# Hakai Nearshore Marine Ecology
# Seagrass Survey Methods

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# Introduction

The Hakai Nearshore research program documents long term observations of ecosystem change in nearshore habitats, and addresses the factors driving spatial and temporal variation in these systems. Seagrass habitats are critical components of nearshore ecosystems throughout the world's coastlines and in British Columbia. Seagrass provide critical nearshore habitat for a wide variety of marine species, as well as a range of ecosystem services including provision of habitat, reduction of land-borne diseases, coastal protection, nutrient cycling, and carbon uptake (Costanza *et al.* 1997, Orth *et al.* 2006, Duffy *et al.* 2006). These ecosystems have become increasingly threatened by anthropogenic disturbances (i.e., climate change, development, pollution), which have led to large scale declines in seagrass meadows worldwide (Waycott *et al.* 2009). Hakai Nearshore's research program provides key information regarding the drivers of long-term change in seagrass communities on BC's Central Coast, where seagrass knowledge is data deficient, and assists in future conservation and management decisions at local, Provincial, and Federal levels. This document focuses on methods for documenting change for the most common temperate seagrass species, eelgrass (*Zostera marina*).

The dynamics of seagrass communities in the Hakai region are governed by a combination of bottom-up (i.e., temperature, salinity, nutrient, wave exposure effects on primary production) and top-down (i.e., predation, herbivory) processes. To elucidate the relative strength of these drivers in an understudied region, an ecosystem approach is needed. This includes understanding the trophic and bioengineering role of top predators (Hessing-Lewis *et al.* 2017), including the sea otter (*Enhydra lutris*), re-introduced to this region. Other potential disturbances to seagrass habitats include disease (e.g. *Labyrinthula zosterae*), and direct physical alteration of habitats from storms and other human activities. Climate change effects on key parameters that affect seagrass growth, productivity, and distribution may also act as stressors, often in interactive ways. Despite the multifaceted drivers of change, seagrass resilience can be conferred through plant properties (i.e. physiology, genetic structure) and supportive ecosystem characteristics.

Hakai Nearshore Ecology connects long term observations of spatial and temporal dynamics, across key nearshore habitats, with focal studies on the mechanistic drivers and mitigators of change. We conduct seasonal and annual surveys of Central Coast seagrass meadows, sampling the associated communities governing seagrass dynamics, quantifying surrounding water characteristics, and meadow disturbance, and conducting targeted studies to tease apart these interactions. This broad-based seagrass program is conducted in concert with research observations in other nearshore habitats (i.e. kelp forests, unvegetated soft sediment, rocky

intertidal), and connected to land-based terrestrial and freshwater ecosystems as well as offshore pelagic ecosystems studied by the Hakai Institute.

# Hakai Seagrass Research Objectives

1. Document change in seagrass meadows & habitat-associated diversity
   (i.e., long-term spatiotemporal variability in ecosystem structure)

2. Determine the drivers of change in seagrass systems
   (i.e., factors governing dynamics and variability in seagrass ecosystem parameters)

3. Assess resilience of seagrass to disturbance and stress
   (i.e., response to climate change, predation, herbivory, disease, substrate, etc.)

4. Quantify functional ecosystem attributes of seagrass habitats in BC
   (i.e., evaluate ecosystem services i.e. nursery effects, carbon storage, etc.)

5. Understand connectivity in seagrass systems
   (empiricize linkages with other coastal habitats & between seagrass meadows)

# Hakai Central Coast Seagrass Sites

Seagrass beds (n=9) are monitored from Calvert Island to the McMullin Group (Fig. 1, red squares). A subset of seagrass meadows (Koeye River, Pruth Bay, and Choked Passage) are monitored year-round, while the remaining meadows are surveyed once annually.

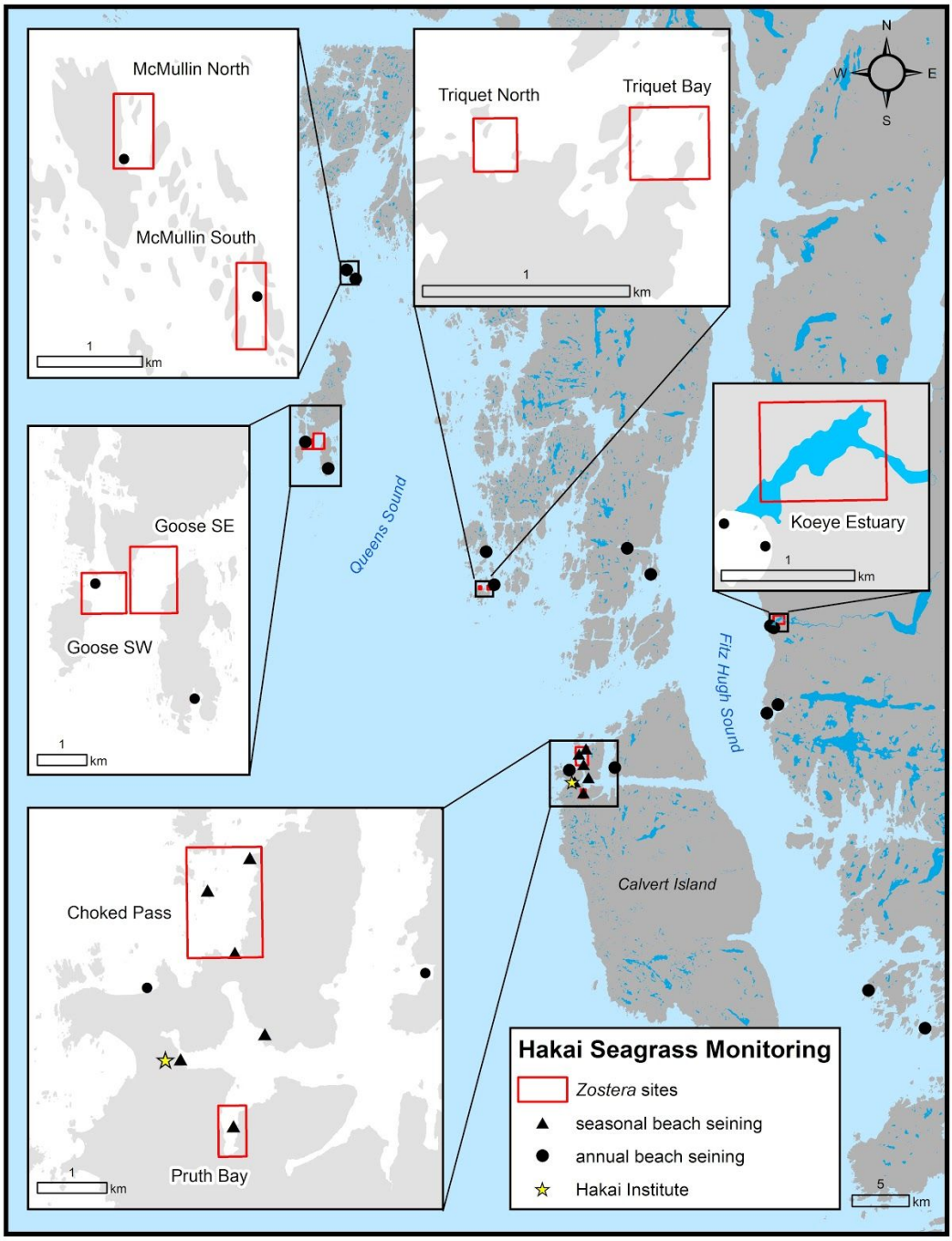



Figure 1. Map of seagrass beds surveyed (red) on the Central Coast of BC. Choked Pass, Triquet, Goose, and McMullin sites have been surveyed since 2014, while Pruth Bay and Koeye have been surveyed since 2016. Black dots represent fish surveys conducted by beach seine that occur seasonally (triangle) and annually (circle). The yellow star indicates the Hakai Institute Calvert Island Field Station.

