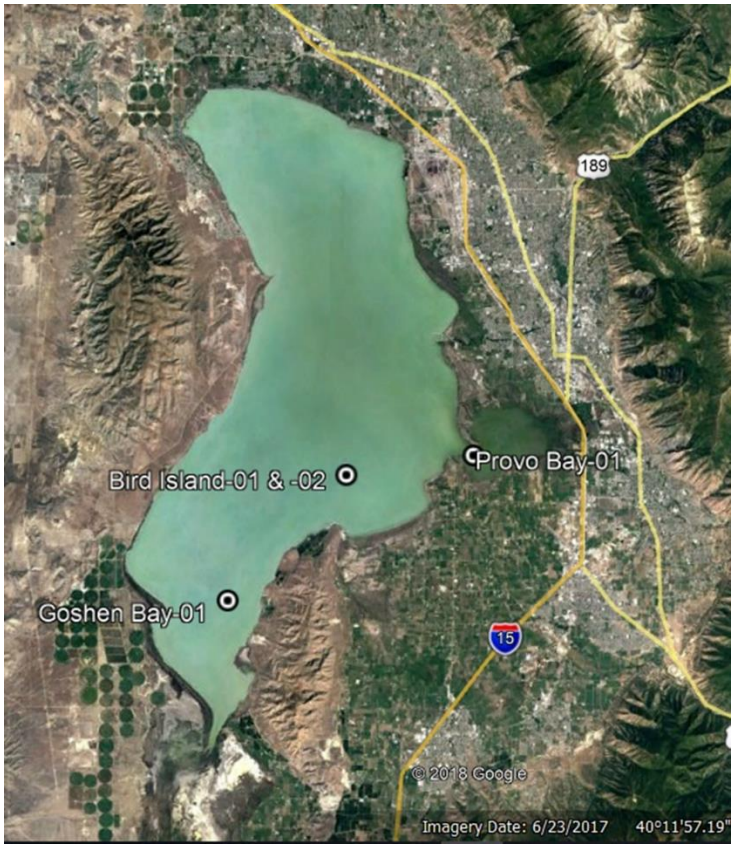
Utah Lake Paleolimnological and Paleoecological Study

Sampling and Analysis Plan



A joint research effort from Utah State University and The University of Utah

Brahney, J., Power, M., Brothers, S., July 5, 2019.

Table of Contents

Table of Tables

Introduction and Background Information

Introduction

This Sampling and Analysis plan was prepared by Dr. Janice Brahney with assistance from Dr. Mitchell Power at the University of Utah and Dr. Soren Brothers at Utah State University. Determining the historical nutrient and trophic condition of Utah Lake was prioritized by the Utah Lake Water Quality (ULWQS) Science Panel to assist the Utah Department of Environmental Quality (DEQ), Division of Water Quality (DWQ) in the development of site specific (Utah Lake) nitrogen and phosphorus water quality criteria.

Background

Utah Lake is a large shallow eutrophic lake located in north central Utah. In recent years, the occurrence of toxic cyanobacteria blooms has brought new urgency in understanding the cause and effects of eutrophication in Utah Lake. As agencies work towards reducing nutrient concentrations and improving water quality, there arises some uncertainty as to the trophic status and ecology of the lake prior to the settlement of Utah Valley. Because of Utah Lake's unusual features as a shallow remnant of the ancient Lake Bonneville, it may be difficult to assume anything about its historic P concentrations by comparing it to other regional lacustrine systems. However, defining historical phosphorus, nitrogen, silica, calcium, and iron concentrations and how these have changed will assist DWQ in defining numeric nutrient targets while reconstruction of diatom, macrophyte, and zooplankton communities will assist in understanding how the trophic status and ecology of the lake has changed.

