Questions & Answers Part 4

Please type your questions in the Question Box. We will try our best to get to all your questions. If we don't, feel free to email Juan Torres-Pérez (juan.l.torres-perez@nasa.gov), Amber McCullum (amberjean.mccullum@nasa.gov), Britnay Beaudry (britnay.beaudry@nasa.gov) and/or Sativa Cruz (sativa.cruz@nasa.gov).

Question 1: The assumption of testing the eDNA to detect the existence of a species is that those species left something on that sample of soil. What radius from that sample soil can they infer certain species exist based on the sampled soil?

Answer 1: This is one of the recognized disadvantages of eDNA. For example, plants don't shed DNA much, so even if you collect the sample at the base of a tree, you might not get a signature for it. This is why it is so important for us to map biodiversity using many different techniques (eDNA, field observation, and remote sensing). The challenge is understanding how these different observations relate so that we can use them together to get the complete picture.

It's also going to vary based on slope, aspect, and velocity of water, as well as the percent clay (which binds the DNA and keeps it). There is a model called EDITH that estimates how eDNA moves around in space that we are trying to apply with our BIOSA

Question 2: Do you think that hypothesis regarding hydrologic relating to diversity hold in areas that are not water limited eg., tropical systems?

Answer 2: That's a really good question. I'm not sure. We started in Mediterranean climates because that's where we opportunistically had data, BUT based on the literature, it would be reasonable to hypothesize that life still organizes around water availability; however it's worth testing because not all species need to get water the same way, so what that means and how it "looks" from remote sensing view could be very different.

Question 3: Could you please repeat the name of the Python package?

Answer 3: There is no name yet! We welcome your thoughts on what you think it should be called. Expect it in Summer 2024. Make your suggestions in our Naming Survey.

Question 4: How will you publicize the Python based tool when it is ready?
Answer 4: We will publish it with our manuscript, and publish it through Zenodo or JOSS and likely get it reviewed by PyOpenSci. The package will be hosted on Github to enable community contributions and support for maintenance through time. Expect it in Summer 2024. Follow on Twitter: @DrWKID or LinkedIn: https://www.linkedin.com/in/enstavros/

Question 5: Is it possible to plot genetic evolution, mass migration and this kind of spatial pathways into GIS?
Answer 5: To my knowledge there is potential with eDNA to understand genetic evolution through time, however, I am not an expert on this topic.

Question 6: What is the minimal sample spacing when working with eDNA and how can the risk of missing species be mitigated? How do you know that data is missing?
Answer 6: This is a tough question. We collect 6 mL of soil or sediment, and about 500 mL of water. It's very low impact, unless the hike in and out has impact.

Question 7: Is the eDNA laboratory analysis expensive? How is it performed?
Answer 7: We have a 6 metabarcodes workflow that from collection tubes to providing sequencing results costs $172/sample (plus 56% indirect that the university collects). https://pgl.soe.ucsc.edu/addnap.html
Other companies such as Jonah Ventures can do eDNA metabarcoding at similar costs. eDNA metabarcoding involves several rounds of PCR and then library preparation and sequencing. Here is a paper about our projects and the metabarcoding process: https://escholarship.org/uc/item/5qk7q93p

Question 8: What was the maximum water depth in the Monterey Bay study in California? Up to what depth of water can a sensor like PRISM measure?
Answer 8: Water depth at the Elkhorn Slough varies between ~1.5m to ~4.5m depending on tides and location within the slough. The central portions of the slough are deeper than the nearshore zones. The depth at which the sensor (PRISM and any other optical sensor) can be used to detect benthic components will depend on the clarity of the water.

Question 9: What is flowCam?

Answer 9: FlowCam is a flow imaging microscope. The FlowCam combines digital imaging, flow cytometry, and microscopy. By running water samples through the FlowCam, imaging microscopy can take images of the microscopic organisms in the water, namely phytoplankton. This enables us to identify the phytoplankton functional types and to count them (enumeration).