Seagrass meadows were chosen to encompass a range of sizes, depths, tidal and freshwater/marine influences, as well as variation in sea otter occupancy times and active foraging activity (Table 1). These seagrass meadows represent the range of seagrass habitats

observed in British Columbia, however our sampling does not capture small, fringing beds. Annual monitoring involves paired sites, within an island group/region, with contrasting sea otter foraging activity.

Table 1. Seagrass Site Characteristics.

| Monitoring | Site | Tidal Exposure, Mean Chart Depth | Water Inputs | Size ($m^2$) | Sea Otter Occupancy Time | Mean Grain Size (µm) | % Organic Carbon |
|---|---|---|---|---|---|---|---|
| Seasonal | Koeye River | Subtidal, 3.0m | Estuarine | 25,000 | NA | 775.60 ± 212.22 | 0.33 ± 0.11 |
| | Pruth Bay | Intertidal/ Subtidal, -0.5m | Freshwater | 8,900 | NA | 247.93 ± 131.51 | 0.50 ± 0.12 |
| | Choked Passage | Subtidal, 3.5m | Marine | 355,000 | 2014 | 277.73 ± 33.33 | 0.19 ± 0.05 |
| Annual | Triquet Bay | Subtidal, 0.5m | Marine | 31,000 | 2011 | 423.90 ± 178.57 | 0.60 ± 0.26 |
| | Triquet North | Subtidal, 3.0m | Marine | 8,900 | 2011 | NA | NA |
| | Goose SE | Subtidal, 2.5m | Marine | 569,520 | 1980's | NA | NA |
| | Goose SW | Subtidal, 3.0m | Marine | 191,076 | 1980's | 341.58 ± 177.46 | 0.26 ± 0.03 |
| | McMullin S | Subtidal, 1.5m | Marine | 16,900 | 1996 | NA | NA |
| | McMullin N | Subtidal, 0.5m | Marine | 18,500 | 1996 | 343.07 ± 65.82 | 0.84 ± 0.35 |

# Observational Surveys

## Frequency

### Seasonal

Each seasonal site (Koeye River, Pruth Bay, and Choked Passage) is sampled monthly (approximately), through the summer season. In the winter, Pruth Bay and Choked Passage sites are sampled opportunistically approximately every 2-3 months (ie: November and February). Koeye sampling frequency is lower due to its distance from the Calvert Island Observatory. 3-5 days are required to survey the three sites: 1 site per day, involving sample collection via SCUBA in the morning and processing in the afternoon or evening. Koeye sampling is dependent on access during the highest tide, and is spread over 2 days. Additional days and staff may be required to help process samples. Shoots for growth are marked during surveys, and require retrieval 10 days after the monitoring work to allow for a sufficient growth interval.

### Annual

All 9 sites are sampled once during the summer field season in a 2-3 week period. Sample collection and processing are scheduled on alternate days as both require considerable time. Each dive day typically consists of two dives (60-70 minutes each) at one site. A minimum of two divers and one dive tender are needed. Additional staff may be required to help process samples in the lab. Note: An extra visit to each site is needed, 10 days before/after time of survey, depending on when shoots are marked for growth measurements.

## Survey Design

All sites have 6 permanent transects: 3 interior and 3 edge transects within a seagrass meadow (Fig 2.). Each replicate transect is 30m long. Transect distribution aims to capture variability across each seagrass site, and spacing is relative to bed size and configuration. This design also allows us to investigate edge effects.

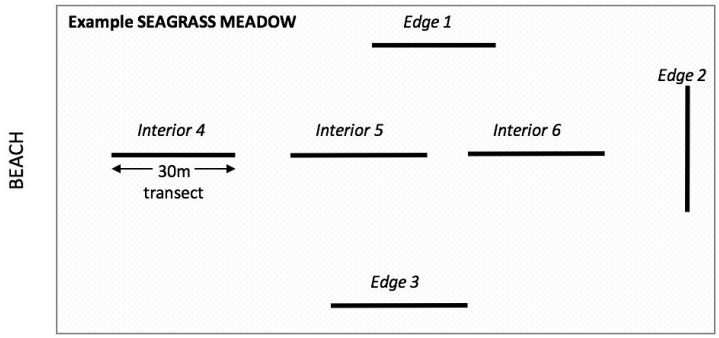


Figure 2. Example survey design in Hakai seagrass meadows

# Survey Protocol

## Overview

Seagrass-specific parameters and seagrass-associated macrophyte and faunal communities are observed annually and seasonally (near monthly). During each seasonal seagrass site (Table 1) visit, SCUBA divers conduct transect-level surveys to record Zostera *meadow* characteristics, and *Fish* and *Macroinvertebrate* abundances and diversities (Table 2). SCUBA divers collect *Biomass* samples of seagrass, and their associated epiphyte and epifaunal communities for lab analysis, and pickup Zostera shoots that were pin-pricked for *Growth* measurements. YSI measurements are taken on each survey day. Annually, additional surveys are added to capture *Clams*, *Below ground* metrics, and *Sediment disturbance* (via sea otter digging).

Table 2. Surveys conducted in seagrass meadows and respective metrics obtained.

| Survey | Frequency | Level | Parameters Collected | |
|---|---|---|---|---|
| | | | ***Field*** | ***Lab*** |
| Habitat | Seasonal Annual | Transect | Record: Depth, adjacent habitat, next dominant macrophyte, substrate, patchiness | |
| Density | Seasonal Annual | Quadrat | Record: Depth, seagrass density, flowering shoots, canopy height | |
| Fish | Seasonal Annual | Transect | Record: Species, abundance, size | |
| All Macro-invertebrates | Seasonal Annual | Transect | Record: Species, abundance, size | |
| Biomass | Seasonal Annual | Shoot | Collections: *Zostera* shoots, 10 sec Video Panorama | Biomass: Zostera shoot, rhizome, epiphyte, mesograzers; Zostera morphometrics, Wasting disease; Voucher Samples |
| Water Chemistry | Seasonal Annual | Site | Record: Temperature, conductivity, salinity, pH | |
| Growth | Seasonal Annual | Shoot | Collections: *Zostera* shoots | Growth rate |
| Crab CPUE | Annual | Meadow | Record: Abundance, size | |
| Belowground | Annual | Transect | Collections: Sediment cores | Seed abundance and viability, belowground biomass |
| Clams | Annual | Transect | Collections: Clams on sediment surface | Clam length, abundance |

| | | | |
|---|---|---|---|
| Sediment Disturbance | Annual | Site | Record: Number and size of otter pits |

# Field Protocols

## A) Habitat Survey

Habitat surveys are conducted to collect long-term observations of seagrass meadow characteristics, condition, and distribution. This survey is conducted in conjunction with the density surveys, fish and macroinvertebrate surveys, and seagrass biomass collections.