There is evidence to suggest that sometime in the recent past; Utah Lake underwent a regime shift transitioning from a clear water macrophyte dominated system to a turbid algal dominated system. Early Latter Day Saint (LDS) settlers have described the clarity of the lake in their journals, lamenting the loss of submerged vegetation(Bushman 1980). Further, early paleolimnological analyses indicate a recent shift to a greater proportion of planktonic taxa as well as the historical presence of epiphytic taxa (Bolland 1974). At present, it is unclear whether such a regime shift has taken place, and if it has, whether the shift was primarily associated with carp introduction, nutrient increases, or both. The current state of Utah Lake is heavily affected by both non-native fish introductions and significant effluent loads from multiple wastewater treatment plants. In recent years, toxic algal blooms have limited access to the lake and compromised downstream uses of water, and only 2 of the two of the thirteen native fish species that inhabited the lake are currently present, one of which is critically endangered and endemic to the lake.

This study will reconstruct historical lake water composition and trophic status as well as the plant, and algal communities within using a combination of well-established and novel paleolimnological techniques. The study will provide a clear historical framework for the timing of environmental shifts as they may relate to natural variability of anthropogenic forcing in the catchment and the lake basin itself.

This SAP outlines the study design, sampling and analytical protocols, safety considerations, data management and reporting requirements.

Objectives and Design of the Study

Specific objectives of the study

The study aims to reconstruct historical nutrient and ecological conditions within the lake. Specifically the project will address the following objectives to support the needs of the Utah Lake Water Quality Science Panel. These are,

- What were the historical phosphorus, nitrogen, and silicon concentrations as depicted by sediment cores?
- What does the diatom and macrophyte community in the paleo record tell us about the historical trophic state and nutrient regime of the lake?
- What do photopigments and eDNA in the paleo record tell us about the historical water quality, trophic state, and nutrient regime of the lake.

Sampling design

Utah Lake is a large (385 km^2) shallow lake with multiple point and nonpoint sources of pollution and other influences. As such, reconstructing the limnological changes that have occurred in recent history of such a large lake will require multiple sediment cores to evaluate changes in the distinct sub-basins. We will use existing sediment cores from 4 locations including Provo Bay (PB), Goshen Bay (GB), Bird Island (BI), North of Provo Bay (NPB), and potentially collect a new core in the northern reach (N) in the main body of the lake (Figure 1). These sites were chosen as representative of the basin for four reasons; i) Provo Bay is a shallow distinct bay that has a unique chemistry and biology(Javakul et al. 1983), ii) Harmful Algal Blooms (HAB's) occurring frequently in Utah Lake often begin in Provo Bay or Goshen Bay *(pers. Comm. S. Daly*), both of which are expected to have the lowest effective fetch, iii) the Bird Island site is the deepest area of the lake and represents a pelagic region with a higher probability of minimizing wind mixing, iv) site NPB will provide reference for the northern basin.

Tasks

1. Core Retrieval and Archiving
2. Analyze Cores
3. Infer historical condition
4. Prepare technical report

Sediment core proxies

1. Chronological data (^{210}Pb, ^{137}Cs, Radiocarbon)
2. Carbon and nitrogen mass and isotopes
3. Phosphorus reconstructions
4. Elemental composition
5. Diatom community composition
6. Photopigments
7. Fossil zooplankton data
8. Fossil macrophyte data
9. eDNA
10. Pollen data
11. Charcoal
12. Production modeling

Table 1 Coring locations and associated analyses

	Core 1	Core 2	Core 3	Core 4	Core 5	Short Cores (total of 8)
	Bird Island (BI)	Goshen Bay (GB)	Provo Bay (PB)	North of Provo Bay (NPB)	North Basin (N)	Lake Perimiter
Latitude	40.1712	40.1087	40.1817	40.2318	TBD	TBD
Longitude	-111.7998	-111.8751	-111.7181	-111.754	TBD	TBD
Archiving	X	X	X	X	X	
Age-Model	X	X	X	X	X	
C/N mass & isotopes	X	X	X	X	X	
P reconstruction	X	X	X		X	
Elemental Composition	X	X	X		X	
Diatom Analysis	X	X	X		X	
Pigment	X	X	X		X	
Zooplankton		X	X		X	
eDNA		X				
Pollen				X		
Charcoal				X		
Production modeling						X

