🌐 Quantification of Total Biomass in Ground Coral Samples

Rowan Mclachlan[1], Kerri Dobson[1], Andrea G Grottoli[1]

[1]The Ohio State University

Apr 22, 2020

| 1 | *Works for me* | dx.doi.org/10.17504/protocols.io.bdyai7se |

Rowan Mclachlan
The Ohio State University

ABSTRACT

This protocol outlines a method for quantifying the total biomass of Scleractinian coral samples which have been ground into a homogenous paste consisting of aragonite skeleton, coral host tissue, and endosymbiotic Symbiodiniaceae cells. There are four parts to quantifying total biomass: 1) grind coral fragments into a homogenous paste, 2) partition the biomass subsample, 3) quantify the ash-free dry weight [AFDW], and 4) standardize AFDW to the colony surface area.

This method has been reported in several publications by Grottoli's team (e.g., Rodrigues & Grottoli 2007). This protocol was written by Rowan McLachlan (03-19-20) and was reviewed by all co-authors.

> 📖 Rodrigues LJ, Grottoli AG (2007). Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnology and Oceanography, 52(5), 1874–1882.

MATERIALS TEXT

LEGEND

[1] for Grinding of Coral Fragments

[2] for Ash-Free Dry Weight Procedure

Reusable materials:

Mortar and pestle [1]

Plastic spatula [1]

Metal spatula [1]

Mechanical pencil [1]

Disposable materials:

Nitrile gloves [1]

Aluminum weighing pan (two per sample) [1]

50 ml polypropylene centrifuge tube [1]

Equipment:

-80 °C freezer [1]

Weighing balance accurate to 4 decimal places [1,2]

Drying oven [2]

Muffle furnace [2]

Software:

Microsoft Excel [2]

SAFETY WARNINGS

Safety warnings:

1. Use nitrile or gloves throughout the procedure.
2. Wear a lab coat and safety glasses throughout the procedure.

BEFORE STARTING

Baking pans

Bake aluminum weighing pans in a muffle furnace at 450°C for two hours. These items do not need to fully cool overnight before removing but can be removed shortly after the baking cycle ends. Caution as items may be very hot and may cause burns to skin.

Grind coral fragments into a homogenous paste

1   **Label pans.** Using an unleaded mechanical pencil, engrave the base of a pre-baked aluminum weighing pan with the coral sample ID (Fig. 1). This pan is hereafter referred to as "pan A".
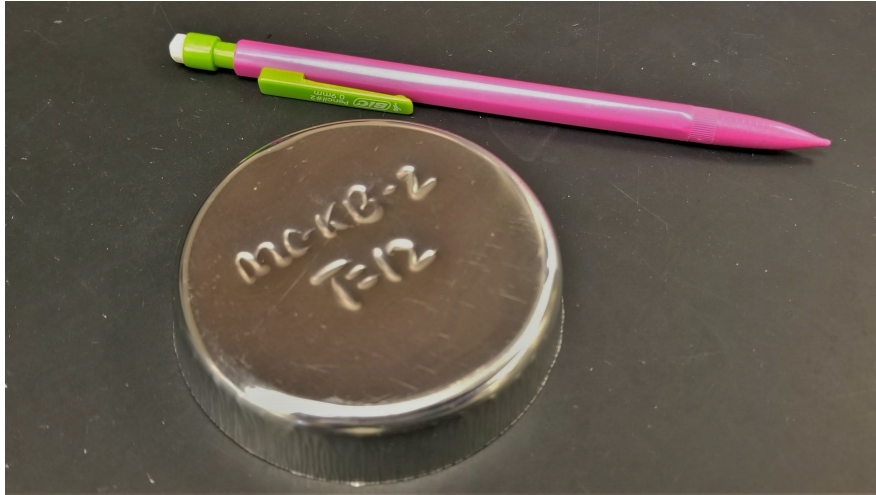
Fig. 1. Pan A is a pre-baked aluminum weighing pan engraved with the sample ID.

