020; Douglas et al. 2022; Greiner et al 2013; Poppe and Rybczyk 2018; Postlethwaite 2018; Prentice et al. n.d.; Prentice et al. 2020; Rohr et al. 2018) is more common than coring to depth of refusal (Murray 2017; Postlethwaite 2018; Prentice et al. 2020; Stephens and Eckert 2018), likely due to difficulty in determining refusal depth in subtidal and intertidal ecosystems.

Coring with a diameter of 5 cm or 10 cm is common in seagrass meadows; larger diameters can reduce the smear effect (the pulling of sediments up the corer wall) and compaction, while making it easier to penetrate dense sediment (Spooner 2015). However, the larger the diameter, the more expensive the core is, likely because of an increase in PVC or Acrylic piping costs.

Core and Sample Storage

A key concern for storage of seagrass cores relative to salt marsh cores is saltwater inundation. Excess water may be drained off the top (Murray 2017) or retained in cores (Spooner 2015). Transport and sub-sectioning of cores is similar to salt marsh techniques; cores are either transferred upright to a laboratory intact (Howard et al. 2014; Poppe and Rybczyk 2018; Prentice et al. n.d.; Prentice et al. 2020; Stephens and Eckert 2018) or they are sub-sectioned in the field immediately following their extraction (Douglas et al. 2022; Postlethwaite 2018; Prentice et al. 2020). If it is not possible to maintain an upright position for transport, dividing sediment cores into sections in the field is preferable (Howard et al. 2014). Since most coring is done in cylindrical piping, cores are typically extruded with a piston device and subsampled using collars of desired thickness, or cores are frozen prior to subsampling. An example of a piston device is provided by Prentice et al. n.d., comprised of a > 1.5 m metal pole on a wooden base platform, with a custom-sized piston attached to the top. Collars of various heights are placed on top of the core, and the core is pushed down on the piston until sediment is even with the top of the collar. The sediment section is then sliced off using a piece of plexiglass or another thin surface. Sediment subsections are frozen or kept on ice. Water is typically left in the coring tube if intact cores are to be frozen, and frozen cores are sliced into subsections.

Core Sub-Sectioning

In a global survey of seagrass meadow sampling methods, Kennedy et al. (2010) determined that core sampling for carbon analysis is usually restricted to, or more frequent, in the upper 5 - 10 cm of the sediment column. A more recent survey of seagrass sampling techniques by Prentice et al. (2020) reported a variable range of 2 - 10 cm sections across studies. Table 4 is a compilation of reported sampling section intervals included in seagrass meadow publications most applicable to Canadian seagrass ecosystems.

Table 4. Sampling intervals for seagrass cores reported in studies reviewed for this report.

Core Depth (cm)	Interval (Section Thickness, cm)	Publication(s)
0-10	1	Douglas et al. 2022 Spooner 2015
10-20	2	Spooner 2015
>20	5	Spooner 2015
0-20	2	Murray 2017
0-25	5	Rohr et al. 2018
20-50	5	Murray 2017
>50	10	Murray 2017
Throughout	1	Gailis et al. 2020 Postlethwaite 2018 Greiner et al. 2018
Throughout	2	Poppe and Rybczyk 2018 Stephens and Eckert 2018

Sediment Compaction

Several factors influence the degree of compaction in cores including sediment porosity, composition, texture, water content, and the shape of sediment grains, etc. (Morton and White 1997). Sediment compaction during coring should be measured at least twice Prentice et al. n.d. per core and compensated for when calculating carbon density or completing sediment dating. There are several ways to account for compaction in carbon analyses. Callaway et al. (2012) rejected sediment cores with greater than 3 cm of compaction. Most publications measured compaction depth and calculated a compaction co-efficient to apply to future carbon calculations, assuming that compaction is uniform throughout the sediment column. However, compaction is unlikely to be uniform, and as a result, minor displacement of sediment depths can result in underestimated sedimentation rates, by as much as 2-3 times lower relative to those calculated for uncompacted cores (Morton and White 1997). Analytical data derived from compacted cores can only be properly interpreted if vertical patterns of compaction are established and incorporated into analysis (Morton and White 1997). The applied methods to account for core compaction in both salt marsh and seagrass ecosystems are outlined in Table 5.

Table 5. Methods for accounting for core compaction in salt marsh and seagrass ecosystems applied in publications reviewed for this report.

Variable	Method	Publication
Core Compaction	Depth reached by corer – length of core sample Difference in elevation inside and outside coring pipe	Callaway et al. 2012 Short et al. n.d. Prentice et al. n.d.
Compression Factor	Length of recovered core / length of corer penetration	Stephens and Eckert 2018 Howard et al. 2014
	Length of penetration / length of recovered core	Gailis et al. 2020
Corrected depth	Compression factor x Core Depth Interval * assumes all core sections compacted equally	Gailis et al. 2020 Howard et al. 2014

Additional considerations to reduce the impact of compaction when field sampling:

• Vibracoring is considered impractical for taking intermittent measurements of compaction because it is a continuous process, and the long pipe sections that are involved make simultaneous outside and inside measurements impractical.
• Piston coring and almost any underwater coring technique prevents measurement of compaction and therefore cores are assumed undisturbed.
• Large-diameter tubes cause less core shortening than small diameter tubes, and friction is reduced by tapering the cutting edge at the head of the core barrel.
• A modified large-diameter corer featuring a piston and sharp cutting edge of razor blade strips is considered a good choice for marshes.
• Lubricant is a viable option to reduce coring friction only if it does not interfere with chemical analyses.
• Using a slow rate of core barrel penetration is known to reduce compaction.

Morton and White (1997) have published a detailed assessment of the patterns of core shortening, and their relative likelihood, for further reading.

CARBON ANALYSIS OF FIELD SAMPLES

Methods Synopsis

Biomass and sediment samples are analyzed for carbon separately to estimate both carbon pools. Once field samples have been processed, they are oven-dried and weighed to determine bulk density. Dried samples are then homogenized and sub-sampled to determine total carbon, inorganic carbon and/or organic carbon content. Carbon analysis of subsamples typically follows the same protocols for both salt marsh and seagrass

samples, and the method used depends on accessibility of necessary equipment, and cost. The most common method of carbon analysis is per cent loss on ignition (LOI). This method also requires several subsamples to be sent for elemental analysis, the results of which are used to convert LOI data into per cent organic carbon. Quantification of sediment depth, dry bulk density, and sediment organic carbon content are all considered necessary for the determination of carbon stocks (Howard et al. 2014).

Biomass Sample Preparation

Harvested living plant material (from both above- and below-ground samples) is first rinsed with freshwater. Dead biomass is not counted towards carbon storage, or if desired, may be counted in a separate carbon pool (Howard et al. 2014). All samples are oven-dried to a constant weight and homogenized (Douglas et al. 2022). After processing, live plant material samples undergo the same carbon analysis techniques as sediment samples.

Sediment Sample Preparation

Shells, rocks, fauna, roots, and large rhizomes are removed from sediment sections prior to drying to a constant weight, homogenizing and sub-sampling for carbon analysis (Greiner et al. 2013; Kauffman et al. 2020; Postlethwaite 2018; Prentice et al. 2020; Rohr et al. 2018). For seagrass sediments, several studies left non-seagrass organic material in the sediment samples to contribute to the sediment organic carbon pool (Prentice et al. 2020; Rohr et al. 2018), which can account for approximately 50 per cent of the pool (Oreska et al. 2017). Various methods for homogenizing dried samples found in the literature reviewed are summarized in Table 6.

Table 6. Homogenization methods for dried samples found in the literature reviewed for this report.