**Equipment**

- Dive slate
- Habitat Datasheet (Appendix 1)
- Transect tape (30m)

**Survey**

Using SCUBA, the diver will start at the 0m mark, and record the following parameters for every 5m section along the transect line (n = 6 observations at 0-5m, 5-10m, 10-15m, 15-20, 20-25, 25-30m):

- *Depth*: Record depth of water
- *Adjacent Habitat*: Record bordering habitat: *Nereo kelp, Macro kelp*, *flat kelp*, or *sand*
- *Next Dominant Vegetation*: Record the next dominant vegetation growing in the seagrass bed, including algae species, and estimate *% cover*.
- *Substrate*: Silt, Sand, Shell Hash, Pebble, Cobble, Boulder, Bedrock
- *Patchiness*: Note size of sand patches within the transect bin: *<1m, 1-2m, 2-3m, 3-4m, 4-5m*
- [1] *Sediment Disturbance*: Along the entire transect, within a 4m total swath (2m each side of transect, count and measure all disturbed areas of the seagrass meadow. Disturbance is characterized by pits with rhizome's clearly dug up. When pits are encountered, record length and width.

[1] Indicates data collected annually only.

Additionally, survey start and finish time must be recorded, so that depths can be corrected for tide heights.

## B) Density Survey

Density surveys are conducted to collect long-term observations of seagrass meadow characteristics, condition and distribution.

### Equipment

- Dive slate
- Density Datasheet (Appendix 2)
- Transect tape (30m)
- $0.25m^2$ x $0.25m^2$ quadrat ("Bident", Fig. 3)

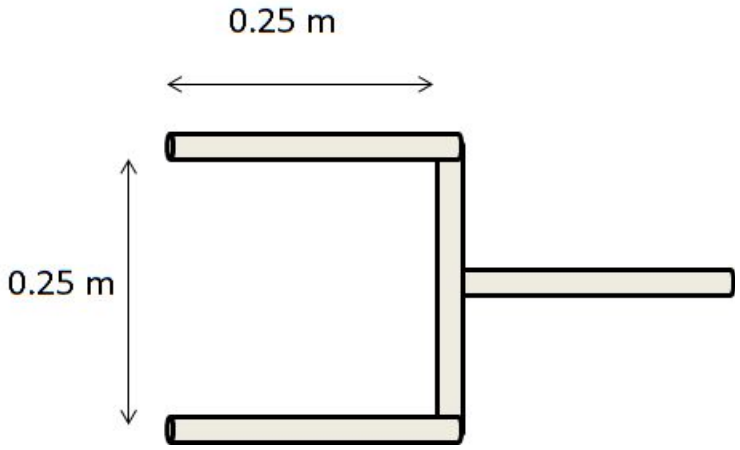



Figure 3. Example of bident used for quadrat surveys

### Survey

Using SCUBA, the diver will start at the 0m mark, and record the following parameters every 5m along transect line (n = 7 quadrats per transect at 0m, 5m, 10m, 15m, 20m, 25m, 30m)

- *Depth*: Record depth of water
- *Zostera Density*: Count and record the number of shoots per quadrat
- *Phyllospadix density*: Count and record the number of shoots per quadrat
- *Flowering Shoot Density*: Record number of flower shoots in the quadrat
- *Canopy Height*: Use slate (with ruler) to measure average height of seagrass blades

Additionally, survey start and finish time must be recorded, so that depths can be corrected for tide heights.

## C) Fish Survey

Fish surveys are conducted along a standardized transect: 30m long x 4m wide (2m swath on each side of transect) x 4m high (water column height). During each site visit to a site, fish surveys are conducted by each diver on transects (n=2 per transect, n=12 per site).

### Equipment

- Transect tape
- Dive slate (marked with cm ruler on top edge).
- Datasheet (see Appendix 1-2)

### Visual Survey

Standardize observation effort to 5-minute swim along the 30m transect.
1st Diver records fish 2m on either side of transect line (4m total swatch) by 4m above bottom surface.

- *Species*: Identify to the lowest taxonomic resolution
- *Abundance*: Record number of fish. With schools, estimate to 10's or 100's.
- *Estimate size*: Use ruler (cm) on slate to measure size of fish (total length)

Record start and finish time, as well as current so survey effort and conditions can be accounted for. The second diver follows, conducting the macroinvertebrate survey (see below), and repeats the fish survey on the return swim.

## D) Macroinvertebrates Survey

Macroinvertebrate surveys are conducted along a standardized transect: 30m long x 2m wide (1m swath on each side of transect)). During each visit to a site, repeat invertebrate surveys (n=2) are conducted by 2 divers on each transects: 1 survey of all macroinvertebrates and 1 survey of cancer crabs and bivalve siphons only. A total of n=12 surveys are conducted at each site.

### Equipment

- Dive slate marked with cm on top of the slate.
- Datasheet (Appendix 1-2)

### Visual Survey

The 1st diver records all macroinvertebrates >1cm, 1m swath on either side of transect line (2m total swath).

- *Species*: Identify to the lowest taxonomic resolution
- *Estimate size*: Use ruler (cm) on slate to measure size of invertebrate
- *Abundance*: Record number of individuals

Record start and finish time, as well as current so survey effort and conditions can be accounted for. 2nd diver repeats survey after diver 1 is complete, but records only cancer crab species >1cm and bivalve siphon counts and identification when possible.

## E) Crab Trap Survey

The following protocol was designed for the 2017 study 'Ecosystem assessments of sea otter impacts to eelgrass' spanning Alaska - BC - California. 2-4 traps should be soaked for 24 hours (± 2-4 hrs) near the seagrass transect in the middle of the bed, time of deployment and retrieval recorded (to calculate CPUE). All crabs are identified to species, sexed, measured for carapace width and then released. If fish are captured in the traps, place in bucket of fresh seawater, process (ID and measure), and release first before invertebrate work. Identify all fish to the lowest taxonomic unit possible and measure for total length. All other invertebrates should be identified to the lowest taxonomic unit possible, counted, and measured. All animals can be released after measuring and counting.

**Equipment**

- Fukui traps (x2) with openings modified to 200mm
- Shrimp pots (x2) with openings modified to 130mm
- Calipers (x2)
- Measuring tape (x1-2)
- Datasheet (Appendix 3)
- Container for bait (1 per trap)
- 2 lbs of frozen anchovies or similar bait (containers stuffed full of bait)
- Surface floats with lead line for marking traps (x 2- 4)
- Waterproof camera (please take a site photo at each site).
- Tidbits (x2)
- Buckets to hold live samples while processing (x2)
- YSI (water temp and salinity)

Fukui crab trap set at the at a seagrass meadow in the Goose Group.

## F) Seagrass Biomass

Along each transect, SCUBA divers collect seagrass shoots every 10m. A total of n=4 shoots per transect are collected and n = 24 shoots per site (n = 28 for Choked Pass due to additional transect).

