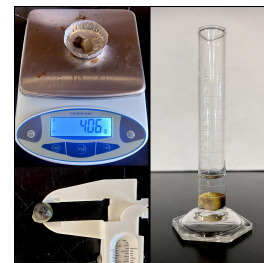


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Standardized Protocol for Measuring Wet Weight, Volume, and Body Dimensions of Sea Anemone Polyps

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Anemones at War Proto...



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We use this protocol and it's working.

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Abstract

This protocol outlines four non-destructive methods for measuring the size of polyps of the aggregating anemone *Anthopleura elegantissima*: **wet weight, volume, and body column diameter and body column height.**

Although designed for the relatively small *Anthopleura elegantissima*, the methods can be adapted for smaller or larger species, such as *Anthopleura xanthogrammica*, by adjusting the size of the aluminum weigh pans, graduated cylinders, and stainless-steel sieves used.

The protocol consists of four sections:

1. Preparation of *Anthopleura elegantissima* polyps
2. Measurement of polyp wet weight in air using a balance
3. Measurement of polyp contracted body column dimensions using calipers
4. Determination of polyp volume via seawater displacement

Step 1 (preparation of polyps) is required before proceeding with any of the other steps, while steps 2–3 may be performed in any order. It is recommended that body volume be measured last, since this step may reintroduce water into the coelenteron that was expelled during preparation.

This protocol is adapted from the methods described in Ford (1964), Francis (1976); Francis (1979); and Sebens (1980).



Materials

Disposable Materials:

- Powder-free nitrile gloves
- 43 mm diameter aluminum weighing dishes [1 per polyp]
- A4 paper data sheet (template provided in protocol)
- Filtered seawater (natural or artificial) [~20 ml per polyp]

Reusable Materials:

- 1 × 150 mm length Vernier calipers (analog used, but digital is recommended)
- 1 × 25 ml volume borosilicate glass graduated cylinder with fine (0.2 ml) graduations
- 1 × 1000 ml volume borosilicate glass Erlenmeyer flask
- 1 × 600 ml volume borosilicate glass beaker
- 1 x Nylon filtration fabric NITEX (mesh opening 20 µm) glued to 2-inch PCV pipe
- Clean lint-free cloth towel/rag [1 per polyp]
- 1 x round-tipped precision forceps or tweezers
- Mechanical pencil
- >10 cm diameter stainless-steel sieve (pore size between 1–5 mm)

Equipment:

- Scientific scale or top-loading balance (ideally with an accuracy of ≥ 0.01 g and resolution of 0.001 g)
- Computer (PC or Mac)

Software:

- Microsoft Excel^(R)

Safety warnings

⚠ Electrical Hazards Warnings: Scientific scales and other laboratory equipment are powered by high-voltage outlets. Keep all liquids away from cords, plugs, and outlets to prevent electrical shock or short circuits. Always ensure hands and work surfaces are dry before handling electrical equipment.

Broken Glass and Sharps Warning: Glassware is fragile and may break easily. Handle with care to avoid cuts. Never dispose of broken glass in regular trash — always place it in a designated sharps or glass disposal container.

Biological Hazard Warning: While nematocysts of *Anthopleura* spp. are typically unable to penetrate human skin, repeated or prolonged handling of live specimens has been reported to cause sensitization and subsequent allergic responses in some individuals.

Ethics statement

All *Anthopleura elegantissima* specimens used in this protocol were collected in compliance with state regulations under the Oregon Department of Fish and Wildlife's Scientific Taking Permit (Permit number 28795, Permit Applicant: Maya Watts, Organization: Oregon Institute of Marine Biology). Collection and handling procedures followed institutional guidelines for the ethical use of invertebrates in research and teaching. All efforts were made to minimize stress and potential harm to the animals, and only the number of specimens necessary to achieve the educational and methodological objectives was collected.

Before start

Preparation of Datasheet

- **Create hardcopy datasheet.** Create and print a hardcopy datasheet to record data (download template datasheet here  Protocol Example Spreadsheet.xlsx 10.4KB). Record all collected data on the printed datasheet in pencil. After completing the protocol, digitize the records by entering the data into a computer using Microsoft Excel^(R).