2 **Prepare equipment for grinding corals.** Prepare a mortar and pestle within an ice bath (Fig. 2). Mortar and pestle can additionally be chilled using liquid nitrogen or by placing in -80 °C freezer ~20 minutes prior to use.
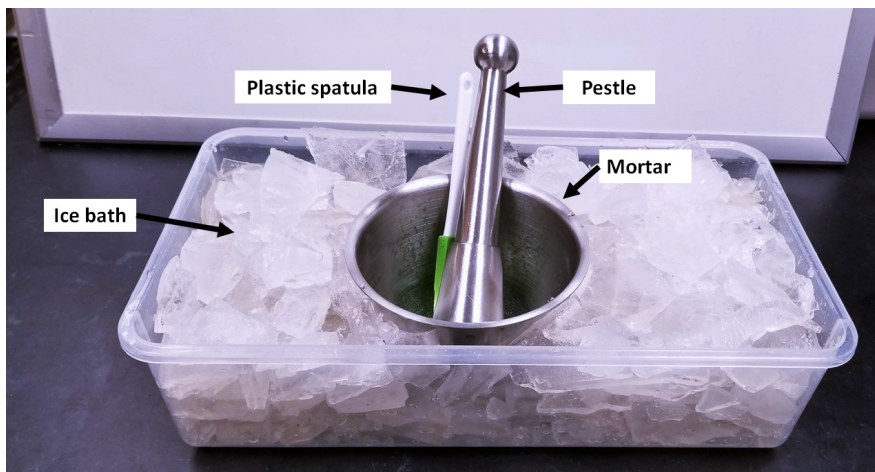

Fig. 2. Equipment used for grinding coral fragments into a homogenous paste.

3 **Grind frozen coral fragments.** Remove a coral fragment from the -80 °C freezer and place it within the mortar (Fig. 3A). Next, using the pestle, break the fragment into smaller ~ 1cm^3 pieces (Fig. 3B). Place one hand over the top of the mortar to prevent pieces of coral from being ejected. Continue to crush the frozen coral pieces into smaller (Fig. 3C) and smaller (Fig. 3D) pieces. Next using circular movements grind the coral pieces into a homogenous paste until the mixture resembles hummus (Fig. 3E). Finally, use the pestle to gather all of the coral paste together at the base of the mortar (Fig. 3F). Try to grind the coral from start (Fig. 3A) to finish (Fig. 3F) as quickly as possible (in no more than 5 minutes) to prevent the sample from defrosting and degrading.
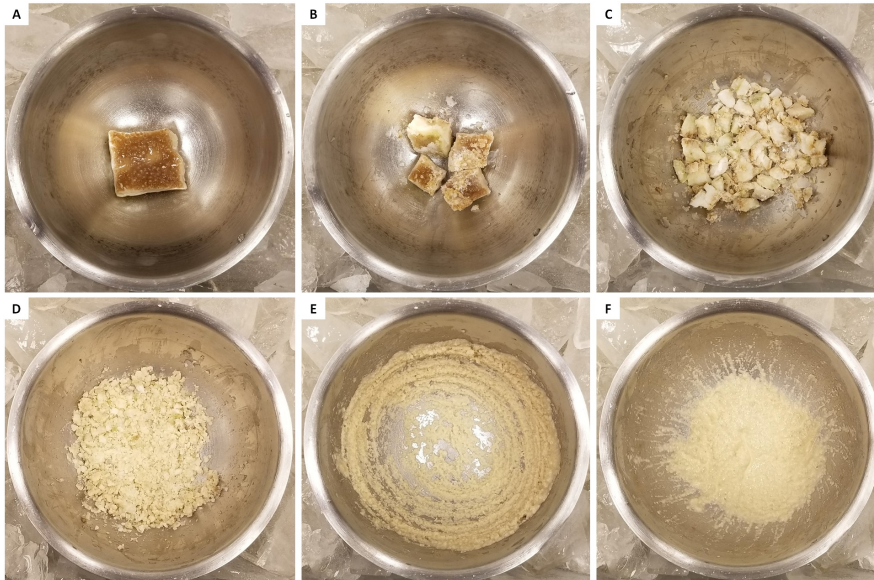
Fig. 3. Sequential photos showing the inside of the mortar during the process of grinding (A) the coral fragment into (F) a homogenous paste.