Table 2 Timeline for sediment core data generation

		2019		2020		
TASKS	Completed	Summer	Fall	Winter	Summer	Fall
Age-Model Construction (NPB)						
Age-Model Construction (GB)						
Age-Model Construction (BI, PB)						
Carbon/Nitrogen Mass Isotope Data (NPB, PB, GB)						
Carbon/Nitrogen Mass Isotope Data (BI)						
Phosphorus Reconstruction Data						
Elemental Composition Data (XRF) (PB, BI)						
Mineral Composition (XRD) (PB, NPB)						
Rock-Eval (BI, GB, PB)						
Diatom taxonomic record						
Diatom interpretation						
Historical Macrophyte Production						
Photopigment Data (GB)						
Photopigment Data (BI, PB)						
eDNA Data						
Pollen/Charcoal Data (NPB)						
Fossil zooplankton Data (PB, GB)						
SACore						
Age-Model Construction (N) *(Brahney)*						
Carbon/Nitrogen Mass Isotope Data (N) (Brahney)						
Phosphorus Reconstruction Data(N) (Brahney)						
Elemental Composition Data (XRF) (N) (Brahney)						
Mineral Composition (XRD) (N) (Brahney)						
Rock-Eval (N) (Brahney)						
Diatom record (N) (Brahney)						
Photopigment Data (N) (Brahney)						

Special precautions and safety plan

Brahney: All SOP's for field and lab contain the associated safety information. Please see details in specific SOP's below.

Brothers: For the in-lake retrieval of nearshore sediment cores from Utah Lake, special precautions will be taken by the principal researcher (Brothers) to ensure the safety of all field participants. Prior to entering the field, Brothers will share with any field assistant(s) a field safety plan describing potential hazards associated with field work, including emergency contact information for all participants and advance knowledge of the nearest medical services, if necessary. In accordance with Utah State University (USU) and the Department of Watershed Sciences policy, an emergency first-aid kit will be taken to the field, and if any sampling is to be carried out more than 30 minutes from the nearest medical services or out of cell phone range, an emergency inReach (Garmin) GPS device will be brought along to call in case of emergency. Near-shore sediment cores will not require boats for retrieval; our primary safety concerns will therefore be to ensure that the weather is favorable (i.e. no signs of storms/lightning) and that potential harmful algal blooms are avoided.

Power: All participants working with the Power Paleoecology team will sign a University of Utah safety liability waiver and take all necessary safety precautions during field-based sediment extraction process as well as laboratory analysis. First-aid kit will be available during both field and lab research. Emergency contact information for each participant will be registered at the Natural History Museum of Utah prior to participation. The use of watercraft during field research will require participants to be wearing a Type I or Type II US Coast Guard approved floatation devices at all times on or near the water. Primary safety concerns will emphasize favorable weather forecasts and proper functioning of personal floatation devices.

Field sampling methods and documentation

The field collection of sediment cores for Provo Bay (PB), Goshen Bay (GB), and Bird Island (BI) were taken using protocols outlined in the SOP from Brahney. The sediment core collected from North Provo Bay (NPB) was taken using protocols outlined in the SOP from Dr. Power. Any additional cores taken in the northern portion of Utah Lake will be conducted under joint SOP's from Dr.'s Power and Brahney. The near-shore short sediment cores will be taken using protocols outlined by Dr. Brothers.

Brahney: Field Sampling Methods and Documentation

Brothers: Field sampling methods and Documentation

Power: Field Sampling Method and Documentation

Laboratory sampling methods and documentation

Links to Standard Operating Procedures (SOP's) are provided below. Not included are operations from external labs and methods under development. These include, carbon and nitrogen mass and isotope analyses, which are conducted in the Newell Lab at Utah State University, minimal preparation (weighting) is required prior to submission. Photopigments are analyzed in Peter Leavitt's lab at the University of Regina. Procedures used in the Leavitt lab are documented in the literature (Leavitt and

Hodgson 2002). The development of novel methods for phosphorus reconstruction protocols is explicitly part of this research and are this not currently available, however, we do provide SOP's for standard phosphorus sequential extraction methods. The sampling and preparation of sediments for eDNA analyses is described in the core collection protocol. Finally, diatom community composition for this study will be conducted at BSA Environmental Services and not in the Brahney or Power lab.