Preparation of *Anthopleura elegantissima* polyps

- 1 Remove any undigested food particles from the sea anemone's gastrovascular cavity.** If the polyp has been recently collected from the wild or fed large prey items (e.g., mussel, squid) in the laboratory, this step is necessary to ensure an accurate wet weight measurement. Otherwise, this step can be skipped. To remove food, the mouth can be gently encouraged to open using the approach described in Bedgood *et al* (2020). Briefly, use tweezers or forceps to touch a food item to the tentacles gently. This typically triggers a feeding response and will cause the mouth to open, allowing for the removal of any undigested material, using blunted or round-tipped forceps.
- 2 Dry the sea anemone polyp.** Gently blot dry the polyp with a clean, lint-free towel, applying light but firm pressure to expel water from the coelenteron (Fig. 1). Use the towel to remove excess mucus. Avoid rubbing the tissues during blotting, as this can cause damage to the polyp.

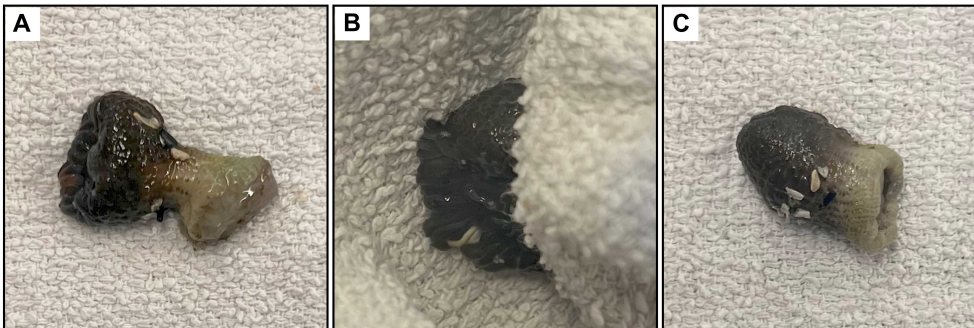


Fig. 1. Drying procedure for an *Anthopleura elegantissima* polyp. (A) Initial appearance of the polyp before drying. (B) Polyp wrapped in a clean towel and gently blotted. (C) Final appearance after removal of excess water.

- 3 Remove debris from the external surface of the sea anemone.** Polyps of *A. elegantissima* often adhere particles such as sand and shell fragments to tubercles/verrucae on their external body wall. Similarly, the pedal disk may adhere to encrusting algae. To obtain accurate size, weight, and volume measurements, all debris must be removed before proceeding. Using tweezers or forceps, carefully remove debris from the body column (Fig. 2A). Clear away shell fragments, loose particles, and any lint remaining from the drying step from the body column (Fig. 2B). Any encrusting algae on the pedal disk can be gently removed by carefully scraping it away with a fingernail (Fig. 2C–D). Take care throughout this process to avoid damaging the underlying tissue.

Note

Fig. 2. Cleaning procedures for an *Anthopleura elegantissima* polyp. (A) Gentle removal of shell fragments and debris from the body wall using forceps. (B) Polyp after removal of external debris. (C) Encrusting algae on the pedal disk being carefully scraped away with a fingernail. (D) Final appearance of the cleaned pedal disk.

