**Detailed Sampling Methods for Waimea Watershed Sampling Project**

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**Marine**

**GENERAL**

Marine sites are sampled along the shore, targeting habitats that are suitable for the target host organisms (i.e. macroalgae, coral, crustose coralline algae and benthic invertebrates) and benthic substrates (sediment, rock swabs). Sampling is conducted during the summer months to avoid large north swells and to complement terrestrial sampling; winter sampling is tractable but requires more flexible scheduling to sample between swell events. A typical sampling event comprises 2 days targeting 3 sites per trip; each site is a single dive of 25-40 minute duration. To facilitate collection, divers are provided with pre-labeled Whirl-pak bags and tubes. Each diver targets a sample type (e.g. coral) and attempts to collect 3 replicates of each species/morphotype; 3 to 7 species/morphotypes are targeted at each site, prioritizing the most widespread species to facilitate between-site comparisons (e.g. the same 3 coral species are sampled at every site, while for macroalgae and invertebrates effort is made to sample 2-3 species at every site and 2-5 species haphazardly site-dependent). Divers keep track of field identifications and descriptions of species/morphotypes using dive slates and underwater photography when possible. Samples are returned to the dive boat, recorded by the field assistant, and stored in a cooler with dry ice. Sampling sites are approximately 20m x 20m. Photo vouchers are taken once samples are returned to the lab to assist with expert identification. All samples except water are stored -80C on return to the laboratory; -20C is acceptable.

**WATER**

Materials:

1L autoclavable plastic bottles

Niskin sampling device with rope and messenger weight

Collect two saline water samples at each marine site using sterile, autoclaved 1L bottles. Fully submerge a 1 L bottle just below the surface until full. Collect a 1 L sample from just above the ocean floor via Niskin Bottle deployment with messenger. Bottles should be triple-rinsed with sample water before collection. Store water in a cooler with dry ice. Keep water isolated from the dry ice to prevent freezing. Once samples are returned to the lab, filter 500ml of water from each bottle using 0.2 µm filters to collect bacteria and fungi and store filter dry at -80C.

**HARD SUBSTRATE SWABS**

Materials:

Sterile polyester swabs

Sterile tubes with attached caps, 15ml

Identify 4 visually distinct turfing algal areas at each site to maximize site representation. For each turf area, 3 replicate swabs are taken. Use a sterile swab to scrub an area the size of two adult hands. Continuously rotate swab while scrubbing. Place the entire swab into sterile 15ml tube. Once samples are returned to boat, empty out excess water from the tube before placing in cooler.

**BENTHIC INVERTEBRATES**

Materials:

Sterile Whirl-pak bags, 200 ml

Ziplock bags, gallon

Identify up to 7 distinct species or morphotypes of marine invertebrates. For each species or morphotype, collect 3 individuals. When possible, use Whirl-pak bags for sample collections. For larger organisms, attempt to collect in a gallon ziplock bag. If organism is too large, collect the smallest representative portion of the individual possible.

**CORAL AND CRUSTOSE CORALLINE ALGAE (CCA)**

Materials:

Bone shears

Sterile Whirl-pak bags, 200 ml

Identify 3 species of coral, and 3 morphotypes of CCA**.** For each species or morphotype, collect 1-2 cm of material from 3 individuals using bone cutting shears and place in pre-labeled whirl-pack bags.

**ALGAE**

Materials:

Sterile Whirl-pak bags, 200 ml

Identify up to 7 species of algae and attempt to collect 3 replicates of each species**.** Attempt to collect enough material to loosely fill the Whirl-pak bag.

**SEDIMENT**

Materials:

Sterile 50ml tubes

Collect sediment at 3 points within each marine site. Push a sterile 50ml tube into the topmost 2cm layer of sediment. Once samples are returned to the boat, pour off excess water prior to storing in cooler.