Brahney: Initial Core Description (includes sample prep for dating)

Brahney: Fossil zooplankton and other organic microfossils (including macrophyte oospores)

Brahney: Elemental composition (complete or sequential digest)

Brahney: Phosphorus fractionations using a modified SEDEX protocol

Power: Laboratory Sampling Methods and Documentation: Pollen and Charcoal

Brothers: Laboratory sampling methods and documentation

Brothers: Historical production modeling

All analytical methods, quality control, and documentation procedures are included in SOP's/

Data analysis, record keeping, and reporting requirements

The project includes the collection of physical samples and the generation of analytical data. Physical samples are stored in a walk-in fridge at the EBPL or LacCore Facility where they will be permanently archived. Archive halves of select cores are stored at the LacCore facility at the University of Minnesota. Sediments from the North Provo Bay core are archived at the University of Utah and nearshore short cores will be stored in the Brothers Lab at Utah State University.

Data will be compiled and stored in Microsoft Excel spreadsheets, Word documents and comma separated values files, and are backed-up to external hard drives and USU's Box cloud storage daily, which allows for centralized and secure file management. The EBPL maintains GoogleDrive storage where data are also archived. Chronological and elemental data with uncertainty will be reported and archived according to recommended protocols (Mustaphi et al. 2019) including deposition of data into the Neotoma, Interdisciplinary Earth Data Alliance (IEDA) and the Linked Paleo Data (LiPD) repositories where appropriate (Mustaphi et al. 2019).

1. **Dissemination methods**

The data and results of analyses generated in this project will be disseminated through publishing in peer-reviewed and widely available scientific journals and through presentations at national and local conferences by the project PI, Co-PI, and graduate and undergraduate students. Data tables generated through the project will be published as supplementary information along with journal publications. Post-publication data and articles will be archived through the Utah State University open access Digital Commons website (http://digitalcommons.usu.edu/) managed through the Merill-Cazier Library.

2. **Policies for data sharing**

Prior to archiving, data and any remaining archived samples will be considered for sharing with other researchers as requested. All interested sample and or data users will be required to sign a sample and data use contract that will require them to acknowledge as deemed appropriate the investigators in any resulting publication or presentation

3. **Plans for Archiving and Preserving**

All remaining physical samples will be permanently archived at EBPL and stored in temperature controlled low humidity environment. Similarly, at the time of publication or two years following the completion of the project, geochemical data will be archived in the Integrated Earth Data Applications EarthChem database (http://www.earthchem.org/), and ecological data will be archived in Dryad (http://datadryad.org/), and DataOne (http://www.dataone.org/), as appropriate. All additional data will be archived through USU's Digital Commons, where all files are backed up at multiple sites, including cloud storage and preservation copies are stored in Amazon Web Services, with redundant storage across multiple facilities and are regularly verified for the integrity of data using checksums.

4. Roles and responsibilities

Dr Brahney will be responsible for maintaining all physical samples and ensuring her and her students appropriately store and archive dust and associated data including, physical properties, biogeochemical data, and experimental results. Generated data will be backed up to external hard-drives and through automatic daily updates to cloud storage through USU's Box subscription.

Dr. Brothers will ensure that all students are properly trained in the relevant data analysis techniques. Any computer files containing data calculations or primary production models will be personally verified by Brothers to minimize the chance of errors. Students working will be required to keep detailed lab notes of the progress of their work, and all data generated by these analyses will be stored and backed up on multiple computers and/or hard drives, and will be available upon request. Upon completion of the project, the results of these objectives will be included in the official report to the Division of Water Quality.

Dr. Power is responsible for the all data collection, archiving, interpretation, and dissemination from the North Provo Bay Core.

Level of Effort and Budget

Table 3 Level of Effort

Task	Deliverable	Brahney	USU Student	Power	UofU Student	Brothers	USU Ugrad
1	SAP	8	0	4	0	2	0
2	Collect/Preserve/sample Cores	50	120	20	40	8	0
3.1	Dating cores	8	40	10	40	0	0
3.2a	Nutrient - Phosphorus	130	1490	0	0	0	0
3.2b	Elemental	16	32	16	80	0	0
3.3c	Isotope	20	40	20	80	0	0
3.3d	Fossil Zooplanktoin	40	680	0	0	0	0
3.3e	Macrophyte fossils	40	680	10	40	16	680
3.3f	Production model	0	0	0	0	170	0
3.3g	Diatom record	30	20	0	0	0	0
	Charcoal and LOI	0	0	200	1200	0	0
3.3g	Pollen	0	0	20	500	0	0
3.4	Photopigment	16	190	0	0	0	0
3.5	eDNA	36	850	0	0	0	0
4	Interpretation	80	100	80	80	40	40
5	Report	80	100	80	80	40	40
Total	Hours	**554**	**4342**	**460**	**2140**	**276**	**760**