### Equipment

- Dive slate with Datasheet (see Appendix 1 for Habitat Datasheet)
- Mesh collection bag
- Large Ziploc bags (uniquely labelled) per transect (n=4 per transect)

### Collections

1. Starting at 0m, use a Ziploc bag to collect 1 seagrass shoot every 10m; outside of quadrat area used for density counts
2. Break the seagrass shoot below the first node, and package into the Ziploc bag as gently and quickly as possible, to minimize loss of mesograzers, diatoms, etc.
    - *For seasonal sampling*: Break shoots at the first rhizome internode, near the base.
    - *For annual sampling*: Remove ~10cm (7cm is needed in lab) of rhizome attached to the shoot
3. Record Ziploc bag ID on datasheet with corresponding site, transect ID, and distance.
4. To keep track of biomass data, mesograzer data, and epiphyte data pertaining to specific shoot morphometrics, assign a unique ID (UID) to each shoot (UID tagging conducted by surface team).
5. Return sample to lab and store in fridge for lab processing (pg. 18)

Diver collecting seagrass shoot in Ziplock bag.

## G) Seagrass Growth

At each site, haphazardly select three bundles of 5 intact shoots from 1 edge and 1 interior belt transect to be marked (n=2 bundles per transect during seasonal sampling, n=2 bundles per 1 edge and 1 interior transect during annual sampling). Mark shoots at the 0m end of each transect for easy collection finding. Tie the bundle with flagging tape and reference with surface float attached to an anchor.

### Equipment

- Pins (dissecting or hyperdermic needles work well)
- Flagging tape
- Surface floats

### Shoot Marking Method (adapted from Nash & Martinez 2015, Duffy *et al.* 2014, Short & Gaeckle 2002)

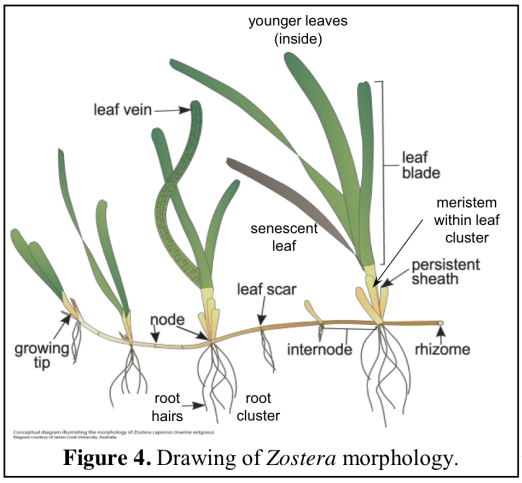
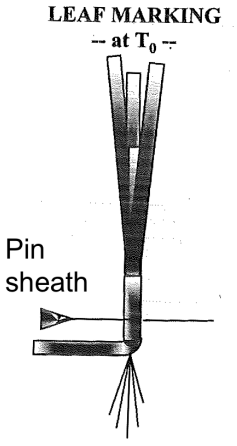



**Figure 4.** Drawing of *Zostera* morphology.




1. Mark shoots in the field (T0_date_marked). Choose shoots that don't exhibit signs of recent or imminent flowering.
2. Make 3 parallel pin prick holes through the outermost leaf sheath to create a leaf scar on all blades present at time of marking using a dissecting needle. The outermost sheath should contain a bundle of shoots including intact, fully extended blades. If blades/leaves are smaller (i.e <5mm), punch 2 holes. Keep marks consistent and well defined to avoid confusion with other natural leaf damage.
3. Holes should be 1 cm below the top of the outermost sheath mark. To determine where the bundle sheath is located, peel back the blades from the middle of the shoot bundle. The sheath forms a bundle around blades, and prevents peeling of blades down to rhizome. There may be another, younger inner sheath that is higher up than the outermost sheath. Be careful not to rip the sheath mark.
4. The outermost blades are often in the process of sloughing and many will not be retained through the growth interval. Only mark blades within the sheath bundle.

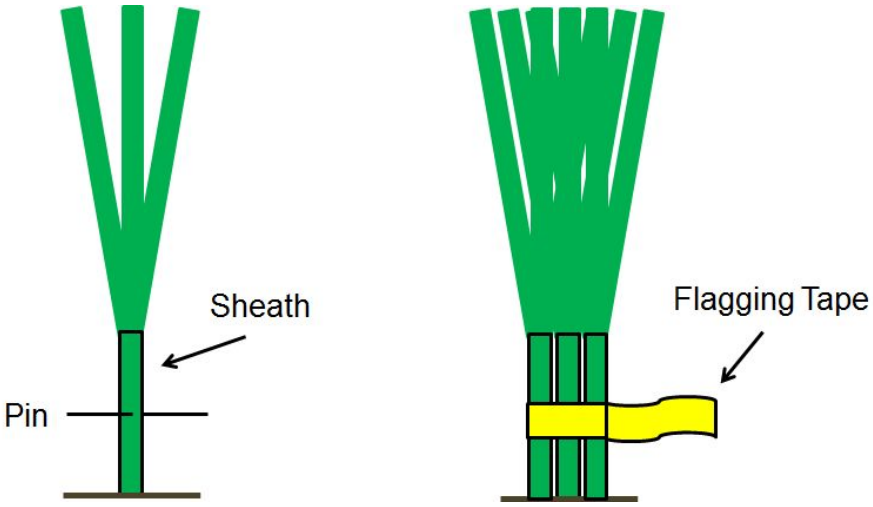



Figure 5. Pricking seagrass shoots 1cm below sheath and marking with flagging tape.

## Shoot Collections

1. Marked shoots should be left to grow for ~10 days or sufficient time to allow for a plastochrone interval: the time for production of a new blade/leaf. Note date collected to calculate growth period.
2. Re-locate marked shoots in the field and carefully bundle into large Ziploc bags. Create loose bundles by holding shoots at their rhizomes. Avoid tight bundles or folds of the plants to decrease blade loss and blade damage. Keep water in bags to prevent tangling. Bring to lab for growth measurements.
3. Keep shoots in water and cold prior to growth measurements. Rinse shoots prior to measurements.
4. If processing won't occur within 2-3 days of shoot collections, scrape any epiphytes from the shoots and freeze to process at a later date (drain water prior to freezing). Note that freezing makes growth processing difficult and should only be done if no other option is available. See page 20 for lab methods.

## H) Below ground Biomass Cores

Once a year at each site, SCUBA divers collect sediment cores every 10m along each transect. A total of n=24 cores are collected at each site (n=28 for Choked Pass due to additional transect). , Sediment core collections are paired with the Habitat and Density Surveys (pg. 8),

### Equipment

- Modified clam gun (10cm diameter x 10cm height)
- Ziploc bags
- Dive slate
- Datasheet (see Appendix 1)
- Unique IDs

### Methods

1. Using SCUBA, insert clam guns to 10cm depth and plug suction hole found on handle or barrel (hole location will vary depending on model used)
2. Pull sediment core out and place contents into Ziploc bag
3. On datasheet, record Ziploc ID number and distance under corresponding transect information
4. Bring to surface where each Ziploc bag will be given a unique ID on the boat. See pg. 24 for lab methods.