Homogenization Method	Publication
Mortar and pestle	Callaway et al. 2012
	Gailis et al. 2020
	Rohr et al. 2018
Electric coffee grinder	Connor et al. 2001
Food processor	Yu and Chmura 2009
Stainless steel spatula in glass jar	Johannessen et al. 2003
Ground through mesh screen 0.425 mm 2.5 mm	Poppe and Rybczyk 2018 Magenheimer et al. 1996

When preparing seagrass meadow sediment samples, a key consideration is the likely presence of coarse gravel, which cannot be ground during the homogenization step. Higher sediment gravel content can result in more varied carbon analysis results. Postlethwaite (2018) accounted for this by removing gravel and subtracting the weight of gravel, which was determined after its removal, from the organic carbon values. Grain size analysis can also be conducted to provide additional information on the sample. The size fraction of clay and silt (0 – 63 μm) has the highest organic matter content and can be used as a proxy for degree of study site

exposure (Rochette 2020). For this approach subsamples are weighed, wet-sieved for 10-20 minutes using a 63 μm sieve, oven-dried and weighed again (Murray 2017). Alternatively, a particle size analyzer, such as a Malvern Mastersizer, may be used (Rohr et al. 2018) for grain size analysis. However, the majority of seagrass sediment analyses from a global review were completed on bulk samples without grain size analysis (Kennedy et al. 2010).

Dry Bulk Density

Dry bulk density (DBD) of soil is calculated as the mass of soil that has been dried to constant weight divided by the initial volume of the sample. Constant weight is achieved when the weight of the sample does not significantly change between drying periods. Drying to constant weight can be completed by allowing samples to dry for at least 24 hours, cooling to room temperature in a desiccator for 1 hour, weighing again and repeating the process until weight differences between weighing sessions are less than 4 per cent. This typically requires 48-72 hours (Howard et al. 2014).

In the literature reviewed herein, drying temperatures varied from 55 °C to 105 °C (Table 7). Drying samples at or over 105 °Cis not recommended, as this may oxidize organic matter, resulting in an underestimation of organic carbon. Determining dry bulk density for each depth interval from a sub-sectioned core is the most practical approach for this analysis (Howard et al. 2014). However, some studies removed a small sample of known volume from each section of the sediment core (e.g., 1 cubic centimeter) instead of determining bulk density on the entire sediment layer (Chastain et al. 2021; Murray 2017).

Table 7. Temperatures and corresponding drying times that were provided in the publications reviewed for this report.

Temperature (°C)	Duration (hours)	Publication
55	72	Murray 2017
60	72	Chastain et al. 2021 Gailis et al. 2020 Chastain 2017
60	>96	Postlethwaite 2018
60	Constant weight	Howard et al. 2014 van Ardenne et al. 2018 Stephens and Eckert 2018
65	Constant weight	Kauffman et al. 2020
85	72	Callaway et al. 2012
100	24	Orson et al. 1998
105	6	Rohr et al. 2018
Freeze-dried	Constant weight	Gonneea et al. 2019 Connor et al. 2001

Soil Carbonates and Sample Acidification

Calcium carbonate, or inorganic carbon, is not included in the estimation of blue carbon stocks. Therefore, inorganic carbon must be removed from samples prior to carbon analysis to avoid biasing results, if the amount of inorganic carbon could be significant (Howard et al. 2014). Removal of inorganic carbon is especially relevant for seagrass sediments, as there can be high levels of calcium carbonate in seagrass samples, especially when epiphyte material has not been removed. This process may be required in salt marsh soils, but inorganic carbon levels in salt marsh samples are usually negligible. Often, studies will test for inorganic carbon in a random sample to determine if constitutes a sufficiently high fraction to require removal (Kauffman et al. 2020). If inorganic carbon is not removed, results can be published as total carbon rather than total organic carbon Murray (2017). Or, if it is negligible then total measured carbon can be assumed to be equivalent to total organic carbon (Kauffman et al. 2020).

Soil carbonates are most often detected by acidification. Effervescence in the presence of hydrochloric acid (HCl) signifies significant presence of calcium carbonate within the samples (Craft 2007; Howard et al. 2014). Samples with carbonates can be pretreated with diluted hydrochloric acid to strip inorganic carbon from the samples before they are analyzed (Craft 2007; Howard et al. 2014; Greiner et al. 2013; Johannessen et al. 2003; Kauffman et al. 2020; Kennedy et al. 2010; Rohr et al 2018). Studies have used 0.1N (Craft 2007) and 5N (Gonneea et al. 2019) HCl to treat samples, however the most common HCl concentration used is 1N (Howard et al. 2014; Johannessen et al. 2003; Kauffman et al. 2020).

The general process for acidification consists of (Howard et al. 2014):

- Covering a sample with dilute HCl, then agitating the sample until CO2 is no longer produced.
- Decanting the acid and drying the sample overnight, followed by weighing the sample.
- The difference in pre- and post- acidification weights provides an estimate inorganic carbon content in the sample.

Alternatively, samples can be fumigated with concentrated HCl for over 24 hours to remove inorganic carbon prior to analysis (Wang et al. 2010). Both acidification and fumigation techniques result in a variable fraction of organic carbon lost to the process; alteration of isotopic composition is also possible with this method (Kennedy et al. 2010). Therefore, acidification and fumigation can result in underestimates of organic carbon content. To quantify inorganic carbon without acidification, samples may be run through an elemental analyzer. More detail is provided in the Elemental Analysis section of this report.

Organic Carbon Content Analysis

Loss on Ignition

Loss on ignition (LOI) is the most cost effective and accessible way to analyze samples for carbon content. However, the method provides only a semi-quantitative estimate of organic carbon. LOI results represent the loss of organic matter (including C, H, N, O, S, and other elements), inorganic carbon and structural water through combustion. Factors such as sample size, exposure time, position of sample in the furnace, and which laboratory is doing the measurements have been found to affect LOI results (Heiri et al. 2001). Replicate samples are often run for precision, and a third replicate can be run when an acceptable variance of < 10 per cent is not met (Yu and Chmura 2009). To reduce error in interpreting results, the LOI method should be consistent among samples for a study and all relevant method details should be included in publications.

To complete precise determinations of organic carbon content, the choice of appropriate ignition temperature depends on the sample soil characteristics. Combustion temperature and exposure time are crucial to maximize the accuracy of method. If the temperature is too low and the duration too short, the

incineration of carbon compounds may be incomplete and result in an underestimation of organic content. Conversely if the temperature is too high and the duration too long, inorganic compounds may be lost and organic compounds overestimated (Wang et al. 2011). One study found that LOI conducted at 550 °C was more susceptible to these errors than LOI conducted at a temperature of 950 °C (Heiri et al. 2001). Wang et al. (2011) compared combustion methods and developed suggestions for specific types and sources of soils:

- Combustion for 12 hours at 500 °C was acceptable for samples from a wide range of sediment sources, excluding marine sediments.
- Combustion for 12 hours at 800 °C is optimal for inorganic carbon determination.
- Combustion at 475 °C is adequate for wetland and stream sediment samples.
- Combustion for 12 hours at 550 °C was necessary for marine sediments.
- Precise results for most sediment sources could be obtained when sample weights ranged from 2.0 - 4.0 g in size.

Froelich (1980) found that 66 ± 9 per cent of organic carbon is combusted at 250 °C, 90 ± 4 per cent at 500 °C, and 72 ± 8 per cent by low-temperature ashing. They concluded that complete combustion of organic carbon was not possible below 1000 °C, but that above 500 °C inorganic carbon would be lost, highlighting the difficulty of separating organic and inorganic carbon amounts through the LOI method. Many recent studies using the LOI method combust samples twice; once at a lower temperature (~500°C) to combust organic matter, then again at high temperature (~1000°C) to burn off inorganic carbonates. Table 8 outlines the LOI combustion times and temperatures for organic and inorganic material from the reviewed literature that provided this information.

Table 8. LOI combustion times and temperatures for organic and inorganic material outlined in the publications reviewed for this report.