Partition biomass subsample

4   **Tare the balance.** Switch on the weighing balance and zero (Fig. 4A). Place a second pre-baked aluminum weighing pan (hereafter referred to as "pan B" on the balance (Fig. 4B) and then tare (Fig. 4C).
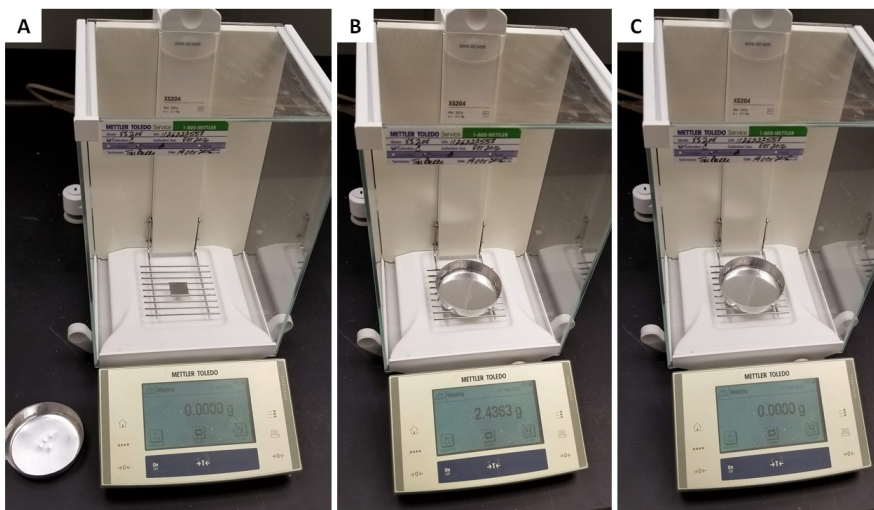

Fig. 4. Photos showing the process of (A) taring the empty balance, (B) weighing pan B, and (C) taring the balance with pan B.

5   **Transfer ground coral paste.** Remove pan B from the balance and place it on the workbench (Fig. 5A). Using a plastic spatula, scrape all of the ground coral paste from the mortar and pestle into pan B (Fig. 5B).
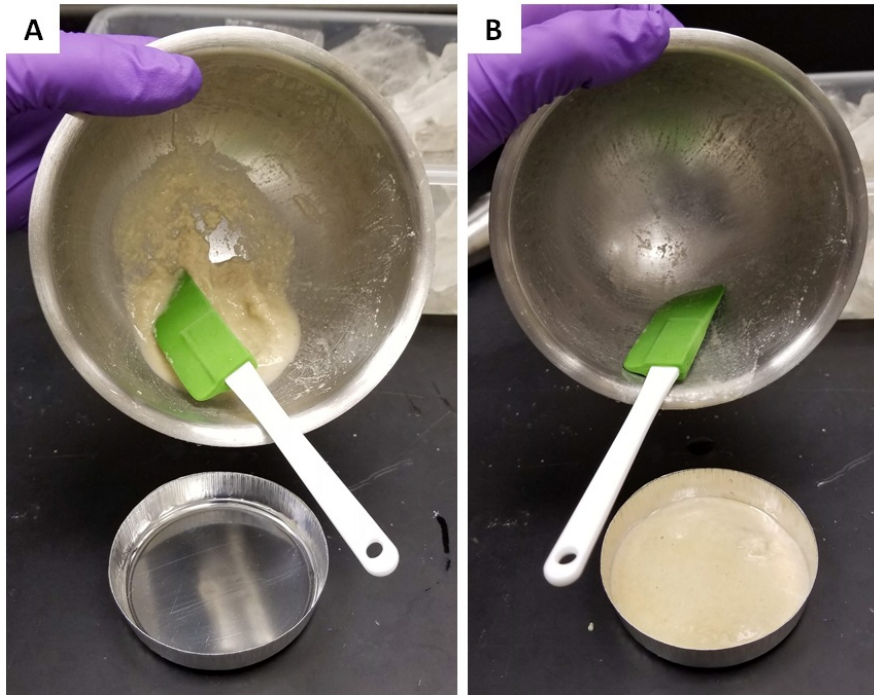
Fig. 5. Photos showing the ground coral paste (A) before and (B) after it has been transfered into pan B.