**Stream**

**GENERAL**

Stream sites are sampled as close to paired terrestrial sites as possible with consideration given for safe stream access. Stream sampling is conducted during periods of low flow (summer months, > 2 days post flood event (Flood defined as >2ft water level on upper Kamananui stream gauge). Stream sampling is conducted within a 100m stretch of stream. This 100m stretch should incorporate a variety of habitat types to increase the chance of capturing biodiversity. When appropriate, consult lists of native species to avoid bycatch. Start at furthest point downstream and work up to avoid contaminating downstream sites. Prior to disruptive sampling (e.g. D-Net), collect water samples. Store all samples on ice. Upon returning to the lab, transfer to freezer or, for water, filter immediately.

**WATER**

Materials:

1L autoclavable bottles

Plastic beaker

Fully submerge bottles in the stream until full. Avoid collecting water from the surface or benthos. Note date and time of sampling and store on ice immediately after sampling. Each water sample will yield 2 filters (0.8 µm and 0.2 µm).

**PRAWNS**

Materials:

Hand nets (2)

Bait (Spam or Sardines)

Ziplock bag, gallon

Place a piece of bait in the hand net. Scan the area for visible tahitian prawns (*Machrobrachium lar*). If no prawns are visible, locate boulder overhangs or piles of rocks in moderate flow areas. Place net containing bait in water just upstream of prawns or suspected prawn habitat. Place second net in water just downstream. Once prawns appear, lure in front of second net. Once prawn is directly in front of second net, rapidly move bait net to scare prawn backwards into second net. Immediately transfer to gallon ziplock bag and bury in ice.

**D-NET (STREAM INVERTS, COARSE & FINE POM)**

Materials:

D-Net

50 ml tubes

2 ml tubes

Sieves

Plastic beaker

White plastic tray

Camera

Fine forceps

Ethanol

Use D-Net to sample at several points within 100m stretch of stream. When sampling, target as many distinct habitat types as possible (pools, banks, high-flow riffles, runs, etc.).

In high flow environments (e.g. riffles): submerge D-Net with the bottom of the net touching the stream bed. Use gloved hands to rub, scrape, and generally perturb the substrate being sampled (rocks, gravel, sand, etc.) such that loosened materials flow into D-Net. Continue to sample until the interior netting of the D-Net is 50% obscured by material. Remove D-Net from water with open side facing up.

In low flow environments (e.g. pools, banks): Roughly perturb substrate with your gloved hands, your feet, or with the end of the D-Net. As material is suspended in the water column, pass the D-Net through it in a swirling pattern so that material is pushed against the interior netting. Once D-Net interior netting is 50% obscured, remove D-Net from water with open side facing up.

Once D-Net has been removed from the water, remove any large non-organic material by hand. Use plastic beaker filled with stream water to rinse material to one side of the net. Invert the net over the sieves (organized with largest diameter sieve on top and descending to smallest diameter sieve). Use plastic beaker and stream water to rinse material into topmost sieve. Continue to rinse until all material has been transferred to sieves. Rinse material on sieves to ensure all particles are separated by sieve diameter. Collect organic material from top sieve (e.g. leaves, twigs, etc.) into 50 ml tube for Coarse POM. Carefully rinse contents of smallest sieve to one side and then invert sieve over tray. Rinse contents of smallest sieve into tray with appropriate amount of water for particle separation. Collect stream invertebrates from tray using fine forceps sterilized with ethanol. Take photos of each invertebrate type encountered and make a note of morphotype on sample spreadsheet. Once invertebrates have been removed, swirl water in tray to encourage separation of organic and non-organic particles. Pour off excess water and then transfer organic portion into 50 ml tube for Fine POM. Drain off excess stream water from fine POM using tube cap or smallest sieve. Maximize habitat types sampled when possible. If certain habitat types do not produce POM (e.g. high flow riffles) or do not yield invertebrates, focus on other habitat types to fulfill sampling requirements.

**SEDIMENT**

Materials:

50 ml tubes

Collect 3 sediment samples. Sample sediment by pushing a 50 ml tube into the streambed until filled. If necessary, move large rocks out of the way to access finer sediment. Spread sampling locations out over entire sampling area.