Table 4 Budget breakdown by PI and analytical cost

Task	Deliverable	Total
1,2,3,4,5	**Brahney and Student** **Task 1 and 2 are completed*	$62,175.50
2	Core retreival and Archiving	$4,200.00
3.1	Age-Model **(with Power)** (3/4 cores)	$6,000.00
3.2	Carbon/Nitrogen Mass Isotope Data **(with Power)** (1/4 cores)	$900.00
3.2	Phosphorus Reconstruction Data	$7,040.00
3.2	Elemental Composition Data **(with Power)**	$6,850.00
3.3	Diatom analysis	$30,000.00
3.4	Photopigment Data	$33,750.00
3.5	eDNA Data	$0.00
3.3	Fossil zooplankton	$0.00
3.3	Macrophyte fossils **(with Brothers)**	$0.00
1,2,3,4,5	**Brothers and Student**	$11,522.96
3.3	Contemporary and historical benthic/algal production modeling	$1,000.00
1,2,3,4,5	**Power and Student**	$44,698.00
3.3	Pollen data (lab technician)	$2,800.00
3.1	Charcoal, LOI, C:N (grad student)	$2,550.00
Total Direct		$213,486.46
Indirect UofU		$5,005.00
Indirect USU		$16,688.83
Total Direct and Indirect		**$235,180.29**

Project team and responsibilities

Janice Brahney, Principal Investigator, Utah State University. PI Brahney is responsible for overseeing the project direction, data analyses, and reporting. Specifically, Dr. Brahney is advising two students, Leighton King, Mark Devey, and Brynn Young, on the collection, sampling, and analyses of sediment core proxies for limnological reconstruction. In addition to overseeing the work performed by graduate students (see below), Dr. Brahney will analyze the diatom community composition once the data is received from BSA Environmental. Dr. Brahney will ensure the timely reporting of all data and analyses to the Utah Lake Water Quality Science Panel.

Leighton King is an MSc student where one half of her project is focused on ecological reconstructions of Utah Lake including historical macrophyte abundance, photopigment analyses, eDNA, carbon and nitrogen mass and isotopes, Rock Eval Pyrolysis, and with the help of undergraduate Brynn Young, zooplankton and other plant microfossil abundance. Under the direction of Dr. Brothers, Leighton is producing benthic versus pelagic production models for Utah Lake.

Mark Devey will focus on the paleo-reconstruction of nutrient concentrations in Utah Lake including standard (sequential extraction) and novel (calcite included phosphate) reconstructions of phosphate concentrations. Mark will also analyze historical elemental concentrations in Utah Lake, including metals and silica.

Mitchell Power, Co-Investigator, University of Utah. Dr. Power is responsible for overseeing the direction and advising of students reconstructing fire history and nutrient loading in Utah Lake, shifts in vegetation, as well as proxies for organic matter in Utah Lake, including loss on ignition and carbon and nitrogen mass and isotopes.

Soren Brothers, Co-Investigator, Utah State University. CO-I Brothers is responsible for the collection and analyses of 8 nearshore short cores to analyze contemporary and pre-settlement macrophyte abundance. Dr. Brothers is also responsible for historical benthic production modeling. Brothers will ensure that all students are properly trained in appropriate field, laboratory, and analytical methods.

Appendix A. SOP's

Brahney: Field Sampling Methods and Documentation: Sediment Coring Utah Lake

EBPL Lab Procedures: Janice Brahney Biotech 3rd floor 435-797-4479 2017

Introduction

Utah Lake is one of the largest freshwater lakes in the western United States. It features several hallmark indicators of a turbid eutrophic lake, including recurrent harmful algal blooms, yet there is no empirical record of whether this lake's waters were historically clearer, and if so, when the transition occurred. In order to examine this question, sediment cores will be taken from the lake for paleolimnological analyses. The analyses will produce a timeline for the history of eutrophication and ecosystem shifts by reconstructing 150 or more years of water quality and community composition of Utah Lake. One core will be Pb-210 dated and analyzed using geochemical and biological proxy methods. A second core will be sub-sampled in the field for eDNA analyses.