## I) YSI Measurements

### Equipment

- Calibrated YSI unit with cord at least 10m long (or max. depth at sites)
- Datasheet (Appendix 4)

### Methods

1. Calibrate the YSI in the morning of fieldwork.
2. In the middle of the seagrass bed, take a discrete depth profile with readings at each metre (ie. surface, 1m, 2m, ..., bottom) with the YSI. At each depth, wait 10sec before taking reading:
    - Temperature (°C)
    - Pressure (mmHg) (Optional)
    - Specific Conductivity
    - Salinity
    - pH

## J) Clam Assemblage

Collections of clams on the sediment surface are made across annual survey sites every 2 years. These clams represent the 'death assemblage' of bivalves and provide data as to the different causes of mortality. Using SCUBA, $1m^2$ quadrats are used to collect surface clams by hand along n=2 transects per site (1 interior and 1 edge). These are conducted along the same permanent 30m long transects.

### Equipment

- Large mesh bags
- Dive slate
- $1m^2$ quadrat

### Methods

1. SCUBA divers collect clams every 10m along seagrass meadow.
2. Place quadrats on one side of the transect.
3. Collect all clams >1cm (whole and fragments) in mesh bag

# Lab Protocols

## A) Seagrass Biomass

Divers collect individual shoots (up to 4 per transect). Each bag will have a unique ID and should contain 1 seagrass shoot, and associated flora and fauna. If a bag contains more than 1 shoot, remove excess material. Process 4 shoot samples per transect (2 minimum). Collect samples for dry weights (biomass) of the following shoot components:

- Seagrass Blades (above ground biomass)
- Seagrass Rhizomes (below ground biomass)
- Invertebrates
- Algal Epiphytes
- "New growth" from one shoot sample per transect
- Filtered Diatoms

### Equipment

- Extra small foil packets for invertebrates (pre-weighed: mg)
- Small foil packets with 0.7 micron Grade F filters (pre-weighed with filter: mg)
- Medium foil packets/whirlpacks for seagrass and epiphytes (pre-weighed: g)
- Spray bottles (1 tap water per person + 1 deionized water)
- Basins
- Large graduated beakers (1L, 1 per person)
- Microscope slides
- Permanent markers
- Corresponding datasheets from the dive (Appendix 1) and lab datasheets (Appendix 5)
- 500 um sieve
- Forceps
- Petri dishes
- Syringe
- Filtering apparatus
- Tape measure (2 m) taped to lab bench for measuring shoot lengths
- Small rulers for measuring invertebrates
- Analytical balance
- Wasting disease datasheet, scanner, and transparencies

### Preparation

1. Pre-assemble and weigh filter packets. Prepare labels with stickers containing information on collection type, site, transect, collection date (YYYY-MM-DD)
2. Pour contents of sample bag through 500um sieve (discard excess water in sink)
3. Place seagrass sample in basin. Collect all visible invertebrates from sieve and bag surface and place in labelled petri dish.

### Morphology

4. Cut blade off at first node and keep everything above this for above ground biomass. Set aside rhizome for below ground biomass processing (annual surveys only).
5. Measure length of longest blade, from first node to tip (measure in cm, to nearest tenth). Note if tip is broken.
6. Measure width of longest blade at midpoint (measure in cm, to nearest tenth)
7. Count number of blades
8. Score for wasting disease, if present scan shoot

### New growth – only 1 shoot per transect (at the 0m mark)

9. Peel back shoot at sheath to expose newest blade, scalpel or scissors can be helpful
10. Separate from the rest of the shoot (from blade tip to 1st node) and place in labelled foil packet (mg) after scraped and rinsed well with deionized water. Label packet with 'NS'

### Scraping

11. Above the first node, cut blades into sections (if needed) and lay out in basin. For annual measurements, save below ground biomass
12. Gently scrape each blade with a microscope slide to remove epiphytes and diatoms
13. Rinse with fresh water over basin and pour through 500um sieve into graduated beaker
14. Save this 'diatom slurry' (<500um) in the beaker for filtration
15. Save all >500um epiphytes and group taxonomically in foil packets (mg) = unfiltered epiphyte biomass
16. Place remaining seagrass shoot into pre-weighed and labelled whirlpak (g) = seagrass biomass

### Invertebrates

17. Group all individuals into taxonomic groups (Appendix 6). Record abundance (where applicable), trait-based ID, average length of the group (mm), as well as the minimum and maximum lengths (mm) of individuals, packet UID, and foil weight on data sheet
18. Place each group in labelled foil packet to be dried in oven.

### Diatom filtration

19. Dilute diatom slurry in graduated beaker to 500mL with tap water
20. Stir sample. Using syringe or graduated cylinder, extract 50mL of sample and filter through pre-weighed Grade F filter (0.7 micron)
21. Fold paper in half and place in labelled foil packet (pre-weighed with filter).
22. Record packet UID, and foil weight on data sheet

### Below ground annual measurements

23. Trim rhizome 7cm below first node cut.
24. Count number of internodes and measure each one in cm, to nearest tenth. Note transition to winter months (shorter), last year's growth should be darker/brown. Categories for coloration are: Light (white to pale yellow), Medium (beige), Dark (brown).
25. Dry the rhizome, including rootlet material, in labeled foil package for below ground biomass.
26. Record rhizome packet UID, and foil weight on annual lab data sheet (Appendix 7)

### Drying

27. Dry all foil packets in the oven at 60°C for 24-48 hours, until crispy
28. Record all dry weights in Google Drive in the seagrassMonitoringDryWeights sheet.

## B) Seagrass Growth

### Equipment

- Small and medium foil packets (pre-weighed mg and g)
- Spray bottles (tap water)
- Basins/Tray
- Microscope slides for scraping off epiphytes
- Measuring tape
- Permanent marker
- Datasheet (Appendix 8)

### Measurements

1. Find clearly marked pin pricks on 4 shoots per transect, for a total of n=24 per site (n=6 transects per site). Best specimens will have observable scars on all marked blades. Per transect, half the shoots are analyzed for detailed and half for EZ biomass allocation.
2. Record processing date, and name of processor (processor_ID).
3. Record the total sheath length (from 1st node to innermost sheath edge) and width (mid way along sheath).
4. Clip shoot at node insertion point (keep rhizomes for measurement and reference mark for annual measurements. Also clip at Reference mark (Fig. 6C "Cut here"). Record Reference length (distance from 1st node to reference marks).
5. Peel apart leaves from the outside in, like a book. Array shoots in order by age, e.g. youngest to oldest leaf/blade (Fig. 6C). Each leaf has a segment within the sheath. Match leaves with sheath segment. You will have to unfold and peel apart the sheath carefully, using a sharp tool to cut down the margins of the sheath. Some of the inner blades will be very thin so be careful.
6. Check to see if there is a new blade ("Baby blade") within the sheath bundle. If a new blade has been produced (i.e. Leaf 0/Baby blade Fig.6C), indicate "Baby Blade" on spreadsheet. Note length (=growth) for this blade from Node 1/insertion point to tip, and keep it separate for biomass measurements.
7. Work from oldest to youngest for leaf measurements. For all leaves, except "Baby Blades" measure new growth from reference mark to pin prick scar (New Growth). For young leaves with no scar, measure from reference mark cut to tip of the leaf (Fig. 6B L2). For reference blade, mark "NA" for growth. If may help to cut blade at pin prick – the growth segment will be kelp for biomass.
8. For length measurements, measure length from insertion point to leaf tip. If there is no sheath mark, measure from 1st node/insertion point (Fig. 6C L2). If it's a "Baby Blade" new growth = length (insertion point to tip).
9. Figure C shows what a side branch would look like. Note "branch #" in blade ID for these.
10. If blade is broken, indicate "B" for broken. If the tip is not rounded, this is considered broken. Note if blade is new (new_blade = no mark = Y)