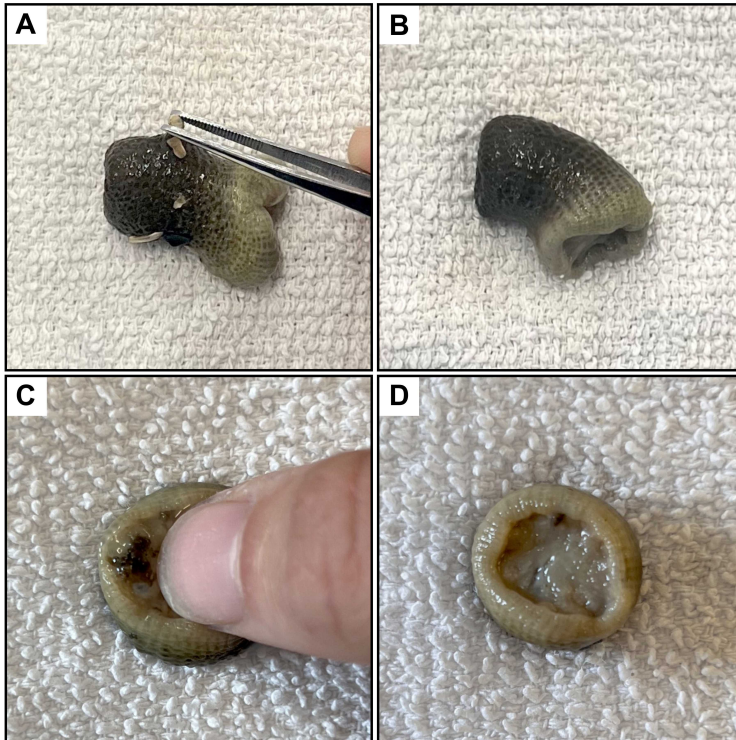


Fig. 2. Cleaning procedures for an *Anthopleura elegantissima* polyp. (A) Gentle removal of shell fragments and debris from the body wall using forceps. (B) Polyp after removal of external debris. (C) Encrusting algae on the pedal disk being carefully scraped away with a fingernail. (D) Final appearance of the cleaned pedal disk.

Measurement of polyp wet weight in air using a balance

- 4 **Measure the sea anemone wet weight.** Turn on the balance, place a clean, empty aluminum weigh dish on the pan, and tare the balance to zero (Fig. 3A–C). Carefully transfer the dried and cleaned *Anthopleura elegantissima* polyp into the dish and record the wet weight in grams on the hardcopy data sheet (Fig. 3D).

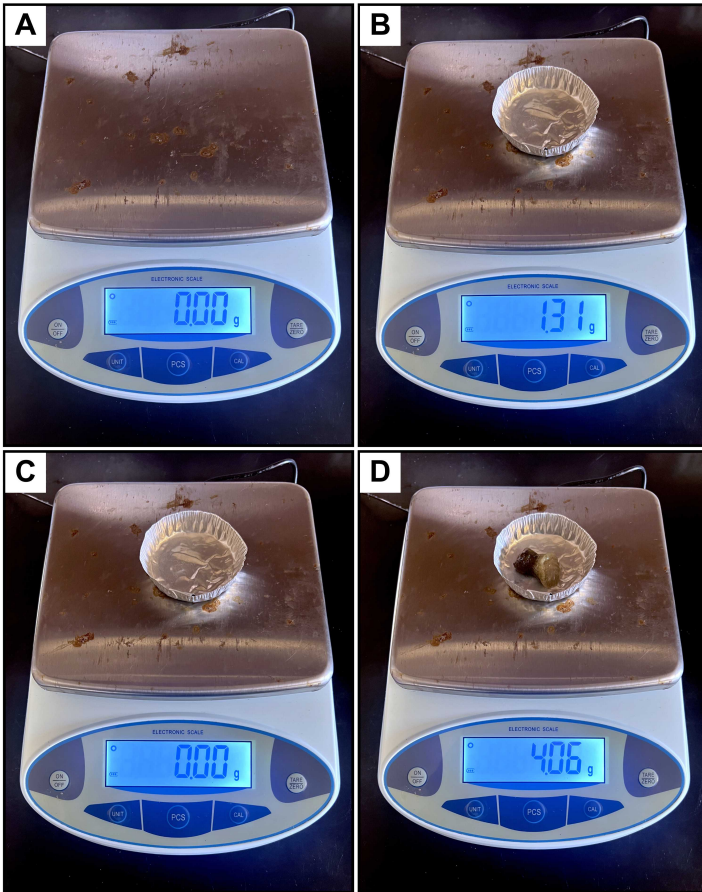


Fig. 3. Procedure for measuring the wet weight of an *Anthopleura elegantissima* polyp. (A) Balance zeroed without an aluminum weigh dish. (B) Aluminum weigh dish placed on the balance. (C) Balance tared with the dish in place. (D) Final wet weight of the polyp measured in the aluminum dish.

