| Chlorophyll A and C Extraction | |
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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, ankle-length pants, gloves, long hair tied back | |
| Purpose: | |
| * Measurement of the amount of chlorophyll present per within symbionts * Procedure is adapted from Jeffrey and Humphrey (1975) | |
| Materials: | |
| Part 1   * Thawed samples * Gloves * 90% acetone (located under the hood) * Acetone Disposal * 1mL pipette * 1mL pipette tips * Pipette disposal * Vortex * Centrifuge   Part 2   * Lab Coat * Cart * Gloves * Kim wipes * Tip disposal * Acetone disposal * Ice bucket/ ice * Ethanol 70% * Regular waste disposal * Glass Cuvettes * DI water * 1mL pipette * Cuvette holder * Pipette tips * Datasheet (computer or clipboard or both) * Samples (thawed and centrifugated for 2 minutes at 5000rpm) * Pen * Paper towels * Parafilm (pre-cut into small squares) * 90% acetone * Aluminum foil/cleaning tray * SOP | |
| Part 1: 24 Hours Before | |
| * Thaw the sample, vortex, and then aliquot a subsample (1 mL) and place into a 1.5 mL Eppendorf tube (may already be done from coral processing). * Centrifuge at 9000 rpm for 1 minute and remove and dispose of supernatant   + With 2 people, 1 person can remove supernatant liquid and 1 person can add acetone to the tube with the pellet * Add 1 mL of 90% Acetone.   + Acetone is in the yellow hazardous cabinet if you need to make more. The 90% acetone bottle is located under the hood. * Vortex for 30 seconds and place in the freezer (use cardboard boxes) for 24h.   + Cardboard boxes ensure no light interfering with Chlorophyll counts | |
| Part 2: 24 Hours Later | |
| * Remove samples from freezer and place on ice * Remove plastic cover and turn on spectrophotometer - (button on back, needs 15 minutes to turn on). * Centrifuge samples at 5000rpm for 2mins.   + All zooxanthellae will be in the pellet and the chlorophyll will be in the supernatant.   + Store samples on ice and cover (use a black ice bucket, need to stay in the dark, no light) for transport into the CORE lab | |
| Using the Spectrophotometer | |
| * Turn on computer * Open Soft Max Pro on computer- if this screen opens click “Done”. * Make sure that the “SpectraMax M3” button in the top right corner on the program is a green checkmark (not red circle)   + If it’s red, click the “SpectraMax M3” button, click “COM1-SpectraMax M3”, then press “OK”   + Proceed once that button is green, red means the spec is not connected to the computer   + Drawer will open after every sample, click “Drawer” on spec to close it * Select “New Cuvette Set” * Select “Settings” on the top in the “Cuvette Tools” bar * When settings open, Read Mode should be ABS, Read Type should be Endpoint   + Change Number of Wavelengths to “2” and change to Lm1 to 630 and Lm2 to 663.   + For protein analysis set wavelengths to Lm1 235 and Lm2 to 280.   + Then click “OK”   + Double check the settings saved on the right-hand corner of the screen   + From here on out, process samples 1 by 1 because the acetone will evaporate and light will affect the readings, keep samples covered in black ice bucket * Every 7-10 samples you will run a “reference point” or “Ref”   + Use 2 mL of 90% acetone as the “ref”   + The “ref” won’t give any data   + Keep the same cuvette for all the reference samples, will need to re-fill with acetone every time because it will evaporate   + Place parafilm over the top of cuvette and wipe with kimwipe   + Place cuvette into the spec with the clear side facing the arrow and close the lid   + Press the “ref” button located next to the “read” button.   + Now the reference point is set, and the spec is ready to read samples * Add 1 mL of the sample into cuvette. Add 1 mL of acetone into cuvette. (If the sample is clear then do not add the 1mL of acetone, just make sure to record for the dilution factor later).   + **Be cautious to not chip the cuvettes when taking them out of the tray**   + Watch for pipette tip contamination, use a new pipette tip every time * Cover the cuvette with parafilm. * Invert cuvette 3 times - CAREFULLY * Wipe with kimwipe and place into spectrophotometer, being careful to NOT get any liquid in the spectrophotometer * Take CHL reading at 630nm and 663nm by pressing the “Read” button on the computer.   + All buttons should be on the computer (“Read” and “Ref”), not the spec, except the “drawer” button is the only button that should be pressed on the spec * Use the blank (2 mL of 90% acetone) to zero every 7-10 samples   + Place 2 mL of acetone into the spec as you did the first time (cover with parafilm, wipe with kimwipe)   + Press the “ref” button. Now you can start the next 7 samples.   + Repeat this for every 7-10 samples   + DISCLAIMER: sometimes when you set the new reference point, it changes the previous samples slightly (~0.001). This is ok, continue with reading the next 10 samples at this new calibration and do not change the readings of the previous samples. | |
| Clean Up: | |
| * For cuvette cleaning between samples, set-up aluminum foil or cleaning tray and wash with 70% ethanol, wipe dry and place upside down in cuvette holder to finish drying, repeat after every sample, keep cleaning/ethanol area separate from the samples * At end of day, turn off spec and log out of the computer * Please take everything with you from the spec room including any trash. | |
| Once done, on the data sheet for chl: | |
| * Standardize units chl a/chl c   + CHL per cell   + CHL per surface area (surface area will be calculated using the 3D scanner) * Calculate the dilution factors with surface area to get each reading | |
| 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* Reference for chlorophyll pigments (i.e., acetone) are conducted every 5 samples to ensure the spectrophotometer is reading correctly.  The spectrophotometer is inspected, calibrated, and/or maintained in accordance with the manufacturer’s instructions. All other instruments are calibrated according to calibration procedures described in the instrument manuals.  Lot numbers and expiration dates for consumables are recorded by personnel performing the testing on datasheets or logbooks, as appropriate. Reagents or standard solutions are not used beyond the expiration date printed on the label. All supplies, equipment, and consumables procured for the analysis of this study are documented, inspected, and accepted in accordance with the requirements of each. | |