6   **Weigh ground coral paste.** Place pan B (filled with ground coral paste) back onto the balance, and record the *total wet weight* (grams) in excel (Fig. 6).
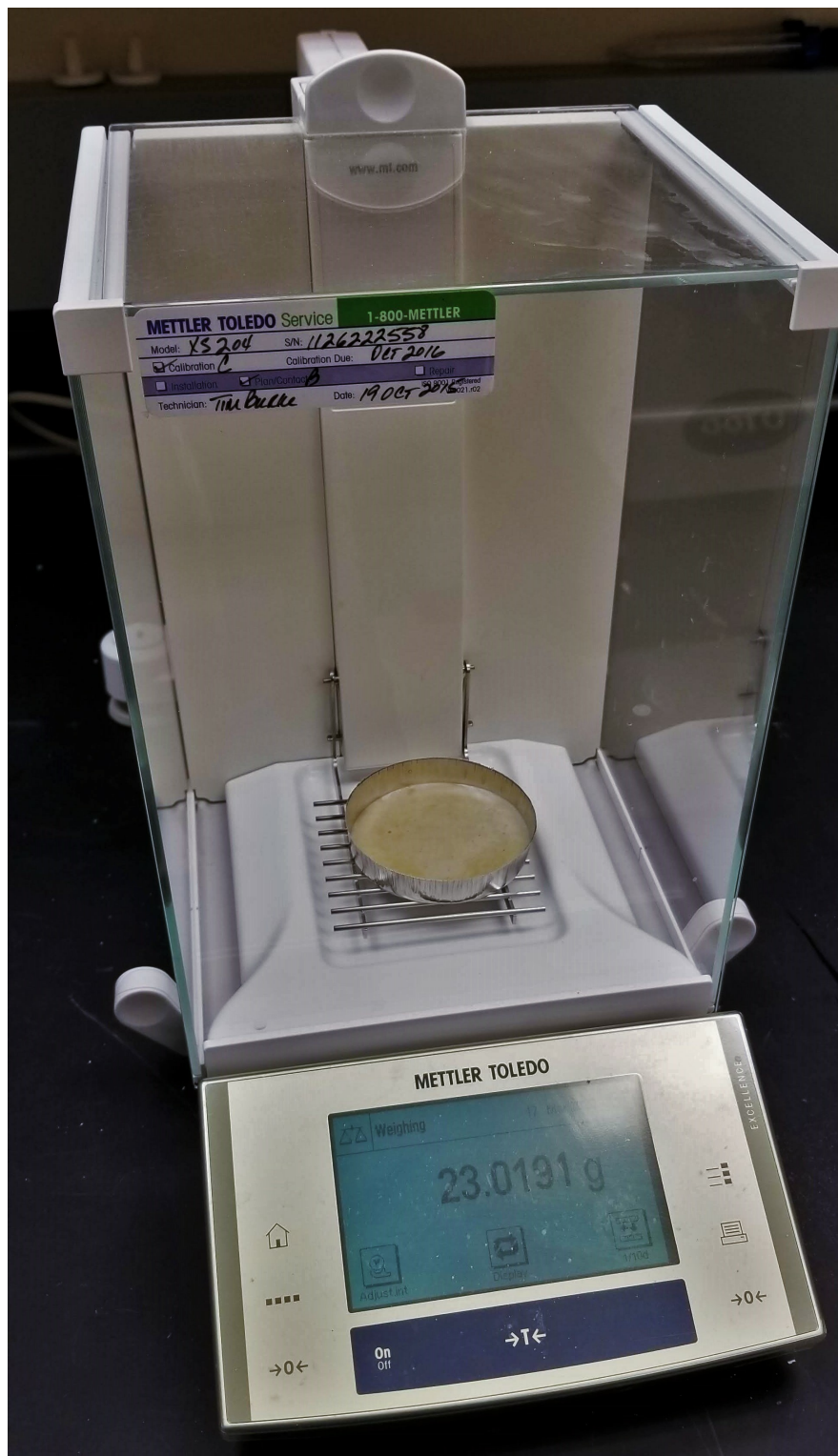
Fig. 6. Photo showing the *total wet weight (g)* of the ground coral sample.