Temperature (°C)	Time (hours)	Variable Measured	Publication
Salt Marshes			
375	4	Combustible organics	Orson et al. 1998
450	4-8	Organic matter	Howard et al. 2014
500	12	-	Artigas et al. 2015 Wang et al. 2011
550	4	Organic matter	Chastain et al. 2021 Gailis et al. 2020 van Ardenne et al. 2018 Chastain 2017 Heiri et al. 2001 Connor et al. 2001
550	5	Organic content	Douglas et al. 2022
600	6	weight LOI	Emery and Fulweiler 2014
950	-	Carbonates	Heiri et al. 2001

950	2	Inorganic content	Douglas et al. 2022
1000	2	Carbonates	Chastain et al. 2021 Chastain 2017
Seagrass Meadow Samples			
450	-	Organic matter	Stephens and Eckert 2018
500	6	Organic matter	Greiner et al. 2013
500	24	Organic content	Poppe and Rybczyk 2018
440-500	-	Organic matter	Kennedy et al. 2010
520	4	Organic content	Rohr et al. 2018
550	4	weight LOI	Postlethwaite 2018
500-550	-	weight LOI	Fourqurean et al. 2012
950	-	Inorganic matter	Stephens and Eckert 2018

Since LOI provides a semi-quantitative estimate of organic carbon content, further steps are required to obtain a quantitative estimate. Two approaches are commonly used to achieve this. The first, more popular and accurate technique, is to send a subset of samples to a laboratory for elemental and coulometric analysis, which can measure total carbon and inorganic carbon directly (see Elemental and Coulometric Analysis section below). Alternatively, when this is impractical, a linear regression equation from the published literature may be used to convert LOI data to per cent organic carbon. The accuracy of using literature values depends on how similar the study sites are to those used in the source. The most widely used formula is published by Craft et al. (1991) and is provided in the literature estimates section of this report, along with other published equations and their respective sampling site locations.

Walkley-Black Method

Also known as Dichromate Digestion, the Walkley-Black method for determining organic carbon content is scarcely used and faces criticism for possible inaccuracy of results. Since the majority of studies use LOI to estimate organic carbon, the Walkley-Black method is not readily comparable to other studies, as it yields total carbon results that tend to be slightly different than those measured using LOI or elemental analysis (Wang et al. 2011). Detailed methods can be found in Wang et al 2011.

Elemental and Coulometric Analysis

Elemental analysis (EA) provides a quantitative measure of carbon content (Howard et al. 2014). It is the most expensive and precise method of measuring the carbon content of samples and is recommended and most suitable for routine analysis. However, samples must be sent to an appropriate lab for analysis and analyzed as fee for service. Inorganic carbon may be removed beforehand by acidification of samples (Greiner et al. 2013; Johannessen et al. 2003; Poppe and Rybczyk 2018; Rohr et al. 2018), but more often samples are run without prior acidification, and inorganic carbon content of samples must be determined otherwise. Greiner et al. (2013)

acidified only a subset of samples for comparison to non-acidified results. Due to the high cost of sending large volumes of samples to laboratories for EA, many publications performed LOI on all samples, and sent a small subset of samples for elemental analysis to establish a relationship between LOI and EA data and convert all LOI values to a quantitative estimate of per cent organic carbon. The result of that small subset is used to establish a relationship and convert the percentage of organic matter determined by LOI into per cent carbon or per cent organic carbon. Analyzing samples for other elements, such as nitrogen in addition to carbon, is possible using this method (Emery and Fulweiler 2014). Samples may also be analysed for stable isotopes of carbon and nitrogen, and particulate organic nitrogen to determine relative contribution to sediment organic carbon stock with the addition of an isotope ratio mass spectrometer (Greiner et al. 2013; Kennedy et al. 2010; Rohr et al. 2018).

Generally, total carbon is measured on a subset of unacidified homogenized samples through dry combustion CHN elemental analysis of a known mass of soil, expressed as percentage of dry weight. The inorganic carbon component of the same subsamples is measured using a coulometer connected to an acidification module, but acid treated samples can be measured by elemental analysis on an elemental analyzer alone. Once these values have been obtained, organic carbon is calculated by subtraction of inorganic from total carbon (Chastain 2017; Howard et al. 2014; Fourqurean et al. 2012; Froelich 1980; Postlethwaite 2018; Prentice et al. 2020). In salt marshes, inorganic carbon is often found to be negligible in subsamples, and therefore assumed to be zero for carbon calculation purposes. This allows for researchers to skip a step of the process and calculate organic carbon by measuring total carbon of analyzed samples (Chastain 2017). The overall accuracy of measuring organic carbon using this technique has been found to be ± 2 per cent with precision better than ± 0.02 per cent of organic carbon for sediments with low carbonate content (Froelich 1980). This technique is typically not done in duplicate due to high accuracy and cost, except where carbon values are extremely low.

Literature Estimates of organic carbon content

If analyzing samples with an elemental analyzer is not feasible and LOI is completed alone, organic carbon may be determined using conversion factors published in the literature. This approach is challenging for biomass samples as a result of the diversity of plant species present in salt marshes and the limited availability of species-specific carbon conversion factors. While this approach is more common in mangrove ecosystems, Canadian salt marshes do not yet have a wide range of published work to allow this to be feasible in most cases. If the carbon conversion factor of a plant species is known, then the carbon content of this species may be calculated as follows Howard et al. (2014):

Carbon in [component] (kg C m^2) = (estimated biomass (kg) * carbon conversion factor) / area of quadrat (m^2)

Once aboveground estimates of carbon are made, below-ground and total biomass may be produced using a species-specific ratio of aboveground to belowground biomass instead of quantifying the belowground biomass separately. Again, this is rarely feasible in salt marsh ecosystems, as accurate published literature regarding the carbon content of Canadian salt marsh plant species is lacking. In Table 9 and 10, are the published relationships to convert LOI to organic carbon and it is advised to use a relationship from the same or similar geographic location or study sites with similar physical characteristics.

Table 9. Literature published relationships to convert LOI to organic carbon for North American salt marsh study sites.

Relationship	Published by	Location	Used by	Accuracy
$C_{org} = 0.40[LOI] + 0.0025[LOI]^2$	Craft et al. 1991	USA; North Carolina salt marsh	Yu and Chmura 2009 Chmura et al. 2003 Connor et al. 2001 Van Ardenne et al. 2018 Howard et al. 2014	$r^2 = 0.99$
%C = 0.44 (%LOI) − 1.80	Chastain et al. 2021	Clayoquot Sound, BC		-
%C = 0.44 (%LOI) − 1.33	Gailis et al. 2020	West coast marshes (Pacific coast) region		$r^2 = 0.97$
$\% C_{org} = 0.47(\%LOI) + 0.0008(\%LOI)^2$	Johnson et al. in prep	Maine	Howard et al. 2014	$r^2 = 0.98$

Table 10. Literature published relationships to convert LOI to organic carbon for seagrass meadow study sites.

Equation/Relationship	Published by	Purpose	Location	Used by	Accuracy
C = 35% dry weight		Estimating C content of seagrass biomass	Global	Fourqurean et al. 2012 Duarte 1990	-
$\% C_{org} = 0.3134(\%LOI) − 0.1149$	Prentice et al. 2020	%OM to %OC when only %OM data available	NW CAD & Salish Sea		-
$\% C_{org} = 0.40(\%LOI) - 0.21$	Fourqurean et al. 2012	%Corg from LOI where %LOI > 0.2	Global data set	Howard et al. 2014	$r^2 = 0.87$
$\% C_{org} = 0.43(\%LOI) - 0.33$	Fourqurean et al. 2012	%Corg from LOI where %LOI > 0.2	Global data set	Howard et al. 2014	$r^2 = 0.96$

Soil Carbon Density

Soil carbon density is calculated by multiplying dry bulk density and per cent organic carbon to produce an estimate in grams of carbon per unit area (Chastain and Kohfeld 2016; Chastain et al. 2021; Connor et al. 2001; Gailis et al. 2020; Magenheimer et al. 1996; van Ardenne et al. 2018). Soil carbon density is typically calculated for each sample or sediment layer over the length of a sediment core. Alternatively, a representative carbon density for each core may be calculated by averaging carbon density from surface to depth where accumulated carbon is negligible (Poppe and Rybczyk 2018).