Measurement of polyp contracted body column dimensions using calipers

- Step 5: Prepare the anemone for caliper measurements.** The anemone polyp should be fully contracted into a compact, dome-like (hemispherical or short-cylindrical) shape with tentacles completely withdrawn before measuring body column height and width. If necessary, place the polyp on a clean, flat surface and gently shape it into a compact hemisphere, taking care to avoid extension or distortion into an irregular form (Fig. 4A–C).

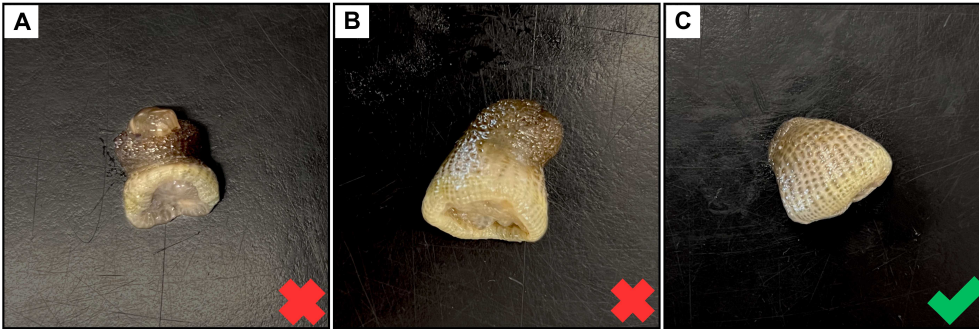


Fig 4. Proper and improper polyp shapes of measurement in *Anthopleura elegantissima*. (A–B) Examples of incorrect body shapes. (C) Correct, fully retracted, compact polyp shape prior to measurement.

- 6 **Step 6: Measure body width.** Place the pedal disk of the *Anthopleura elegantissima* polyp flat on a clean surface. Using calipers, measure the body column diameter by positioning the caliper tips on opposite sides of the column at its widest point, ensuring they just contact the tissue without compressing or distorting the fully contracted polyp (Fig. 5). Keep the calipers parallel to the pedal disk (not angled) to obtain an accurate measurement. Measure pedal disk diameter in the same manner, spanning from one edge of the disk to the other. Record all measurements to the nearest millimeter on the hardcopy data sheet.

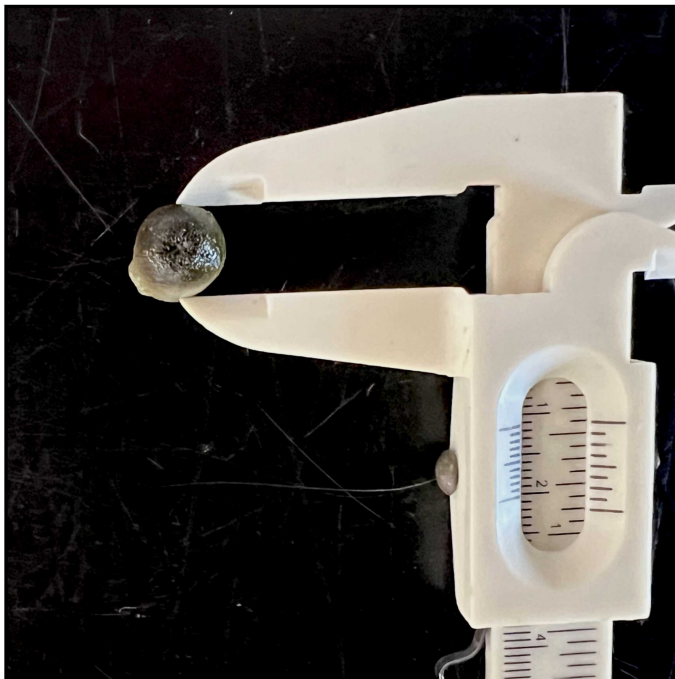


Fig. 5. Measurement of body column diameter in an *Anthopleura elegantissima* polyp using analog or digital calipers.

