| Wax Dipping | |
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| Prepared by: | Bahr Marine Ecology Lab |
| Last Updated: | December 2022 |
| Pre-requisite SOP: Coral Airbrushing Protocol | |
| Safety Precautions: | |
| * Required PPE – Enclosed shoes, gloves * Caution – wax is hot | |
| Purpose: | |
| * Calculation of surface area to standardize responses and account for differences in size across study organisms. * This work follows the methods of Stimon and Kinzie 1991. | |
| Materials: | |
| * Minerva paraffin wax bath * Drying Oven (60°C) * Analytical Balance * Variety of Wooden Dowels * Gulf Wax Paraffin Wax * Small Tweezers * Large Tweezers * Permanent Marker * Aluminum Foil * Digital vernier caliper | |
| Preparing Samples: | |
| Creating the surface area standard curve of mass change of wax-dipped dowels against the geometrically calculated surface area. You want your standard curve to have ab R2 value greater than 0.9   * Make a table with the known dimensions of your wooden dowels to be used as standards, measuring diameter with a digital vernier caliper (mm):  | Sample | Diameter | Initial Weight | Final Weight | | --- | --- | --- | --- | | Standard 1 | 5.6 |  |  | | Standard 2 | 7.6 |  |  | | Standard 3 | 5.4 |  |  | | Standard 4 | 4.9 |  |  | | Standard 5 | 1.7 |  |  |  * Label wooden dowels with a sample number using a permanent marker if they are not labeled already * Turn on the Minerva Paraffin Wax Bath and set it to 65°C for about 5 hours before wax dipping * Take an initial weight of each wooden dowel using the analytical scale to the nearest 0.0001 grams * Using the small or large tweezers, submerge the wooden dowel into the paraffin wax dipping bath for 2 seconds * When removing the wooden dowel, rotate the sample quickly in the air in a circular motion for 10 revolutions * Repeat for each of the wooden dowels * Place wax dipped dowels in a sheet of aluminum foil * Allow the dowels to cool to room temperature before taking the final wax weight (~15 – 20 minutes) * Once the wax dipped dowels have dried fully, take the final weight using the analytical scale to the nearest 0.0001 grams * Enter the initial and final weight into your spreadsheet | |
| Measuring Coral Surface Area: | |
| * After following the airbrushing protocol on fresh or thawed corals, place coral skeletons on labeled aluminum foil in the drying oven at 60°C for 4 hours * Once the coral skeletons are dry (make sure there are no water droplets left), weigh the coral skeletons   to the nearest 0.001 grams   * Using the tweezers, grab the base of the coral skeleton, if you can see where there was dead tissue, use   area to hold onto.   * + You want to make sure you are submerging all areas of the coral skeleton that had cover * Once the wax is liquified (make sure there are no bubbles or solid pieces of wax left in the bath), fully submerge each coral skeleton into the bath for 2 seconds, remove the coral skeleton and rotate the sample quickly in the air in a circular motion for 10 revolutions * Place dipped coral skeletons onto labeled aluminum foil * Allow coral skeletons to dry to room temperature, weigh each coral sample to the nearest 0.0001 grams * Make a table to calculate the surface area of your individual samples  | Sample | Diameter | Initial Weight | Final Weight | | --- | --- | --- | --- | | Coral 1 |  | 4.1123 | 4.4472 |  * Use the surface area script to create an output file with your samples surface area values | |
| Completing in Surface Area Script: | |
| * Using the diameter (mm) of your wooden dowels, calculate the radius * Radius (r) = diameter (D) / 2 * Calculate the surface area of your wooden dowels using the surface area equation: * SA = 4πr2 * Plot the relationship of the surface area (cm2) on the x-axis to the weight of the wax (g) on the y-axis to check if your standard curve has an R2 value greater than 0.9 * Use the plot, calculate the curve coefficient for the slope and the y-intercept to apply as the   standard (this information will be used later to calculate the surface area of your samples)   * Y=mx+b * X= (y-b)/m | |
| Quality Assurance and Control: | |
| *Proper Training*  Proper protocols and training must be implemented to ensure the quality of data generated in the laboratory. Researchers must ensure that all equipment is accurately calibrated, inspected, and maintained according to the manufacturer’s instructions.  *Data Review*  All laboratory data will be reviewed for completeness and transfer errors. Data will be reviewed by a second individual after entry into Excel spreadsheets by comparing the entered, electronic data to the original records (e.g., hand-written datasheets or laboratory notebooks). Data will be summarized as descriptive statistics and in tabular and graphical form to allow visual inspection and verification, and comparison to expected or target values.    *Data Verification*  Data will be checked for compliance with the procedures outlined in the SOPs. Any deviations from those procedures and the impact on the quality of the data will be assessed and discussed with Task Members. Any laboratory data outliers will be flagged.    *Data Validation*  Once the data has been reviewed and verified, it will be assessed to determine the overall acceptability of the objectives of the project. Blank samples, such as water quality testing, will be used to determine any biases or instrument calibration issues during the sample collection and analysis processes. Control samples will be used to determine the condition of the experimental test specimens in the absence of experimental treatments or exposures. Any errors in datasets detected will be discussed with lab members and project leads to determine the impact on the data and its use for the project. If there are any limitations to the data, they will be disclosed as part of the published literature.  *Procedure Specific QA/QC Methods* Procedures for wax dipping measurements follow published procedures (Stimson and Kinzie, 1991). Wax dipping measurements are verified and controlled by conducting a standard curve of known sizes using wooden dowels. Multiple known varieties of sizes of wood dowels are weighed, dipped in wax, and weighed again. The standard curve is then built by graphing the change in mass (y) of the wax versus the calculated surface area (x) of the dowel. Once the standard curve had an R2 value > 0.9, the coral fragments will be wax dipped following the same protocol. | |