Carbon Stocks

Carbon stocks represent the amount of carbon within a defined area and soil depth, calculated by summing carbon stored in carbon pools within an ecosystem. Carbon stock estimates allow for the determination of an area's value in terms of stored blue carbon. Stocks ideally include multiple carbon pools such as above and below ground biomass and soil organic carbon. The entire soil depth profile calculated to depth of refusal is necessary for providing an estimate of all site-associated carbon accumulated since the formation of the ecosystem (Chastain et al. 2021). Accuracy of carbon stock estimates improves with increased sampling sites and additional measurements of depth of refusal across the study area. Total site carbon stock is impractical to obtain where depth of refusal is not reached, or where a study area is sufficiently large and relatively few core samples are taken. For biomass pools, sample pool biomass is multiplied by an organic carbon conversion factor and averaged across all samples to give carbon pool for a given plot size (Poppe and Rybczyk 2018).

To estimate carbon stock from soil cores, soil carbon densities for each core section are summed throughout a core. In case of missing core subsections, the average of depth subsections immediately preceding and following are averaged to produce an estimate (van Ardenne et al. 2018). A simple estimate of carbon stock is produced by averaging the carbon stock per unit area derived from all cores for each study site, multiplied by the study area (van Ardenne et al. 2018). Carbon stocks are reported in megagrams per hectare and in total megagrams carbon to allow for cross-study comparison (Chastain 2017).

Carbon stock estimates rely on appropriate soil core sampling locations where variation in topography is taken into account. Variation can be accounted for with appropriate spacing of cores such that every section and habitat or study area is represented (van Ardenne et al. 2018). Large gaps in soil cores and clear geomorphic differences between transect locations reduces stock accuracy. Alternatively, van Ardenne et al. (2018) found that a 5-core subset produced a similar average carbon stock to averaging all cores within a marsh area, signalling this may be a possibility where time and resources are limited.

Comparison of carbon stocks among studies

Although quantifying carbon stocks by coring to the depth of refusal is ideal, carbon stocks may be quantified to a defined depth, based on the assumption that the highest carbon variability, and most carbon stored, resides in the surface portion of sediments (Howard et al. 2014). When doing so, estimates are corrected to uncompacted depth, and often cores not reaching a specified depth are excluded (Chastain et al. 2021). Calculating stocks to a standard depth would facilitate comparisons across sites and studies. In the literature reviewed for this report stocks have been calculated by summing carbon up to 50 cm depth (van Ardenne et al. 2018), 25 cm depth (Howard et al. 2014; Prentice et al. 2020), and to 20 cm depth (Chastain et al. 2021; Douglas et al. 2022).

To compare across studies, carbon stocks may also be normalized to a specific age horizon instead of a specified depth; this allows for more accurate comparison between sites with different carbon accumulation rates (Chastain et al. 2021). To do this, sediment cores must first be dated (see Radioisotope Dating section in this report), and it is suggested to use the oldest ^{210}Pb date that is shared between all dated cores. For Chastain

et al. (2021), this resulted in the production of a 30-year carbon stock estimate, which expresses the amount of carbon stored in the top 30 years-worth of sediment.

GIS AND INFORMATION LIBRARIES

Areal estimates of carbon stocks are useful to determine the carbon value of an ecosystem or to compare carbon stocks across studies. Carbon data can be analysed with GIS and ArcGIS software to support the estimation of carbon stocks where core data is available. For example, the carbon stocks of locations where only soil depth is recorded can be estimated using regression modelling in ArcGIS that relates soil depth to carbon stock, and interpolation may be used to estimate carbon stock values between core locations (van Ardenne et al. 2018). Estimation is improved through the addition of soil depth data pulled from literature within the described study location of interest (van Ardenne et al. 2018). Gailis et al. (2020) describe a geostatistical method to estimate marsh volume using QGIS tools along with depth profiles, which is deemed more accurate than multiplying a bounded area by the average of uncompacted core lengths. Multiple remotely-sensed data sources at the regional, national and global scale can support GIS analysis of carbon stocks, including data sets on ecosystem extent, plant species, biomass, and average carbon stock values. A subset of these data sets is summarized in Table 11.

Table 11. Data sets available to support the analysis of carbon stocks.

DATA SET	POTENTIAL USE	AVAILABLE FROM
LANDSAT	Produce vegetation indexes based on absence/ presence and abundance of vegetation Vegetation cover in tidal salt marsh and some seagrass ecosystems	US Geological Survey (USGS) web portal
MODIS	Biomass plots over large areas Daily time series data for vegetation cover 250m resolution	NASA's MODIS websites
STRM	Map watershed and mangrove environments	USGS EarthExplorer sites
PALSAR	Elevation maps Topography data Coastal vegetation	Alaska Satellite Facility (ASF)
ICESAT / GLAS	Estimate of canopy height within few-meter accuracy	National Snow and Ice Data Center

CARBON SEQUESTRATION

SEDIMENT ACCRETION RATE (SAR)

The first step of quantifying carbon sequestration is to determine the rate of sediment accumulation across the site of interest. It is often measured as the cumulative sediment accretion rate (SAR), which includes all sediments above a dated depth. Alternatively, it may be measured as a sectional sediment accretion rate, which is between two known ages. In both cases, it is measured as the sediment depth divided by its respective age (van Ardenne et al. 2021).

Using different methods to determine sediment accretion rates limits comparisons among studies due to the tendency for some methods to consistently under or overestimate rates (Chastain et al. 2021). Sedimentation rates may be measured using marker horizons, which require very little equipment, or by using radioisotope dating. With respect to radioisotope dating, recent literature favours estimating sedimentation rates using age models derived from excess ^{210}Pb sediment dating techniques (Douglas et al. 2022; Postlethwaite 2018). Previous research argues using ^{137}Cs dating or marker horizons to establish age models can result in elevated or biased-high SARs and carbon accumulation rates (CARs) in comparison to those calculated using ^{210}Pb results, which is a concern when making global estimates of salt marsh CARs (Chastain et al. 2021).

Marker Horizons

The simplest method for determining sedimentation rate is to use sediment marker horizons (Table 12). Markers are added to the soil, and later retrieved with sediment cores. The amount of sediment accumulated above the marker is measured. The results of this method are subject to post-depositional soil mixing, which often leads to overestimates of carbon accumulation (Chastain 2017). In this method, the rate of accumulation of organic and mineral matter is calculated by multiplying the rate of vertical accretion by soil bulk density and the percentage of soil organic carbon content (Cahoon 1994). This method requires a time lapse of months to years between laying the marker and measuring accretion, making this impractical for some short-term studies.

Table 12. Marker horizon methods found in the literature reviewed for this report.

Marker Horizon	Method	Publications
Feldspar Marker	White feldspar mineral laid in a level layer between vegetation stems, 50 x 50 cm area of marsh, rods placed nearby to mark plot boundaries. Accretion cores collected 6 & 12 months after establishment of marker horizons via single 5 – 10 cm long core using thin-walled aluminum 6 cm diameter core tube. Note: at some spots, feldspar marker layer could not be discerned due to bioturbation by fiddler crabs	Cahoon and Turner 1989 Cahoon 1994 Craft 2007
Clay Marker	Short-term accretion rate A few samples were lost due to erosion	Chmura et al. 2003
Glitter Marker	Coloured glitter applied to 1 m^2 plot on marsh surface, applications repeated with different colours in different years. Sediment cores dissected, and glitter horizons identified by counting individual glitter grains over depth. Study had 5 glitter applications over 32 years	Orson et al. 1998
Weed Pollen Marker	Identification of settlement horizons by increases in weed pollen	Connor et al. 2001

Radioisotope Dating

Dating is usually outsourced to a fully equipped laboratory (Table 13), and intact dating cores are sent to the lab for radioisotope dating analysis. One core from each sampling site is typically dated. It is important to consider the vertical stability of samples and minimize disturbance when dating sediment profiles; therefore, choosing an appropriate coring device is paramount. Compaction correction factors should be applied to core depths, and sediment disturbances should be noted. Callaway et al. (2012) provides guidance for identifying 'excellent' 'good' and 'marginal' cores to help in the process of deciding whether a core is sufficiently intact to perform radioisotope dating. Unsuccessful core dating can result from sample disturbance, bioturbation, or rapid sediment accumulation within the chosen sampling site (Chastain 2017).