7   **Partition biomass sub-sample.** Using a metal spatula, transfer ~ 1 g of ground coral paste from pan B into the pre-labeled pan A (Fig. 7A). Using the metal spatula, spread the ground material out into a thin layer within pan A (Fig. 7B). To ensure that the correct weight of ground coral material was transferred, re-weigh pan B (Fig. 8) and record the *post-partitioning wet weight* (grams) in excel.

The weight of the biomass subsample transferred to pan A is calculated using the equation below.

Subsample wet weight (g) = Total wet weight (g) - Post-partitioning wet weight (g)
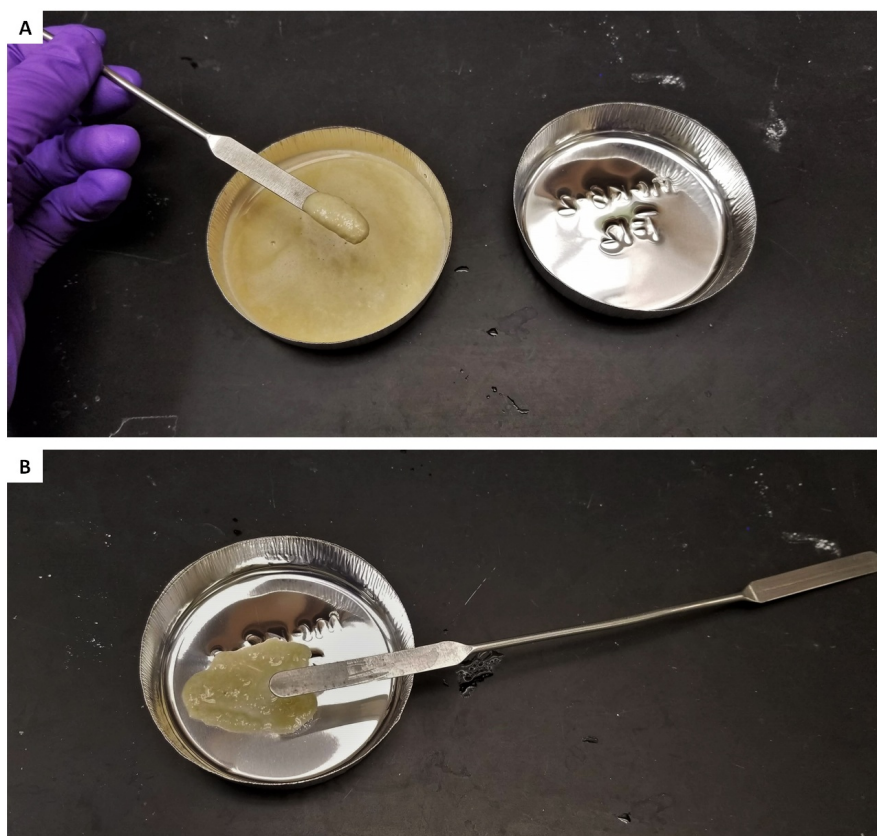