- 7 **Step 7: Measure body height.** Place the *Anthopleura elegantissima* polyp on its side on a clean, flat surface so that the pedal disk is perpendicular to the table. Using calipers, measure the distance from the center of the pedal disk to the center of the oral disk, taking care not to compress or alter the shape of the fully contracted polyp (Fig. 6). Keep the calipers parallel to the table and perpendicular to the pedal disk. Record the measurement to the nearest millimeter on the data sheet.

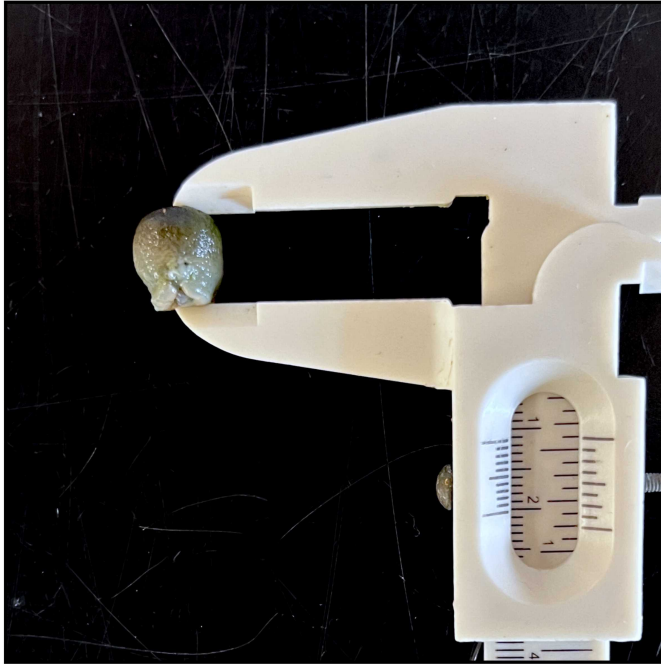


Fig. 6. Measurement of the body column diameter in an *Anthopleura elegantissima* polyp using analog or digital calipers.

Determination of polyp volume via seawater displacement

- 8 **Filter seawater.** Collect unfiltered seawater in a clean beaker or Erlenmeyer flask large enough to hold the total volume required for subsequent polyp volume measurements and comparable in size to the receiving beaker to prevent overfilling (a 1000 mL flask was used in this protocol). Place a 20 μ m NITEX filter over a second clean beaker, ensuring it fits securely just inside the rim without shifting. Slowly pour the unfiltered seawater through the filter into the collection beaker (Fig. 7A), taking care not to overfill either the filter or the beaker (Fig. 7B). Use the filtered seawater for the following steps.

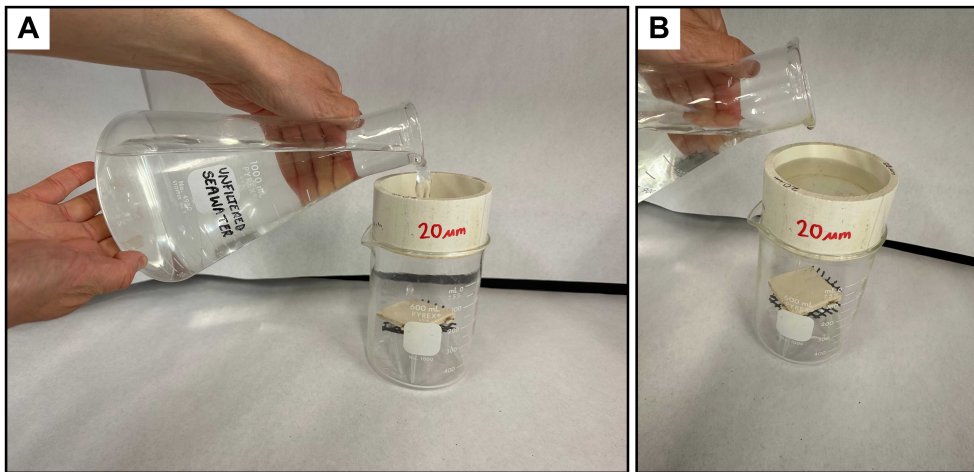


Fig. 7. Demonstration of filtering seawater through a 20 µm filter, (A) pouring the unfiltered water through the filter into a beaker, (B) pausing to allow the seawater to drain through the filter, without overflowing.