The two most common types of radioisotope sediment dating are ^{137}Cs and ^{210}Pb. ^{137}Cs provides date markers whereas ^{210}Pb provides concentration slopes (Jeter 2000). Sediment accretion rates derived from ^{210}Pb and ^{137}Cs dating methods differ (Turner et al. 2006), potentially resulting from soil compaction and decomposition at depth (van Ardenne et al. 2021). Chastain (2017) found that carbon accumulation and sedimentation rates derived from ^{210}Pb dating were 26 and 29 per cent lower, respectively, relative to those calculated using ^{137}Cs. In another study, the ^{137}Cs dating produced slightly, but non-significantly, higher carbon and sediment accumulation rates relative to those derived from ^{210}Pb dating (Chastain et al. 2021). It is often useful to use both methods and compare calculated sedimentation rate results (Jeter 2000). Measurements of both ^{137}Cs and ^{210}Pb can be made on a single sample (Orson et al. 1998). Choice of technique is also dependent on timescale, as ^{210}Pb dating can be applied to longer time periods and thus greater sediment depths than ^{137}Cs dating (van Ardenne et al. 2021). Indicators which suggest the dating of cores is reliable include agreement between ^{137}Cs and ^{210}Pb rates, a significant amount of ^{137}Cs at surface level, and inventory of ^{137}Cs in the core (Chastain 2017).

Table 13. Laboratories which provided radioisotope dating of samples for studies reviewed in this report.

Company	Publication
Flett Research Ltd. Winnipeg, Canada	van Ardenne et al. 2021 Chastain et al. 2021 Gailis et al. 2020 Johannessen et al. 2003
Canberra Inc. USA	Gonneea et al. 2019
University of Delaware, Department of Oceanography, Radioactive Testing Lab	Artigas et al. 2015
Core Scientific International, Winnipeg, Canada	Chastain et al. 2021 Chastain 2017
MyCore Scientific, Dunrobin, Canada	Chastain et al. 2021 Chastain 2017
MyCore Scientific, Winnipeg, Canada	Postlethwaite 2018
GEOTOP Laboratories, Universite du Quebec, Montreal, Canada	Gailis et al. 2020

Western Washington University, Washington, USA	Arias-Ortiz et al. 2018
Keck Carbon Cycle AMS Facility	Van Ardenne et al. 2021
National Ocean Science Accelerator Mass Spectrometry facility, Woods Hole Oceanographic Institution, Massachusetts, USA	Gonneea et al. 2019

[137] Cs

Radioisotope dating of ^{137}Cs provides age 'markers' that correspond to a significant peak in ^{137}Cs activity, resulting from nuclear bomb testing in 1963 (van Ardenne et al. 2021), and the ^{137}Cs horizon in 1954 (Jeter 2000). This dating method relies on the assumption of constant surface material deposition, and that sediment has not been manually removed or added at the study site (Callaway et al. 2021). It has been used as a tool for assessing vertical marsh development since 1954 (Orson et al. 1998). It is useful for chronology studies on decadal time scales, as it cannot be used to date sediments prior to its first appearance in sediments in 1954 (Jeter 2000). [137]Cs is typically measured using a high-resolution gamma ray spectroscope coupled to nuclear data acquisition and reduction system (Orson et al. 1998). This method can determine the age of carbon stored, when most burial occurred, and the annual rate of accretion (Artigas et al. 2015).

Some disadvantages to using the ^{137}Cs method include the fact that results can be highly influenced by unusual weather events, such as storms or flooding, the maximum 1963 concentration can be missing where ^{137}Cs concentrations are low, some cores show more than one maximum concentration, and measurements are less accurate when surface sediments are mixed (Jeter 2000). Difficulties quantifying low concentrations of ^{137}Cs in coastal sediments and its low retention in high organic content sediments limits the applicability of this technique in salt marsh ecosystems (Chastain et al. 2021). Previous research argues age models that are established using ^{137}Cs dating result in elevated sediment and carbon accumulation rates that would be a concern if used in large-scale estimates, such as global salt marsh carbon accumulation rates (Chastain 2017).

[210] Pb

The more prevalent method of dating in recent studies is the use of ^{210}Pb age models to derive a sediment mass accumulation rate (Chastain 2017; Douglas et al. 2022). There is a decreasing trend of ^{210}Pb levels with depth to a constant level that is inherent in the sediment itself, and this trend is caused by radioactive decay of fallout ^{210}Pb with time (Jeter 2000). The estimated rate of decline with depth of excess ^{210}Pb in each core can be used to calculate sediment accumulation rates (SAR), mass accumulation rates, and ultimately carbon accumulation rates (CAR) (Gailis et al. 2020). [210]Pb dating is suitable for establishing chronology of sediment deposits over the past 100 years (Chastain et al. 2021; Jeter 2000) and provides logarithmic profiles (Jeter 2000). It can be applied to longer time periods, is highly retained in organic matter, and has lower detection limits, providing better carbon accumulation rates at greater depths than [137]Cs (Chastain 2017; Chastain et al. 2021; Douglas et al. 2022; van Ardenne et al. 2021). The ^{210}Pb method performs best in relatively quiet deposition areas, which includes marshlands (Jeter 2000). However, this method may be unsuccessful where concentrations in sediments are extremely low (Connor et al. 2001). To acquire the maximum chronology information possible for sediment cores, it is still recommended to use ^{210}Pb dating in conjunction with the ^{137}Cs method (Jeter 2000).

Cores are analysed for dating in sections (typically 1 - 2 cm) from surface level to the depth at which excess ^{210}Pb declines to zero (Poppe and Rybczyk 2018) using alpha or gamma spectrometry. The rate of decline of excess ^{210}Pb is used to estimate sediment age at various depths, which is used to determine SARs, mass

accumulation rates, and ultimately CARs (Gailis et al. 2020). SARs include a compaction correction factor applied to each section increment to estimate uncompacted depth below the surface (Howard et al. 2014). Bulk SAR is calculated for the entire core using uncompacted core depth divided by age of the deepest portion of core containing ^{210}Pb activity (Chastain et al. 2021).

There are two models used to derive ^{210}Pb chronologies: the constant initial concentration (CIC) model, and the constant rate of supply (CRS) model. They are both used to determine the long-term sediment accretion rate for a sediment core. The CIC model provides a single accretion rate for each dated core. CRS provides a separate accretion rate for each sample at various core depths, producing a range of rates within each core which are averaged to produce one accretion rate per core which can be compared to CIC model results (Poppe and Rybczyk 2018). Variants of the decay equation published by Appleby and Oldfield (1978), and a more recent publication by Appleby (200), are widely used as a basis for CRS models (Chastain et al. 2021; Gonneea et al. 2019; Poppe and Rybczyk 2018; van Ardenne et al. 2021).

The Constant rate of supply (CRS) model is used to construct the age-depth relationship and determine mass accretion by several publications (Chastain 2017; Chastain et al. 2021; Gonneea et al. 2019; Postlethwaite 2018; Prentice et al. 2020; van Ardenne et al. 2021). It provides sufficient temporal resolution to assess decadal scale changes over the past century within a marsh location (Gonneea et al. 2019). Sediment accretion rates are taken for each chosen interval, typically 1-2 cm intervals, using its specified date (Prentice et al. 2020). This model has been used to find sediment ages and accretion rates for the past century, and it assumes ^{210}Pb supply to the sediment surface is constant through time but allows for changing sedimentation rates in addition to decay (Gonneea et al. 2019).