Safety Information

Safety is the most important thing while in the field. Be fully prepared and properly equipped. The safety of everyone depends on your knowledge and awareness, on being mindful of potential dangers, and on making smart decisions.

Safety Equipment

First aid kit (including regular medications), GPS, maps, warm/waterproof clothing, sunscreen, hat, food, water filtration device, vehicle/boat emergency kit, flashlight, cell phone, photo identification in case of accident of injury.

The Brahney Lab has an InReach emergency GPS locator. Ensure that the battery is charged and pre-set emails are appropriate in content prior to leaving on the trip.

Working Outside – General

- Sunlight contains UV radiation, which can cause cataracts and skin cancer. Be sure to cover up and bring sunscreen, wide-brimmed hat, and UV absorbent sun glasses.
- Heat: Drink plenty beforehand and bring plenty of water to prevent dehydration. Generally, for a half day trip with activity in the sun, bring 2-3 L or 1L with a filter system. Wear light loose clothing. Take frequent short brakes in the shade. Eat smaller meals.
    - Signs of heat-related illness: clammy, profuse sweating, dizziness. Place an overheated worker in the shade or a cool room. Loosen clothing and apply a cool wet cloth to face and neck. If sweating ceases or vomiting occurs, seek medical attention.
- Always work in teams of at least two. Use the buddy system
- Clothing: Wear appropriate footwear for the activity and loose synthetic clothing. Always be prepared for changing weather conditions. Bring a rain jacket, rain paints, waterproof boots.

Working Near and In Water

- Wear a life jacket at all times!

- Hazards related to working in and near water include: Drowning, which may occur due to shock from immersion in cold water, weight of water logged clothing, no life jacket, incapacity due to injury, fatigue or hypothermia. Mitigate these risks by always wearing a life jacket.
- Be conscious of changing weather patterns. Wind can cause large waves and make boating difficult due to the large size of Utah Lake. You don't want to get caught on the opposite side of the lake when wave conditions increase.
- Working in lakes during thunder and lightning storms is not recommended. If caught in a lightning storm, take precautions as described below.
    - Note: Working with metal coring equipment in the presence of lightning is especially hazardous.

<u>Thunderstorms and Lightning</u>

- If on the water in worsening weather conditions, get out as soon as possible and seek refuge.
- Avoid isolated tall trees. Lightning is likely to strike the tallest object in a given area (try not to be the tallest object). Avoid open areas. Retreat to dense smaller trees, low lying areas, and avoid water. Avoid metal objects like fencing and do not lean on concrete as it may have metal scaffolding inside. Never lie flat on the ground. If possible, crouch on top of life jacket to insulate you from the ground.

Materials

- Livingston/Bolivia Corer
    - 5 Core tubes (marked with arrows designating UP, or label them in the field)
    - Piston
    - Piston cable
    - Collar
    - Drive rods
- Something to remove/tighten the nuts
- Endcaps for PVC (blue and orange)
- Towel to dry core tube
- Duct tape to tape endcaps on.
- Core cutter
- Gloves (the cables can be rough on the hands)
- Something to measure the depth of the water.
- Electrical tape
- Vise Grips
- Extruder
- Spatula
- Rag, bucket for water to clean the extruder after each push.
- Smaller spatula for eDNA subsamples from the middle of the core
- Whirl-paks for samples
- Nitrile gloves (for eDNA sub-sampling)
- Ethanol to clean equipment between uses
- 220 20mL plastic scintillation vials for eDNA
- Cooler with Ice
- Field notebook
- GPS Unit

- Pencils, permanent markers

*Treat gear with care at all times. All gear should be cleaned and dried after use, and then properly stored.

Methods

Use UV sterilizer on all equipment needed for eDNA before leaving for the field.