**ROCK SWABS**

Materials:

2ml tubes

Sterile flocked swabs

Select three submerged rocks in high flow areas (characterized by visibly moving water and lower surface biomass accumulation) and three rocks in low flow areas (stagnant water, high surface biomass). Swab an area of approximately 2 adult hands (~20 cm diameter circle), rotating the swab as you go. If stream flow is relatively uniform, swab three rocks in the center of the stream, and three rocks closer to the banks as a proxy for stream flow. Snap or cut off swab tip into a 2ml screw cap tube

**AQUATIC GASTROPODS**

Materials:

15ml tubes

Locate aquatic gastropods on the surface of rocks (typically lower flow areas). Remove gastropods from rocks using a clean gloved hand and place in 15 ml tube. Gastropods may not be present in all sites.

**Terrestrial**

**TERRESTRIAL PLANTS**

Equipment:

Hand Shears

Knife

Trowel

Paper towels w/ 70% ethanol

50 ml tubes

Sterile flocked swabs

Locate three dicotsand three monocotswithin the plot. If the plant is not immediately identifiable, take a photo voucher and/or press and label a voucher specimen. Attempt to find individuals that have either fruit or flower. For each plant take the following samples (wipe off tools with ethanol between samples):

1. Cut off enough leaf material to loosely fill a 50 ml tube.
2. Swab leaf area approximately equivalent to the palm of your hand. Clip swab tip into a 2 ml extraction tube .
3. Cut off a section of stem (for small plants) or shave bark/stem using a knife and place in a 50ml tube.
4. Use a trowel to expose roots at the base of the plant. Trace the roots back to the plant to ensure they are from the same individual. Clip off enough roots to loosely fill a 50 ml tube.
5. Clip off enough fruit or flower to loosely fill a 50 ml tube.

Locate three lichens, three ferns, and three mosses within the plot. For each sample, use a knife or trowel to remove the entire thallus, trying to remove as much surrounding environmental material as possible and place in a 50 ml tube. For ferns, if the thallus is too large, collect a single frond. Clean knife/trowel with ethanol between samples.

**HARD SUBSTRATE SWABS**

Consumables:

2 ml tubes

Sterile flocked swabs

Locate three bare rock surfaces. For each surface, use a sterile swab to scrub an area the size of two adult hands. Rotate swab continuously while scrubbing. Snap or cut off the swab tip into a sterile 2 ml screw cap tube.

**LEAF LITTER INVERTEBRATES**

Materials:

Burlese funnels

Pillow cases

1 cm sieves

Forceps

70% ethanol

Heat lamp

Scintillation vials

Petri dish for sorting

Teasing needles for sorting

At the sampling site, demarcate a 2m x 2m sampling area. Gather all dead surface detritus material and sieve through a 1 cm sieve. Sieved material should be collected into a washed thin pillow case. Once done, tie up the pillow case and place into a cooler with an ice pack. Do not allow the bags to touch the ice pack.

After transporting samples back to the lab, set up the Burlese funnels on the same day. Fill the collection chamber of the funnel up to near the top. Set up two chambers per site. Place the collection chambers underneath a heat source and run the chamber for 1 week. Check every day to make sure that the arthropods dropping into the collection vial of 70% ethanol are submerged and transfer collected arthropods to a scintillation vial. Replace with fresh 70% ethanol daily. Continue for 1 week.

**SOILS**

Materials:

Quart Ziplock bags

Paper towels soaked in 70% ethanol

Glassine weighing paper

Plastic disposable spatulas

Soil corer, 3cm

Slide hammer

At the sampling spot after the detritus has been cleared, use a 3 cm corer and core deep to 30 cm. Upon retrieval, the core should be divided into 3 portions with gaps of 3-4 cm between portions. Label the respective portions shallow, middle, and deep (7-10 cm each). Place each layer into a quart ziplock bag and place into a cooler with ice After each sample, the corer should be cleaned with paper towel soaked in 70% ethanol. The first wipe will help clean the corer, and a second wipe is necessary to completely clean out any soil remnant on the corer. Once samples are returned to the lab, refrigerate immediately and process within 24 hours. In the lab, homogenize the soil core within the bag by hand massaging thoroughly for 30 seconds. Place the homogenized soil in a -20 °C freezer for storage.