## Growth Biomass (Dry Weight)

A) Detailed Biomass (n=2 per transect)

*Goal: compare all growth measurements (see below)*

- If there's a baby blade, weigh and package separately.
- For the third shoot, separate New, Old and Sheath biomass and dry
- For other plants, separate remaining New, Old and Sheath biomass and dry

Package prep

1. Baby blade (node to tip)
2. 3rd Old (scar to tip)
3. 3rd New (ref to scar)
4. 3rd Sheath (node to ref)
5. Old Bio (all leaves scar to tip)
6. New Bio (all leaves ref to scar)
7. Sheath Bio (all leaves segments within sheath/below reference length)

B) Easy Biomass (n=2 per transect)

*Goal: compare sheath length to plastochrone interval method*

- Package 3rd blade separately from the rest of the blades (combine these)

**Package prep**

1. EZ 3rd blade (node to tip)
2. EZ blade bio (all remaining plant parts)

Figure 6. A) Leaf order for measurements by Ruesink (modified from Gaeckle & Short 2002, Nash & Martinez 2015, and Emmett et al. 2014) and B) Leaf order for measurements by Nash and Martinez (2015). C) Assemblage of blades when processing for growth.

**Figure A**

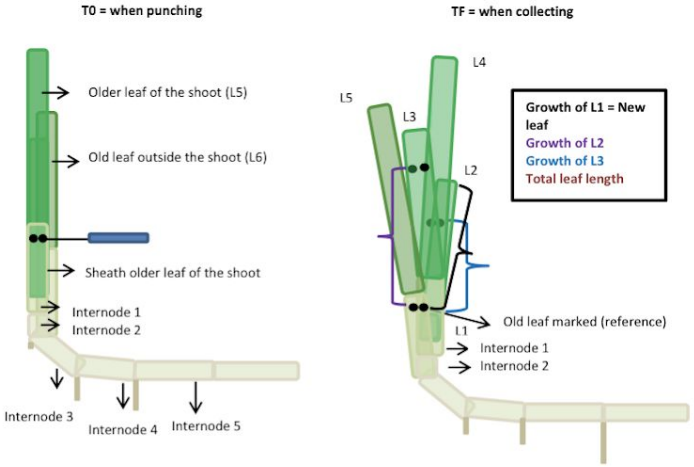



**Figure B**

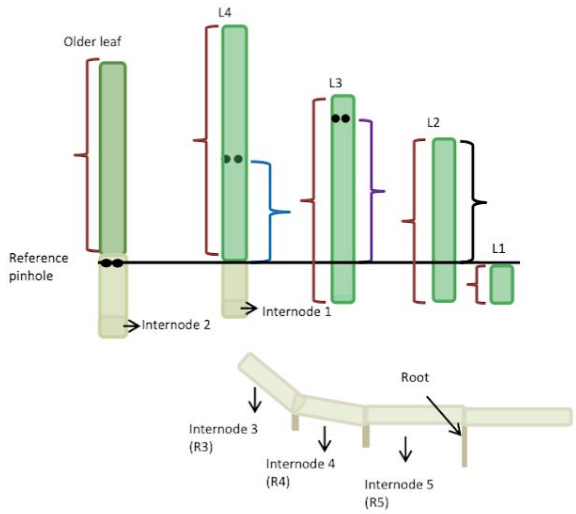
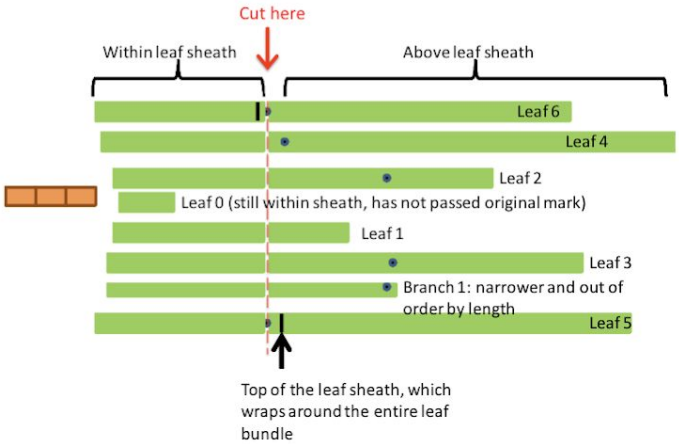



**Figure C**



## C) Wasting Disease

From each biomass collection (see Field Methods Section E for collections, and Lab Methods for preliminary processing), temporarily take the 2nd oldest blade for assessment of seagrass wasting disease (*Labyrinthula zosterae)* prevalence (presence/absence) and severity (proportion of blade infected). From each site, a total of n = 24 shoots will be assessed. Be sure to return the blade for biomass measurements (Lab Methods - Section A Seagrass Biomass).

### Equipment

- Microscope blade for scraping
- Lab Datasheet (Appendix 9)
- Scanner
- Transparencies
- Printed rulers (google drive)
- Permanent marker

### Measurements

1. Take the clean 2nd oldest blade from biomass collections for assessment, cut at the first internode. Keep track of site, transect ID, and date.
2. Measure length and width of blade.
3. Carefully look for wasting disease characterized by translucent lesions outlined by a black band.
   See the Wasting Disease Handbook in the Nearshore Google Drive for wasting identification and/or consult with Krystal Bachen or Angeleen Olson.
4. Record if there are lesions present or absent. If present, cut out the section containing lesion(s) for scanning (Fig. 7).
5. Scan all lesions from the same site together using transparencies, if possible, but keep track of which blade (i.e.: transect) it came from. Label each transparency, generally, with site and date of collection, and each blade with transect ID.
6. Return each blade to their respective labelled biomass packets to be dried.


Figure 7. Example scan of wasting disease.

## D) Below Ground Biomass Cores

### Equipment

- Coarse sieves (2000um mesh) and fine sieve (500um mesh)
- Small and medium foil packets (pre-weighed mg and g)
- Permanent marker
- Datasheet (Appendix 10)
- Petri dish

### Measurements

1. With the sieves nested, rinse sediment through the coarse 2000um sieve collecting everything that falls through in the 500um mesh. From the coarse sieve, keep all rhizome material (rootlet hairs and rhizomes), but discard everything else.
2. Rinse sediment thoroughly through the 500um sieve. Keep all rhizome material and search for seeds. Categorize and count viable (hard) vs. unviable (squishy) seeds. Note colour: light (white/green) or dark (black/brown), firmness: hard or soft, and if only a husk (empty seed casing).
3. Thoroughly rinse all rhizome material. Package in pre-weighed foil packet with UID, record on data sheet, and dry in drying oven.