The constant initial concentration (CIC) model uses a cumulative sedimentation rate downcore, a decay constant of ^{210}Pb, and change of excess ^{210}Pb activities with depth to estimate sediment accretion rates (Callaway et al. 2012). The CIC model is used to calculate downcore distribution of ^{210}Pb, assuming negligible migration of ^{210}Pb and associated radionuclides in sediments, constant input of ^{210}Pb from the atmosphere, and a constant proportion of ^{210}Pb trapped in sediments by weight (Callaway et al. 2012; Gailis et al. 2020). This model is also applied under the assumption that excess ^{210}Pb in sediments deposited at surface will be the same regardless of site sedimentation rate (Callaway et al. 2012) and SARs remain constant throughout time (Gailis et al. 2020). A study by Callaway et al. (2012) found that uncertainty resulting from the use of this model ranged from < 10 per cent for surface samples (<10 cm depth), to 15 per cent for deeper samples. The CIC model is ultimately recommended by Gailis et al. (2020) as it is considered to provide more accurate estimates of salt marsh age than CRS models.

When cores do not show a complete excess ^{210}Pb profile, sediment chronologies may be estimated using the Constant Flux: Constant Sedimentation model, per Krishnaswamy et al. (1971), as implemented in a recent study by Chastain et al. (2021).

^{14}C

The ^{14}C dating method is useful for dating over longer time periods (millenia) (van Ardenne et al. 2021) and greater soil depth than ^{137}Cs and ^{210}Pb. However, the effects of both compaction and decomposition may become larger with increasing depth, causing progressively deeper dates to give lower rates (van Ardenne et al. 2021). As well, van Ardenne et al. (2021) found in some cases dates fell on sections of the ^{14}C calibration curve such that there were multiple possible date ranges.

7Be

^{7}Be measurements indicate if there has been very recent deposition at a sampling location (within the last half year), and whether a sediment core has been taken with surface sediments intact. Detection of ^{7}Be in first 1 – 2 cm of sediment gives affirmative answers to these questions (Jeter 2000). If ^{7}Be is not detected in surface sediments, it could be the result of vertical mixing through bioturbation and physical processes that dilute ^{7}Be (Jeter 2000). Therefore, ^{7}Be measurements can provide supplemental information, but are not for measuring sedimentation rates. It can be measured by direct gamma spectral analysis of sediment sample simultaneously with ^{137}Cs measurements at no additional cost (Jeter 2000).

CARBON ACCUMULATION RATES (CAR)

The carbon accumulation rate is a product of vertical soil accumulation rates and average carbon density of the dated section (Connor et al. 2001) and is determined using the SAR and the soil carbon densities of sediment cores (Chastain et al. 2021; Poppe and Rybczyk 2018; Prentice et al. 2020). CAR is calculated as the per cent organic carbon of samples multiplied by the SAR (Douglas et al. 2022; Postlethwaite 2018). Alternatively, van Ardenne et al. (2021) calculates CAR by dividing organic density (per cent organic matter multiplied by bulk density) by the surface SAR. Inorganic carbon accumulation rates may also be determined by dividing the mean bulk density at the desired depth by the SAR and subtracting the CAR (van Ardenne et al. 2021). Accumulation rates may be calculated cumulatively or sectionally, by using cumulative area accretion rates, or rates applicable to smaller sectioned-off study sites. Core compaction factors must be accounted for in accumulation rate estimates (Chastain 2017).

CAR results are often scaled-up to apply to across a habitat range. Multiplying the CAR by the habitat area gives the total annual sediment carbon burial per habitat (Douglas et al. 2022). The same methods can be used to calculate the inorganic carbon burial rate. Cumulative and sectional accretion rates may be used to calculate cumulative and sectional carbon accumulation for study sites (van Ardenne et al. 2021). Commonly, studies are interested in reporting the amount of carbon stored per year in sediment of various ages. Gonneea et al. (2019) recommend choosing the timescale and sediment interval based on ^{210}Pb chronology. For example, in their study, Gonneea et al. (2019) evaluated salt marsh sediment every 2 cm over the past century, with carbon storage determined at a lower resolution (2-10 cm intervals) on larger timescales (to 557 CE). To estimate storage at such large timescales, a model was used, which assumed marsh accretion was equivalent to relative sea-level rise (Gonneea et al. 2019).

MODELLING

Modeling can also be used to estimate CARs. For example, the Coastal Wetland Equilibrium Model (CWEM), formerly known as the Marsh Equilibrium model, estimates elevation change and organic carbon accumulation of marshes over a 100-year period, including multiple scenarios of sea-level rise, sediment availability, and habitat migration (Moritsch et al. 2022). The model accounts for feedbacks between vegetation biomass, sediment capture efficiency, and accretion rate, making it more realistic than models that use a constant accretion rate across all marsh elevations (Moritsch et al. 2022).

GREENHOUSE GAS FLUX

The measurement of greenhouse gas (GHG) emissions, or flux, records variations in GHG sources and sinks. Three major greenhouse gases of concern in blue carbon habitats are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). The flux of these GHGs may be directly measured in small-scale studies, providing accurate readings over a designated area for a particular length of time. Alternatively, GHG fluxes can be estimated with computer modelling over larger timescales geographic areas.

STATIC CHAMBER SAMPLING

The static chamber technique is the most common method of direct gas flux measurement that was cited in the literature reviewed for this report. It provides a closed-system measurement of ecosystem respiration (Magenheimer et al. 1996). CO_2, CH_4 and N_2O fluxes are commonly determined using closed static chambers (Emery 2018). Chamber collars are inserted directly into the soil at the sampling site and remain in place throughout the sampling season. Gas flux measurement chambers are attached to collars during sampling, and gas samples are withdrawn with syringes during specific sampling intervals. Chambers are commonly in place for between 20 minutes and several hours and sampling intervals vary (Table 14).

Static chamber collars typically remain in place throughout the sampling season. Collar locations should be chosen randomly within representative vegetation stands (Emery 2018), including all major vegetation zones for a desired study area. Sampling periods vary, and those found in the literature for this report include:

- monthly over the course of a year (Chmura et al. 2016),
- weekly from July-September (Magenheimer et al. 1996),
- for one lunar month (Diefenderfer et al. 2018),
- or during growing season (Marsh et al. 2005).

It is customary to include vegetation within chambers in marsh flux studies (Diefenderfer et al. 2018), however this approach is limited by plant height, and chamber size limitations (Emery 2018). It is also important to monitor lighting and temperature conditions in study chambers (Emery 2018).

There are several shortcomings with the static chamber approach to measuring GHG flux. Closed chambers alter wind, temperature, humidity conditions, and can therefore alter the GHG emissions that they are designed to measure (Emery 2018; Rochette 2011). Concerns regarding chamber function include the effects on N_2O production and transport in soils, soil chamber gas transfer, and leakage from contamination of chamber headspace and air samples (Rochette 2011). However, chambers are often necessary, and work well for making comparisons because it is assumed that static chambers affect each study site equally and minimally in most months (Emery 2018). Rochette (2011) recommends the adoption of a standardized methodology to improve reliability of reported gas flux measurements and allow for comparisons across studies.

Lab-made Chambers

Chambers can be constructed with inexpensive and easily obtained items (for a description see Magenheimer et al. 1996 and Chmura et al. 2016). Typically, chambers are made of aluminum or plastic and are often ~18 L in volume, with a diameter of 20 to 30 cm. The addition of a circulation blower in each chamber ensures adequate mixing of the chamber atmosphere, and a reflective black-out cover may be used during the growing season on transparent or translucent chambers to prevent photosynthetic CO_2 assimilation (Marsh et al. 2005). Aluminum foil may be used to cover chambers to minimize temperature increases, and chambers may also include fans, pressure controls, and thermometers. Ponded sites require the use of floating chambers. To minimize disturbance, a length of tubing with a stopper attached to the chamber can be used to sample from a distance at these sites (Magenheimer et al. 1996).

Samples are taken manually from lab-made chambers, and should be analyzed as quickly as possible, or within 24 hours. Extended time periods between gas sample collection and analysis can lead to underestimates in flux due to prolonged exposure time, and possible gas leakage from collection containers (Magenheimer et al. 1996; Marsh et al. 2005). Gas samples are extracted using gas-tight syringes, through the insertion of a syringe needle through a rubber septum (Emery 2018). Gas samples may be transferred to evacuated Exetainer vials before analysis (Chmura et al. 2016; Emery 2018), in which case sample analysis should be done less than a

week after collection to minimize leakage from vacutainers (Magenheimer et al. 1996). Static chamber samples are analyzed in the laboratory for CO_2, CH_4, and N_2O on a gas chromatograph and gas calibration curves are established (Chmura et al. 2016; Emery 2018; Marsh et al. 2005).