**FRUIT FEEDERS**

Materials:

Insect net

Collecting vials

Aspirator

Forceps

Paper towels soaked in 70% ethanol

Bananas

Jars

Mesh screen

At each site, find a fruit that is decaying on the ground. Carefully approach the fruit and lower an aerial net onto the fruit. The flies will go into the net. Collect the flies into a clean vial and place in a cooler with ice packs. Check the fruit for beetles. Collect the beetles using forceps into another vial. Clean the forceps with 70% ethanol prior to using them on other samples. Keep the beetles in a cooler with ice packs.

**LEAF-EATING ARTHROPODS**

**Materials:**

Sweep net

50 ml tubes

Divide the sampling location into 3 divisions (each part contains one of the soil core locations). At each of these parts, use the sweep net to beat understory plants. Periodically check the sweep net for herbivorous arthropods such as grasshoppers and leaf hoppers. Capture herbivorous arthropods in a 50ml tube. Repeat until at least 3 replicate individuals have been collected from each part of the site. Upon returning to the lab, place the collected arthropods into fresh 70% ethanol and store at -20 °C.

**WOOD AND WOOD ARTHROPODS**

Materials:

Emergence chamber (3 per site) (note that the bottom of the chambers should)

Scintillation vials for specimen storage

70% ethanol

At each site, gather enough coarse woody debris to fill 5 cm of the rearing chamber. Target coarse woody debris that is between 0.5-3.0 cm in diameter. The wood should be partially decayed with the bark still on but starting to flake off. Close the rearing chamber and return them to a location where they may be reared with natural light and temperature similar to the field site.

Fill collecting jars with 70% ethanol and checked every week for emergence. Sorted specimens by morphotype and place in fresh 70% ethanol for DNA extraction. Continue collections for one month.

At the end of the one month period, pool clippings of wood from throughout the chamber into a single 50ml tube and store at -20° C until ready for DNA extraction.

**MUSHROOMS**

Collect mushrooms using sterile forceps and 50ml tubes. Preferentially collect flesh fungi over perennial shelf fungi. Multiple adjacent fruit bodies of the same species can be collected as a single sample if fruiting bodies were especially small ( < 2 cm) and are growing closely or in a single attached cluster. Preserve samples on ice in the field, then freeze and freeze dried in the laboratory.

**MOSQUITOES**

Materials:

Vacuum aspirator

Collection cups

50 ml tube

Collect at least 3 mosquitoes using a battery powered aspirator. Place collection cup containing mosquitoes in a cooler with ice. Once mosquitoes stop moving, transfer the bulk collection into a 50ml tube and store the tube in the cooler. Upon returning to the lab, sort individuals by species, sex, and blood content. Store individuals in separate 2ml tubes at -20°C.

**DROSOPHILA**

Materials:

Vacuum aspirator

Collection cups

50 ml tube

Collect up to 18 drosophila using a battery powered aspirator. If drosophila are not abundant, use rotting fruit as bait. Place rotting fruit in a glass jar covered with a fine mesh screen to prevent contact with bait. Clean the screen with ethanol and wait for drosophila to congregate on the screen. Collect drosophila using an aspirator. Once samples are returned to the lab, preserve in fresh 70% ethanol and store at -20° C. Place collection cup containing drosophila in a cooler with ice. Once drosophila stop moving, transfer the bulk collection into a 50ml tube and store the tube in the cooler. Upon returning to the lab, sort individuals by species and sex. Store individuals in separate 2ml tubes at -20°C.