Fig. 7. Photos showing (A) the transfer of the biomass subsample from pan B into pan A and (B) the biomass subsample spread into a thin layer within pan A.
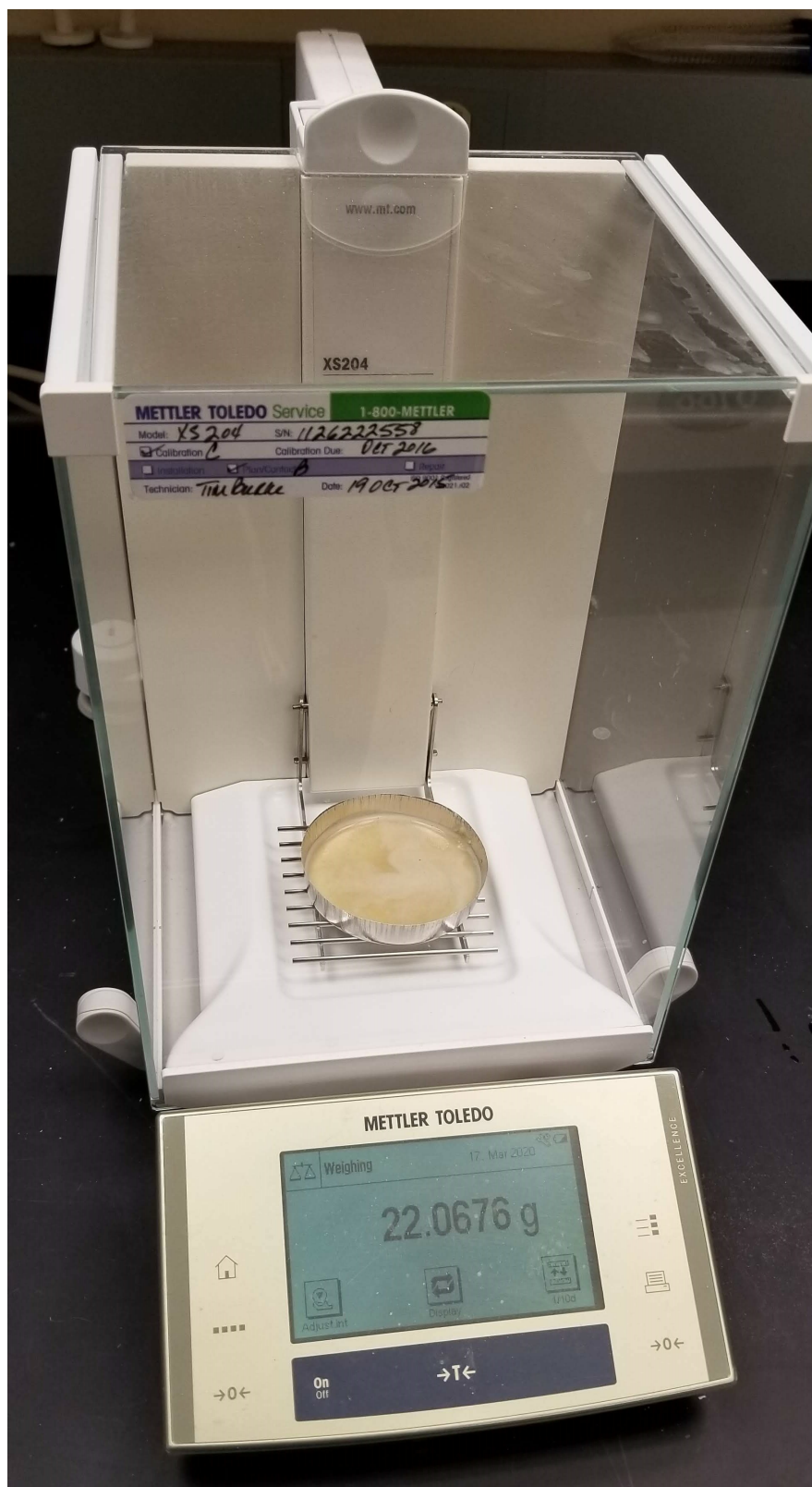
Fig. 8. Photo showing the *post-partitioning wet weight (g)* of the ground coral sample.

Archive remaining ground coral paste. Bend pan B to create a pouring spout, and pour any liquid present into a

8   pre-labeled 50 ml centrifuge tube (Fig. 9A). Next, using a plastic spatula, scrape the remaining coral material into the centrifuge tube (Fig. 9B) until the pan is empty (Fig. 9C). Cap and return the centrifuge tube to the -80 °C freezer for use in future analyses.
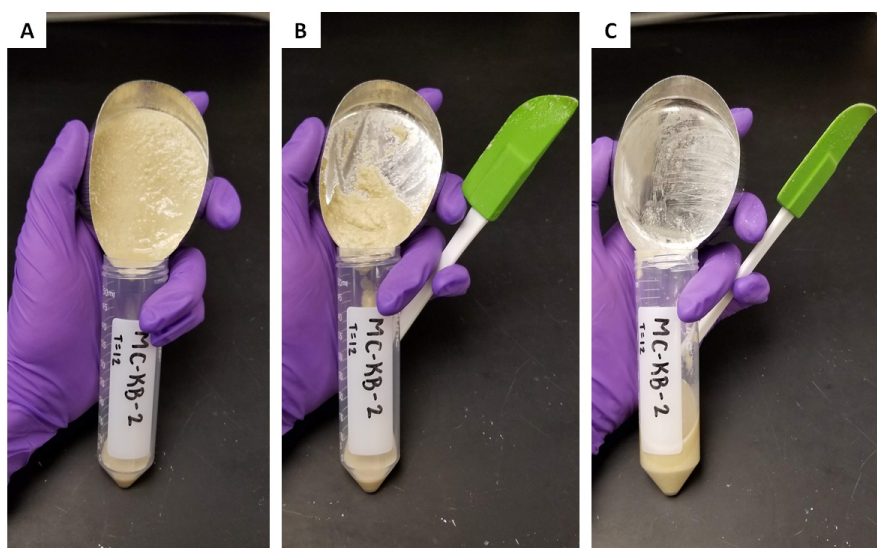