Table 14. Sampling intervals for lab-made static chamber used by studies reviewed for this report.

Sampling Interval	Publication
20-minute intervals over 1 hour	Chmura et al. 2016
15-minute intervals over 1 hour	Marsh et al. 2005
1-minute intervals over 5 minutes	Marsh et al. 2005
Initial air samples upon setup, repeated sampling after 4-5 hours	Magenheimer et al. 1996
6-10 Samples over 25–60-minute period	Emery 2018
Chambers in place for between 20 minutes and 2 hours, during which time gas samples are withdrawn	Poffenbarger et al. 2011

Automated Chamber System

GHG flux can also be measured with automated systems, which are significantly more expensive, but improve sampling efficiency and are likely to provide more accurate estimates (Diefenderfer et al. 2018) (Table 15). Without autonomous deployment fewer samples can be collected, duration of sampling is limited, and labor investments are greater (Diefenderfer et al. 2018). Automated chambers are also the best method for addressing small-scale variability of gas concentrations among study sites (Diefenderfer et al. 2018). However, most soil-flux chamber systems cannot withstand inundation by saltwater, which should be accounted for when determining sampling location. Chambers are programmed to close for a specified amount of time at a specified interval, for example, 10 minutes each hour (Diefenderfer et al. 2018), during which time, instantaneous measurements are made within the chamber for the duration of its closure. One shortcoming of automated chambers is the potential for chamber closure and equipment malfunction, though this is rare. Diefenderfer et al. (2018) found that during their study of 2647 chamber measurements, 156 chamber closure malfunctions were identified.

Table 15. Automated chamber systems used in studies reviewed for this report.

Automated Chamber Equipment	Notes	Publication
eosGP concentration probe Eosense, Dartmouth NS Canada	Calibration range: 0-20,000 umol/mol equilibration time: <90s accuracy: +- 200umol/mol	Branimir et al. 2020
Mini-Pro CH4 Probe Pro Oceanus, Bridgewater NS Canada	Calibration range: 0-10,000 umol/mol equilibration time: 4 minutes accuracy of +- 200 umol/mol	Branimir et al. 2020

Soil CO2 Flux Sensor Eosense Dartmouth NS Canada		Branimir et al. 2020
Cavity Ring-Down spectrometer with recirculation pump model G2508, Picarroo Inc, Santa Clara CA USA	Additional components eosAC non-steady-state flow through chambers, eos MX recirculating multiplexer; eosLink-MP v1.6.6A and eosAnalyze-AC v3.4.0A software (Eosense Inc Dartmouth NS CAD)	Diefenderfer et al. 2018 (As per Crosson 2008)
Cavity ring-down spectrometer analyzer Model G2301, Picarro Inc.	Instantaneous measurement CO2 & CH4	Abdul-Aziz et al. 2018

CARBON TRANSPORT

Quantification of carbon transport between ecosystems, or study sites, is an emergent field that involves tracking organic carbon using tracers, biomarkers, or bulk sediment characteristics. Carbon transport is a relevant consideration for coastal ecosystems; for example, 50 per cent of carbon stored in seagrass meadow sediments is derived from external sources (Kennedy et al. 2010). It is assumed that most of the sequestered carbon in tidal salt marsh systems is directly produced by the plants within the system (Kennedy et al. 2010), however in some settings, there are significant external contributions of carbon derived from adjacent terrestrial or marine ecosystems. Tracking carbon using biomarkers and tracers is complex, and thus in this report only an overview of current work is provided. For more information regarding specific protocols, publication references are provided.

Tracing the origin of carbon stored in a given ecosystem can be done using stable isotopic tracers, such as ^{13}C and [15]N. These isotopes are used to differentiate between terrestrial and marine sources of organic matter and can be used to differentiate among marine source sources with distinct isotopic ratios, such as seagrasses, seston, and macroalgae (Geraldi et al. 2019). However, some blue carbon sources of organic matter have similar ^{13}C values limiting its use as a tracer. Therefore, it is suggested that more specific markers are needed (Geraldi et al. 2019).

Biomarkers, such as n-alkanes and phenolic compounds, have been proposed as taxonomic fingerprints (Geraldi et al. 2019). Molecular distribution of biomarker compounds and organism-specific biomarkers has been used to assess the differences in organic matter transport between estuaries by tracking mixing patterns (Jaffe et al 2001), which has a direct effect on the transport of carbon between sites. Relative biomarker data abundance can be used to signal degradation of organic carbon sources, increased contribution of phytoplankton or terrestrial plant material to the organic carbon pool, and the rate of output or circulation within a small ecosystem-scale study (Jaffe et al 2001). However, the biomarker profile of marine primary producers is required for this approach. Lipids can also provide convenient biomarkers to trace the source and fate of organic carbon (Geraldi et al. 2019).

[226]Ra Groundwater Tracers

Methane flux from salt marsh groundwater has been calculated from mass balances of the conservative tracer [226]Ra in the flow of salt marsh groundwater into adjacent tidal creeks in a study completed by Schutte et al. (2020). High-volume sampling is needed for [226]Ra measurements, which were taken via the establishment of groundwater monitoring sites, constructed with PVC wells across a salt marsh. Each well was sampled bimonthly

or quarterly, and radium samples collected for the determination of ^{226}Ra and ^{228}Ra activities using gamma ray spectrometry. Different wells collected samples from distinct groundwater sources with different residence times and exchange rates, which could be distinguished based on radium mixing curves. The slopes of radium mixing curves were used to estimate relative importance of each groundwater source to total groundwater discharge into an adjacent tidal creek, and the volumetric flux of groundwater into tidal creeks at each study site could be estimated.

eDNA Tracers

Environmental DNA (eDNA) is an emergent method for tracing carbon in blue carbon ecosystems and can be used to track carbon sources to the species level. For example, eDNA analysis can be used for provenance of blue carbon in seagrass meadows (Reef et al. 2017, Geraldi et al. 2019). A recent publication by d'Aurlac et al. (2021) provides an extensive laboratory technique for the handling and analysis of eDNA. Given the specificity of this tool, eDNA could help reduce uncertainty when determining organic carbon sources (Reef et al. 2017, Geraldi et al. 2019).

KELP ECOSYSTEMS

Kelp ecosystems are an emerging focus for blue carbon research. Since kelp forests are limited to rocky substrates, they do not store carbon in sediments where they grow. However, kelp are highly productive and the carbon they sequester in their tissue short-term may end up stored long-term in the sediments of nearby ecosystems or the deep sea (Krause-Jensen and Duarte 2016). The potential for kelp forests to contribute to long-term carbons stores has not been studied extensively in Canada; no publications for Canadian sites were found during this review that focused on the measurement of carbon transport from kelp forests to other ecosystems or the deep sea.

KELP CARBON STORAGE

Some work has been completed internationally to try and quantify kelp carbon storage. Bayley et al. (2017) used Google Earth imagery, field survey data, *Macrocystis thalli* mean wet weight, and carbon per cent estimates to convert kelp biomass to carbon standing stock in the Falkland Islands. Filbee-Dexter and Wernberg (2020) determined the continental-scale contribution of kelp forests to Australian blue carbon using areal extent, biomass, and productivity measures for its dominant kelp, *Ecklonia radiata* from across the entire Great Southern Reef.

Bayley et al. (2017) and Filbee-Dexter and Wernberg (2020) studies are examples of ways for kelp carbon to be estimated, and although not directly applicable to Canadian ecosystems, these give an example of the kinds of estimations that might be possible. However, these methods can rely heavily on literature estimates of kelp biomass, and corresponding carbon mass, the accuracies of which were not always recorded, and should be further investigated. If these estimates can be determined for Canadian species, it is possible that Canadian studies can make these same types of kelp carbon estimates.