**GASTROPODS**

Materials:

50 ml tube

Collect 3 snails and 3 slugs at each site. Use clean gloves or sterile forceps to place gastropods into separate 50 ml tubes. Upon returning to the lab transfer gastropods to tubes containing 70% ethanol. Prior to transferring snails, extract the snails from their shells by pouring boiling water onto the shell. If gastropods are small enough to fit in a 2 ml tube, use entire organism for DNA extraction. Otherwise, cut a cross section from the middle of the gastropod for DNA extraction.

**AIR SAMPLES**

Materials:

3D printer air sampler

Sterile flocked swab

2 ml tube

Set up air sampler to run for 2 days. When sampling run is complete, remove the top from the air sampler and swab the interior funnel of the air sampler with a flocked swab for 30 seconds. Snap or cut off the tip of the swab into a 2 ml screw cap tube. Thoroughly clean the air sampler with 70% ethanol between sites.

**BIRDS**

Materials:

Mist net

Paper bags

Bird bands

Clip scale

Capillary tube

Sterile flocked swab

2ml tubes

Set-up mist net no earlier than sunrise and check at minimum every 15 minutes for birds. Extract the birds with gloved hands. Place the bird extracted from the mist net in a soft, breathable cotton bag or brown paper bag and transport to the banding station. Keep the bird in the bag for no more than 30 minutes before being processed. Ensure that bagged birds remain cool and in a quiet setting to minimize stress. Collect fecal matter deposited in the bag using a flocked swab. Store fecal matter in a 2ml tube on ice. Weigh bird using a clip scale. After removing the bird, weigh the empty bag to determine the mass of the bird. Place an aluminum, federally numbered band on the tarsus of the bird. The number will be recorded and used to identify the bird for the duration of its life. Identify the bird species, sex, and age based on morphological and plumage characteristics.

Collect a surface swab of the bird. Prepare a sterile flocked swab by moistening with sterile 1X phosphate buffer solution. Swab the skin surface and feathers of the bird while continually rotating the swab. Include the chest and back contour feathers, the eye lid, the feet, and the cloaca.

Collect a small blood sample (<100 ul) from the brachial vein. Open the wing and manually restrain by hand. Expose the brachial vein by moving aside feather cover and sterilizing with 70% Ethanol. Use a 27-G needle to puncture the brachial vein. Once punctured, use a heparinized microhematocrit capillary tube to capture the blood. Expel the blood from the capillary tube into a 2ml collection tube using a microcap.

**RATS**

Equipment:

Tomahawk traps

Plastic base/rain gutter downspout splash block link

Consumables:

Peanut butter

Old fashioned rolled oats

Flock swabs

2 mL microcentrifuge tubes, sterile

Sterile scissors

Nitrile gloves

N95 mask

Sterile toothpicks

Isoflurane

Clear bags

Cotton balls

19 gauge needles and syringes

1 gallon Ziplock bags

At each site, place 20-29 Tomahawk traps on top of a plastic base. Set traps under trees, against rocks, and under shrubbery. To increase the likelihood of capturing rats, keep baited traps closed for 3 nights. Add bait to the traps. Roll oats and peanut butter together into quarter size balls and place inside each trap. At dusk on the 4th night, open and re-bait the traps. Check the traps the following morning before dawn.

Euthanize captured rats with isoflurane and exsanguination. Remove traps that are occupied with rats from the plastic base and move them to a processing area. Seal the entire trap in a clear plastic bag containing a cotton ball soaked in isoflurane. Wait for 15 minutes.

Remove the trap from the bag and carefully transfer the rat onto a sterile portable tabletop. Perform a cardiac puncture using a 19-G needle. as a secondary euthanization method. Optionally collect blood samples. Swab the skin and fur of the rat from the length of the tail, the right leg, and the right ear of each rat. Swab each area for 5 seconds with the same flock swab while constantly rotating the swab. Cut the base of the swab with sterile scissors and place the tip in a sterile 2 mL microcentrifuge tube. Collect fecal samples from the plastic base under the trap. Using a sterile toothpick, transfer fresh fecal pellets from the plastic base into a sterile 2ml tube. Double bag the rat carcass in 1 gallon Ziplock bags and place on ice.