*Zostera marina* seeds from Below Ground Biomass Cores

## E) Clam Methods

### Equipment

- Bins for sorting
- Calipers
- Datasheet (Appendix 11)

### Measurements

1. Separate clams into species or functional groups and record abundance
2. Record width (mm) and height (mm along primary growth axis) to determine size-weight relationships.
3. Record fouling of the shell. The fouling score corresponds to the age of the shell, such that the fouled shells are considered old and the unfouled shells are more recent:
    - Score 3 (fouled) - algae or other organisms growing on it
    - Score 2 (unfouled) - shells had no algae growth and should be clear of flesh
    - Score 1 (unfouled) - remnants of flesh are present
4. Record evidence of predation:
    - Smash or shear – indication of sea otter predation by characteristic damage to the shell by tooth or tool (Kvitek *et al*. 1992).
    - Chip – indication of crab predation, as they are known to chip at the edges of clams until they can access the meat (Boulding, 1984)
    - Drilled – indication of Gastropod predation due to the characteristic drill marks on the shell (usually a round hole in the umbo region)
    - Unknown

# Appendix

Appendix 1. Habitat and Fish/Invertebrate Datasheet

**Seagrass Fish & Macroinvert Surveys**

On each side of the line: 2m fish surveys / 1m invert surveys

**Site** *CH PR KY* **Tran** *E I* ____ **Date: 2017 -** ___ - ___ **Diver:** _____

| Transect ID: | | | Transect ID: | | |
|---|---|---|---|---|---|
| F Inv Cr Vis (m): | | Cur: 0 L M H | F Inv Cr Vis (m): | | Cur: 0 L M H |
| Time In: | Time out: | | Time In: | Time out: | |
| Species | Size (cm) | | Species | Size (cm) | |
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**HABITAT SURVEY**

4 m Belt Transect (0-4m from edge)

Adj Habitat: Seagrass, Standing Kelp, Flat Kelp, Sand

Vegetation: Zm, Nereo, Macro, Ptery, Des, Cyma, Agarum, SacLat, Ulva

**Time Start** _____ **Vis** _____ m

**Time End** _____ **Diver** _____

Substrate: Sand, Shell Hash, Mud, Rock, Gravel, Pebbles, Algae

Patchiness: <1m, 1-2m, 2-3m, 3-4m, 4-5m

| Dist (m) | Depth (m) | Adjacent Habitat | Next Dominant Vegetation | Substrate | Seagrass Patchines | Flowering Shoots (#) | Bag ID | Blade UID |
|---|---|---|---|---|---|---|---|---|
| 0 - 5 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |
| 5 - 10 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |
| 10 - 15 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |
| 15 - 20 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |
| 20 -25 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |
| 25 - 30 | | | 1 | 1 | | | | |
| | | | 2 | 2 | | | | |

Appendix 2. Density and Fish/Invertebrate Datasheet

## Seagrass Fish & Macroinvert Surveys

On each side of the line: 2m fish surveys / 1m invert surveys

**Site** *CH PR KY* **Tran** *E I* ___ **Date: 2017 -** ___ **-** ___

| Transect ID: | Diver: | | Transect ID: | Diver: | |
|---|---|---|---|---|---|
| F Inv Cr | Vis (m): | Cur: 0 L M H | F Inv Cr | Vis (m): | Cur: 0 L M H |
| Start Time: | End Time: | | Start Time: | End Time: | |
| Species | Size (cm) | | Species | Size (cm) | |
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## Seagrass Density Survey

At edge sites, 2m into meadow

**Time Start** _____ **Vis** _____ m

**Time End** _____ **Diver** _____

| Dist. | Depth (m) | Quad Density (# shoots) | Flowering Shoots | Canopy Height (cm) | Notes |
|---|---|---|---|---|---|
| 0 | | | | | |
| 5 | | | | | |
| 10 | | | | | |
| 15 | | | | | |
| 20 | | | | | |
| 25 | | | | | |
| 30 | | | | | |

Appendix 3. Crab CPUE Datasheet

**Seagrass Crabs**

| Habitat | Site | plot # | start date | set time | retrieval time | species | sex | size (mm) | comments |
|---|---|---|---|---|---|---|---|---|---|
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Appendix 4. YSI Datasheet

## Hakai Nearshore YSI

**Project:** **Date:**
**Site:** **Processor:**

| Depth | Temp (C) | SPC | Salinty | pH |
|---|---|---|---|---|
| 0m | | | | |
| 1m | | | | |
| 2m | | | | |
| 3m | | | | |
| 4m | | | | |
| 5m | | | | |
| 6m | | | | |
| 7m | | | | |
| 8m | | | | |
| 9m | | | | |
| 10m | | | | |

Entered:

**Project:** **Date:**
**Site:** **Processor:**

| Depth | Temp (C) | SPC | Salinty | pH |
|---|---|---|---|---|
| 0m | | | | |
| 1m | | | | |
| 2m | | | | |
| 3m | | | | |
| 4m | | | | |
| 5m | | | | |
| 6m | | | | |
| 7m | | | | |
| 8m | | | | |
| 9m | | | | |
| 10m | | | | |

Entered

**Project:** **Date:**
**Site:** **Processor:**

| Depth | Temp (C) | SPC | Salinty | pH |
|---|---|---|---|---|
| 0m | | | | |
| 1m | | | | |
| 2m | | | | |
| 3m | | | | |
| 4m | | | | |
| 5m | | | | |
| 6m | | | | |
| 7m | | | | |
| 8m | | | | |
| 9m | | | | |
| 10m | | | | |

Entered:

**Project:** **Date:**
**Site:** **Processor:**

| Depth | Temp (C) | SPC | Salinty | pH |
|---|---|---|---|---|
| 0m | | | | |
| 1m | | | | |
| 2m | | | | |
| 3m | | | | |
| 4m | | | | |
| 5m | | | | |
| 6m | | | | |
| 7m | | | | |
| 8m | | | | |
| 9m | | | | |
| 10m | | | | |

Entered:

Appendix 5. Seagrass Biomass Lab Datasheet

**Seagrass Biomass Lab Datasheet**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lab Date _____ | | | | | Lab Date _____ | | | Tech _____ | |
| Site _____ | | | E I ____ - ____ m | | Site _____ | | | E I ____ - ____ m | |
| | **Bag #** | _____ | **UID** | _____ | | **Bag #** | _____ | **UID** | _____ |
| ZM | L (cm) | _____ | W (cm) | _____ | ZM | L (cm) | _____ | W (cm) | _____ |
| | # blades | _____ | broken? | Y N | | # blades | _____ | broken? | Y N |
| | UID | _____ | foil wt | ____g | | UID | _____ | foil wt | ____g |
| NS | L (cm) | _____ | W (cm) | _____ | NS | L (cm) | _____ | W (cm) | _____ |
| | UID | _____ | foil wt | ___mg | | UID | _____ | foil wt | ___mg |
| filter | UID | _____ | foil wt | ___mg | filter | UID | _____ | foil wt | ___mg |
| meso/ epi | spp x # | min/max/avg | UID | foil wt | meso/ epi | spp x # | min/max/avg | UID | foil wt |
| Lab Date _____ | | | Tech _____ | | Lab Date _____ | | | Tech _____ | |
| Site _____ | | | E I ____ - ____ m | | Site _____ | | | E I ____ - ____ m | |
| | **Bag #** | _____ | **UID** | _____ | | **Bag #** | _____ | **UID** | _____ |
| ZM | L (cm) | _____ | W (cm) | _____ | ZM | L (cm) | _____ | W (cm) | _____ |
| | # blades | _____ | broken? | Y N | | # blades | _____ | broken? | Y N |
| | UID | _____ | foil wt | ____g | | UID | _____ | foil wt | ____g |
| NS | L (cm) | _____ | W (cm) | _____ | NS | L (cm) | _____ | W (cm) | _____ |
| | UID | _____ | foil wt | ___mg | | UID | _____ | foil wt | ___mg |
| filter | UID | _____ | foil wt | ___mg | filter | UID | _____ | foil wt | ___mg |
| meso/ epi | spp x # | min/max/avg | UID | foil wt | meso/ epi | spp x # | min/max/avg | UID | foil wt |

Appendix 6. Mesograzer functional group list.