Question 10: Would the CyanoSCape project also check the Berg and Eerste Rivers Health statistics or only static bodies of water as per slide 52?
Answer 10: We have a small team and are hoping to be able to collect all of the sides in our plan, but we might not be able to collect in all of our sites during the campaign. If we could add additional aquatic scientists to our team, we might be able to expand our measurements. Currently, the requirement to be on-site during the airborne overflight limits us within the appropriate solar geometry (~2-3 hrs). This time will be filled doing the in-water and surface sampling and not leaving enough time to get to multiple sites each day.

Question 11: How are you able to ensure satellite match up with the field sampling? Is this difficult to achieve?
Answer 11: Satellite overpasses over specific sites can be found online. Here's for example the site from the OceanColor Web where researchers can request the information for specific satellites: Overpass Predictions Home (nasa.gov). As mentioned previously, and most importantly for aquatic systems, it is critical to plan the field campaign to be within +/- 1hr of the satellite or airborne sensor overpass since water masses can change constantly.

Question 12: Can you please explain the effect of aircraft angles on calibration on slide 54?
Answer 12: If the flightlines are not optimized for solar geometry, sunglint can saturate the sensor and the data would be beyond correction using sunglint correction algorithms. Data collection optimized for solar geometry can be used for calibrating airborne and satellite sensors and to validate the algorithms that use these remote sensing data collections. For BioSCape/CyanoSCape we'll have the opportunity to test new and emerging hyperspectral algorithms for phytoplankton biodiversity so we want the best science quality data collection from aircraft to exploit the exciting opportunity of both AVIRIS-NG and PRISM imaging spectrometers from the G-III aircraft.

Question 13: How do you obtain pitch-roll-yaw parameters? Do you use control points with X, Y, and Z data?

Answer 13: Pitch, roll, and yaw are variables provided by the navigation sensors onboard the aircraft. Our pilots are well accustomed to flying straight lines (e.g., into and out of the Sun) and keeping the aircraft stable. Wind does cause some trouble, but the pilots are great. Of course if the weather is too rough, the flights won't happen and for coastal sites, there would be white caps and not appropriate for science quality data collection.

Question 14: Slide 56: what is the accuracy/precision of the Azimute (e.g. how much margin do you have when planning a flight path)?
Answer 14: Great question! We generally use +/- 2-5 degrees of tolerance in aircraft heading into and out of the Sun (solar plane). This paper can help you out: Guild L. S., Kudela R. M., Hooker S. B., Palacios S. L. and Houskeeper H. F., 2020, Airborne Radiometry for Calibration, Validation, and Research in Oceanic, Coastal, and Inland Waters. Front. Environ. Sci. 8:585529. DOI: 10.3389/fenvs.2020.585529.

Question 15: How important is sun elevation when planning to collect via Drone/ UAV?
Answer 15: Whether flying a fixed wing aircraft or drone/UAV, the flight planning requirements to avoid sunglint are the same for aquatic targets. If you see sunglint in your data, the flight plan was not optimized for solar geometry.

Question 16: In the Fieldwork, it is collecting data from control zones with no presence of cyanobacteria?
Answer 16: Yes, we'll be collecting throughout the water body and not all water bodies will have algal bloom levels present.

Question 17: Slide 6, what does ACS and BB9 stand for?
Answer 17: These are bio-optical instruments that measure absorption and backscattering in the water column, respectively. ACS is a **Spectral Absorption and Attenuation Sensor** instrument. BB9 measures backscatter at 9 wavelengths and is composed of 3 BB3 instruments each measuring backscatter at 3 different wavelengths.

Question 18: Why is the Floating Algal index so different from the chlorophyll indices? Aren't most of the phytoplankton floating? Is it detecting different spp.?
Answer 18: Each index takes advantage of spectral (hyperspectral) data a bit differently aligned with the spectral libraries associated with floating algae. Chlorophyll is a more

general indicator. Also, each index uses specific regions of the electromagnetic spectrum. How much a particular pigment absorbs into that specific region will influence the “behavior” of the index. Please be sure to access past ARSET trainings on water quality or coastal ecosystems where such topics are covered in detail.