A recent study by d'Aurlac et al. (2021) investigates the use of eDNA for blue carbon tracking, using deep-water coring techniques to retrieve sediment cores. A KC Denmark gravity corer is used, fitted with a weight to force it sufficiently deep into the seafloor. The corer is allowed to freefall 10 meters above the seafloor, and a vacuum created by the release of the top lid to retain sediment during retrieval. A research vessel must be used and equipped with hydraulic cranes and winches capable of lifting the corer (up to 290kg) out of the sediments, and preferably a vessel with a dynamic positioning system. On-vessel, the core is sealed

and secured, and transported with its seawater while being minimally disturbed. d'Aurlac et al. (2021) provide detailed instructions to making and assembling to deep-water corer, along with the equipment needed for sampling. Sampling in deep-water kelp forests is expensive due to the need for durable equipment and a research vessel and crew to access the study sites.

KELP CARBON SEQUESTRATION

Krause-Jensen and Duarte (2016) provide some general estimates of macro-algae carbon sequestration. It is estimated that 0.4 per cent of annual net primary productivity of macro-algae growing in soft sediments is buried and sequestered in the shallow surrounding sediments, while 0.92 per cent is sequestered through burial of particulate organic carbon in deep waters. Total export of particulate organic carbon to the deep sea is estimated at 2.30 per cent of macro-algae net primary production, and sequestration through export of dissolved organic carbon is 7.69 per cent of macro-algae net primary production (Krause-Jensen and Duarte 2016). Filbee-Dexter and Wernberg (2020) were able to use these general estimates to determine an average sequestration rate per unit of area for kelp forests in Australia.

KELP GREENHOUSE GAS FLUX

Gas flux for kelp forests was calculated by Bayley et al. (2017) by converting total carbon standing stock to CO_2, using a conversion factor of 3.67. This conversion factor is based on relative atomic weights.

TRANSPORT

Bayley et al. (2017) provide a range of estimates for the transport of macroalgal particulate organic carbon, but estimates vary by an order of magnitude, highlighting the need for targeted efforts to address the main sources of uncertainty. These efforts should include the determination of area covered by macroalgae, the amount of macroalgal-derived carbon that is sequestered in sediments, and the fate of macroalgal-derived dissolved organic carbon that is exported from the mixed layer (Bayley et al. 2017).

Kelp is often seen washed ashore in the wrack zone of beaches and shorelines. Pedersen et al (2020) estimated the contribution of detrital macroalgal carbon (*Laminaria hyperborea* specifically) to shorelines for a study focused on Norway. Fresh weight biomass was converted into units of carbon by applying a dead-weight: fresh-weight ratio, recorded as 0.163 ± 0.047 for blades and 0.135 ± 0.019 for stipes, resulting in a carbon content of 33.0 ± 3.1 per cent of dead weight for blades, and 29.7 ± 2.6 per cent for stipes (Pedersen et al. 2020). However, these literature estimates may not be applicable to the macroalgal detritus seen on Canadian shorelines.

In terms of carbon tracers, stable isotopes, pigments, and lipid tracers have all been documented to underperform at detecting and identifying kelp carbon in marine sediments. However, eDNA could address the limitations of other tools associated to support the species-specific identification of kelp contributions to carbon stores (d'Aurlac et al. 2021).

CARBON VALUATION

Measuring blue carbon stocks can help provide a tangible or monetary value to blue carbon ecosystems. This has been done in various ways by numerous publications. One popular method determines the potential carbon emissions that would result from ecosystem loss and assigns a monetary value (e.g., Pendleton et al. 2012; Postlethwaite 2018). Potential carbon emissions from conversion of a coastal ecosystem are calculated using areal estimates of extent, the current conversion rate (per cent of area lost per year) and the near-surface carbon stocks that are susceptible to loss in each habitat type. Area may be derived from international

monitoring databases and published literature, and annual area loss derived from recently published literature. A price per area based on the local carbon price per tonne can be used to assign a monetary value to ecosystem loss. Alternatively, to determine carbon value, an area may be priced based on the local carbon price, and an area's current and projected carbon stock, using both carbon storage and sequestration rates from published literature (Postlethwaite 2018). Multiplying the carbon value and the interpolated carbon stock for each area provides a value of the carbon deposit and an estimated value of stored carbon (van Ardenne et al. 2018).

The social cost of carbon (SCC) method considers the monetary value of economic damages associated with CO_2 emissions and the damages avoided by emission reduction. It is defined as the marginal value of economic damages of climate change attributable to an additional ton of CO_2 in the atmosphere in 2020 (Pendleton et al. 2012). Global emissions estimates of each type are multiplied by a recent estimate of global economic cost of new atmospheric carbon ($/ton of CO_2). The economic value of carbon accumulation over time can be estimated through total carbon sequestered under modeling scenarios, such as using the Coastal Wetlands Equilibrium Model (Pendleton et al. 2012).

CONCLUSION

This report summarizes the available published methods used to quantify blue carbon in Canadian and Canadian-applicable ecosystems. However, given that blue carbon research is relatively new, there are areas where blue carbon methods could be improved.

STANDARDIZATION

The primary recommendation made apparent through the completion of this report is the need for standardization of methods. Though the use of different methodologies is likely insignificant on a site-by-site basis, different methods are likely to significantly impact global and national blue carbon estimates. The first step towards standardization of a universal procedure is to test currently used methods for accuracy. There is very little evidence of current testing of method accuracy, and many publications rely on outdated literature as the basis for their methodology. Standardized methods would allow for more reliable comparisons between study sites and publications and would result in a more accurate estimation of current global carbon stocks. Several of the publications reviewed for this study urged that the standardization of methods for measuring sediment carbon stocks and accumulation rates, including field-based and remote sensing methods, should be highly prioritized (Fourqurean et al. 2012; McLeod et al. 2011), as well as the standardization of units during measurement and analysis (Postlethwaite 2018).

A publication by Fourqurean et al. (2012) supplied a set of recommendations for standardized terms that could be used universally in the future, listed below.

- Dry Bulk Density (DBD): the mass of dry matter divided by the volume of the undisturbed soil sample (grams per millilitre).
- Organic matter content (OM): determined by loss on ignition
- Loss on ignition (LOI): the fractional weight loss of dry sediment samples after combustion at 500–550 °C.
- Organic Carbon: determined by measuring the organic carbon content of a known mass of soil using an elemental analyser, expressed as a percentage of the dry weight.

Standardized terms could also be determined for field sampling, carbon sequestration, and greenhouse gas flux measurement processes.

RECOMMENDATIONS FOR FUTURE WORK

Updated global carbon estimates should be prioritized (Postlethwaite 2018). Global and regional estimates are currently limited to the upper 50 cm to 1 m of marsh soil, with very few studies having quantified blue carbon stocks below 1 meter (van Ardenne et al. 2018). Marshes in various geomorphic contexts likely have different patterns in carbon density and soil depth, and thus full depth carbon density estimates need to be performed for marshes in a variety of regions to properly assess these relationships (van Ardenne et al. 2018). Varied core depths and varied assumptions of organic sediment depth inhibits comparisons of carbon stock and accumulation rate estimates between studies and study sites (Postlethwaite 2018).

It would be useful to have a representative sample of carbon estimates from a variety of ecosystem types and species published (Postlethwaite 2018), which could be used when making large-scale estimates or as literature sources in low-budget studies. Data from a variety of locations would ensure researchers are able to choose published carbon conversion estimates that closely resemble their own study site. If carbon content estimations are made for each major species in Canadian salt marsh ecosystems, biomass carbon pools can be estimated without the need for destructive sampling.

Large-scale projects benefit from advancement of remote sensing mapping techniques. The collection of online databases containing publicly available information allows for the determination of blue carbon potential using GIS. Longer-term multi-year assessments of carbon entering long-term storage should be prioritized to provide baselines for the impacts of climate change and human influence on dynamic coastal environments (McLeod et al. 2011).

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