To be added summer 2018.

Appendix 7. Annual Seagrass Biomass Lab Datasheet. Additional sections have been added to include Below Ground Biomass metrics from rhizome collections.

## Annual Seagrass Shoot Biomass

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lab Date _____ | | | | | Lab Date _____ | | | Tech _____ |
| Site _____ | | | E I ____ - ____ m | | Site _____ | | | E I ____ - ____ m |
| | **Bag #** | _____ | **UID** | _____ | | **Bag #** | _____ | **UID** | _____ |
| ZM | L (cm) | _____ | W (cm) | _____ | ZM | L (cm) | _____ | W (cm) | _____ |
| | # blades | _____ | broken? | Y N | | # blades | _____ | broken? | Y N |
| | UID | _____ | foil wt | ____g | | UID | _____ | foil wt | ____g |
| NS | L (cm) | _____ | W (cm) | _____ | NS | L (cm) | _____ | W (cm) | _____ |
| | UID | _____ | foil wt | ___mg | | UID | _____ | foil wt | ___mg |
| RHZ | 1) ____cm____ | 2) ____cm____ | 3)____cm____ | | RHZ | 1) ____cm____ | 2) ____cm____ | 3)____cm____ | |
| | 4) ____cm____ | 5) ____cm____ | 6)____cm____ | | | 4) ____cm____ | 5) ____cm____ | 6)____cm____ | |
| | UID | | foil wt | ____g | | UID | | foil wt | ____g |
| filter | UID | _____ | foil wt | ___mg | filter | UID | _____ | foil wt | ___mg |
| meso/ epi | spp x # | min/max/avg | UID | foil wt | meso/ epi | spp x # | min/max/avg | UID | foil wt |
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| Lab Date _____ | | | | | Lab Date _____ | | | Tech _____ |
| Site _____ | | | E I ____ - ____ m | | Site _____ | | | E I ____ - ____ m |
| | **Bag #** | _____ | **UID** | _____ | | **Bag #** | _____ | **UID** | _____ |
| **ZM** | L (cm) | _____ | W (cm) | _____ | **ZM** | L (cm) | _____ | W (cm) | _____ |
| | # blades | _____ | broken? | Y N | | # blades | _____ | broken? | Y N |
| | UID | _____ | foil wt | ____g | | UID | _____ | foil wt | ____g |
| **NS** | L (cm) | _____ | W (cm) | _____ | **NS** | L (cm) | _____ | W (cm) | _____ |
| | UID | _____ | foil wt | ___mg | | UID | _____ | foil wt | ___mg |
| **RHZ** | 1) ____cm____ | 2) ____cm____ | 3)____cm____ | | **RHZ** | 1) ____cm____ | 2) ____cm____ | 3)____cm____ | |
| | 4) ____cm____ | 5) ____cm____ | 6)____cm____ | | | 4) ____cm____ | 5) ____cm____ | 6)____cm____ | |
| | UID | | foil wt | ____g | | UID | | foil wt | ____g |
| **Filter** | UID | _____ | foil wt | ___mg | **Filter** | UID | _____ | foil wt | ___mg |
| **Meso + Epi** | spp x # | min/max/avg | UID | foil wt | **Meso + Epi** | spp x # | min/max/avg | UID | foil wt |
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Appendix 8. Seagrass Growth Datasheet

| Site | Transect_location | Shoot_no | Processor_ID | Month |
|---|---|---|---|---|
| | yyyy/mm/dd | | | |
| Date_marked | | | | |
| Date_collected | | | | |
| Date_processed | | | | |
| Plastochrone interval (days) [1] | | Unmarked new blades [2] | | |
| Sheath_length (cm) [5] | | Reference Length | | |
| Sheath_width (mm) [7] | | Blade ID: Baby Blade, Blade_#, Branch_# | | |

| Site | Transect_location | Shoot_no | Processor_ID | Month |
|---|---|---|---|---|
| | yyyy/mm/dd | | | |
| Date_marked | | | | |
| Date_collected | | | | |
| Date_processed | | | | |
| Plastochrone interval (days) [3] | | Unmarked new blades [4] | | |
| Sheath_length (cm) [6] | | Reference Length | | |
| Sheath_width (mm) [8] | | Blade ID: Baby Blade, Blade_#, Branch_# | | |

| Blade_ID | New_growth_cm | Length_cm | Broken_blade | New_blade | Width_mm |
|---|---|---|---|---|---|
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| Biomass samples | | | | | |
|---|---|---|---|---|---|
| | E-Z | | Detailed | | |
| Sample type | UID | Foil Wt (mg) | Sample type | UID | Foil Wt (mg) |
| 3rd blade | | | baby blade | | |
| remaining bio | | | 3rd new | | |
| | | | 3rd old | | |
| | | | 3rd sheath | | |
| | | | new bio | | |
| | | | old bio | | |
| | | | sheath | | |

| Blade_ID | New_growth_cm | Length_cm | Broken_blade | New_blade | Width_mm |
|---|---|---|---|---|---|
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| Biomass samples | | | | | |
|---|---|---|---|---|---|
| | E-Z | | Detailed | | |
| Sample type | UID | Foil Wt (mg) | Sample type | UID | Foil Wt (mg) |
| 3rd blade | | | baby blade | | |
| remaining bio | | | 3rd new | | |
| | | | 3rd old | | |
| | | | 3rd sheath | | |
| | | | new bio | | |
| | | | old bio | | |
| | | | sheath | | |

Appendix 9. Wasting Disease Datasheet

## Hakai Seagrass Wasting Disease Surveys

| Date (YY-MM-DD) | Site | Transect ID | Dist. (m) | UID | Length (mm) | Width (mm) | Broken? (Y/N) | Prevalence (0 = None, 1 = Lesions) | Lesion Severity (*see % Cover Categories) | Processor |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
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* % Cover Categories: <1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50 etc.

Appendix 10. Below Ground Biomass Core Lab Datasheet

**Seagrass Sediment Core Lab datasheet**

| Date Collected (Y-M-D) | Site | Transect, Distance | UID | Seed viability and count | | | | | Below ground biomass | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | light, firm | dark, soft | dark, firm | light, soft | husk | Bag Weight (g) | UID |
| | | | | | | | | | | |
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Appendix 11. Sub-tidal Clam Measurement Datasheet

**Subtidal Clam Measurememements Datasheet**

| Date: | Site: | UID: | | | |
|---|---|---|---|---|---|
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| **Species** | **Width mm** | **Height mm** | **Valve** | **Fouling** | **# Fragments** |
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WEIGHTS PER SPECIES:

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