Fig. 9. Photos showing the ground coral paste (A) before, (B) during, and (C) after it has been transfered into a 50 ml centrifuge tube.

Ash-free dry weight (AFDW) procedure

9   **Dry-weigh-burn-weigh.** Place pan A with the biomass subsample (Fig. 10A) and an empty pre-baked pan (hereafter referred to as the "control") into a 60 °C drying oven overnight. It is assumed that any weight change that will occur in the control pan is representative of all pans.

The next day, remove the dried sample in pan A (Fig. 10B) and the control from the drying oven and place them on a workbench. Allow pans to cool, and weigh each pan in triplicate, making sure to tare the balance in between measurements. If the weight of a pan does not stabilize on the balance after 1 minute (i.e. mass is continuously dropping), then remove the pan, and allow it to cool for a further five minutes. When all *dry weights* have been recorded, load pan A and the control into a muffle furnace and burn for 6 hours at 450 °C.

The next day, remove the burnt sample in pan A (Fig. 10C) and the control from the muffle furnace oven and place them on a workbench. Weigh each pan in triplicate, making sure to tare the balance in between measurements. When all *burnt weights* have been recorded, discard of all pans.

Ash-free dry weight (AFDW) of the biomass subsample is calculated using the following equations:

[1] Δ Coral weight (g) = average weight dry coral pan (g) – average weight burnt coral pan (g)

[2] Δ Control weight (g) = average weight dry control pan (g) – average weight burnt control pan (g)

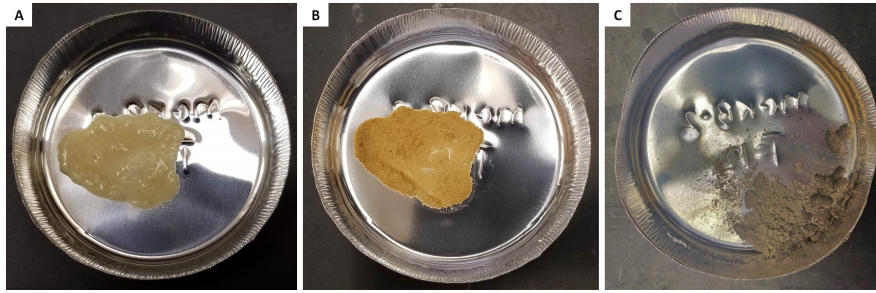*[3] AFDW (g) = Δ Coral weight (g) – Δ Control weight (g)*

Fig. 10. Photos showing the appearance of pan A (A) before it is placed into the drying oven, (B) after it has been dried overnight at 60 °C, and (C) after it has been burnt for 6 hours at 450 °C.

10 **Calculate the surface area of the biomass subsample.** Quantification of the coral surface area is out the scope of this protocol, as there are several methods (foil-wrap, wax-dip, 3d-scan, etc.). This protocol assumes the operator already knows the surface area of the whole coral fragment that was ground into a homogenous paste at the start of this protocol. The surface area (SA) of the subsample used for biomass is calculated using the following equation:

$$SA_{subsample}\ (cm^2) = [Subsample\ wet\ weight\ (g) \div Total\ wet\ weight\ (g)]\ x\ SA\ whole\ colony\ (cm^2)$$

11 **Calculate biomass.** The AFDW is standardized to surface area (cm^2) using the following equation:

$$Biomass\ (g\ /\ cm^2) = AFDW\ (g) \div SA_{subsample}\ (cm^2)$$