- 9 **Fill the graduated cylinder with an exact volume.** Select the smallest graduated cylinder (10–50 mL) that can accommodate the *Anthopleura elegantissima* polyp without risk of lodging. Fill the cylinder with a measured volume of prepared 20 µm–filtered seawater sufficient to fully submerge the polyp (e.g., 5 mL in a 25 mL cylinder for a medium-sized polyp; Fig. 8A). Use a cylinder with appropriately fine graduations (e.g., 0.2 mL for a 25 mL cylinder or up to 1 mL for a 50 mL cylinder). Read the water level at eye level from the bottom of the meniscus (Fig. 8B) and record the initial volume to the nearest milliliter on the data sheet.

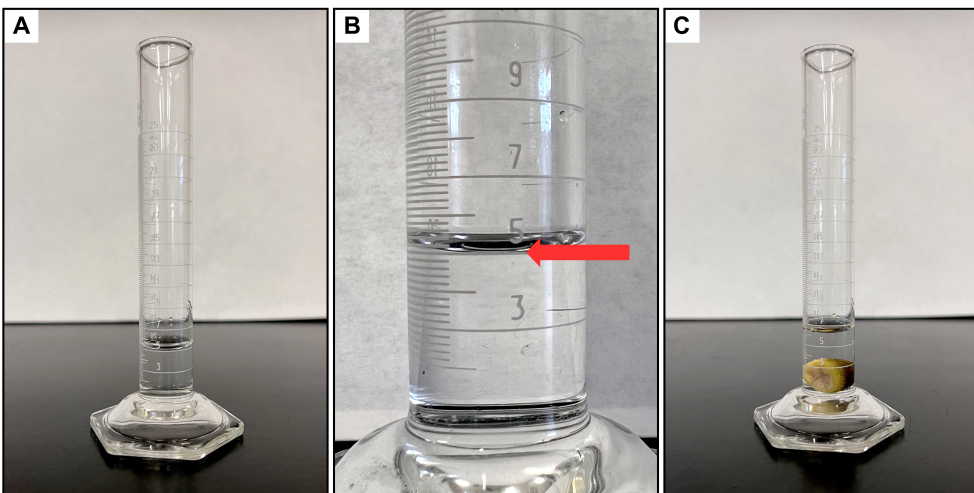


Fig. 8. Volume measurement of an *Anthopleura elegantissima* polyp using seawater displacement. (A) Initial seawater volume (5 mL) in a 25 mL graduated cylinder. (B) Correct technique for reading liquid volume in a graduated cylinder; the arrow indicates the bottom of the meniscus, which should be read at eye level for accuracy. (C) Final volume (6.4 mL) after the polyp is placed in the graduated cylinder.

- 10 **Measure polyp volume.** Gently lower the polyp into the graduated cylinder, taking care to avoid splashing or wetting the cylinder walls, as this would alter the final volume measurement. Once the polyp is fully submerged (Fig. 8C), record the new water volume to the nearest milliliter on the hardcopy data sheet.
- 11 **Return the sea anemone to the water table.** Gently pour the contents of the graduated cylinder into a stainless-steel sieve, allowing the seawater to drain while retaining the polyp. Carefully lift the polyp from the sieve and return it to the water table or to a laboratory holding aquarium.
- 12 **Calculate the volume of the polyp.** Enter the data from the paper hardcopy datasheet into Microsoft Excel^(R). To determine the volume of the *Anthopleura elegantissima* polyp, use the displacement equation:
$$\text{Polyp Volume (mL)} = \text{Final Water Volume (mL)} - \text{Initial Water Volume (mL)}$$

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