*Take note of weather and lake conditions in field notebook (sunny, cloudy, rainy, clear, turbid, etc.).
Record the location and relevant information for each core collected.
Record the number of sub-samples taken from each core, their locations within the core (depth), and any other relevant information.

Collecting the Sediment Core

1. Select appropriate coring location. Typically the deepest part of the lake.
2. Assemble the corer.
   Pass the piston cable through the hole at the top of the collar all the way through the core tube. Once it exits out the other side, turn the tube over and fasten the piston onto it by passing the cable through the eyelet at the top of the piston, securing with an overhand double-knot with ~3cm tail. Use electrical tape, to tape the knot to the center of the piston to prevent it from getting in the way of the piston as it moves through the core tube.
   Reinsert the piston, which provides suction at the top of the tube while sampling, by turning the core tube over and using the ground to push the piston into core tube.
   Pour water through the weep hole to allow the piston to move smoothly through the core tube.
   Test the fit of the piston by pulling up on the piston cable through the tube, it should have several pounds of resistance to it and not move easily by hand. If using a clear tube, you should see a black ring all the way around indicating that the rubber is making contact with the tube. There are two nuts on the piston that can be adjusted to achieve the desired fit. Position the piston so that the widest part of the conical tip is flush with the bottom end of the tube.
3. Measure the depth to drive.
   For the first drive, this will be the water depth, measured from a stable reference point on your platform.
   From that depth, subtract the length of the corer (from the bottom of the core tube to the top of the middle, non-threaded section of the connector).
   The remaining length will be the total length of drive rods you will need. Count out this number of drive rods (use the same rods for each drive and keep them in order) and mark the depth on the last rod.
   > *For example: Depth to drive= 9.82m, length of corer= 1.41m, total drive rod length needed= 9.82-1.41=8.41 m. So you need four whole drive rods (4 x 2m) plus 41 cm of the fifth. Mark 41cm from the bottom of the fifth rod, with Sharpie or colored electrical tape.

   Decide whether you're going to count the top or the bottom of the tape as the mark, and keep that the same. Don't forget to take the tape off after the drive, or use a new symbol next to your mark if using pen so you don't get confused on the next drive.
4. Position the corer.
   One person will lower the core tube, one will add drive rods (always in the same order) as necessary, and one will keep tension on the piston cable to prevent the corer from sliding down the square rod. Stop at the desired depth (i.e. when the mark on the last drive rod reaches your reference point) of where you will start coring, which will be the top of your first drive.
5. Setting the piston.

To fix the piston at this depth, secure the piston cable while maintaining tension to a secure object. The best way is to use a climbing figure-8 attached to a carabiner and clip it onto a fixed object (such as an eyebolt on the deck). Alternatively, you can use a pair of Vice Grips or some similar tool to clamp the piston cable securely to an immobile object on the deck, but this can be damaging to the cable.

6. Collecting the core.
   *The marker tape should be 1 m above the reference point.
   Ensure that the corer is vertical.
   Push down the drive rods until your tape mark is at your reference point, allowing the piston to stay in place and the core tube to move past it and take the core. Complete the full drive in a single action if possible; if not, push again to try and complete the drive.
   If using a figure 8, someone should tightly hold the loose end of the cable at a right angle to the direction of the fixed end of the cable.
7. Bringing the core to the surface.
   Release the cable and bring up the core, disconnect drive rods along the way. Hold the drive rods and cable together and pull up at the same time, otherwise the piston might slip and allow part of the core to fall out the bottom.
   After you remove the last drive rod, pull up the core tube capping the bottom end before it breaches the surface of the water (someone needs to be ready for this, it's a 3 person job).
   * Endcaps are blue for the top (blue=sky) and orange for the bottom.
   Dry the core tube with a towel and tape the endcap securely on with tape.
   Remove the piston, cap, dry and tape the top.
   Label the core with the name and length of the core.
   Keep the core cool and out of the sun.
8. Clean all equipment prior to collecting the next sediment core.

Helpful Videos:
https://ca.water.usgs.gov/projects/repeat_videos_3.html
https://ca.water.usgs.gov/projects/repeat_videos_5.html
https://www.youtube.com/watch?v=DcolL_DkWuM

Sub-sampling the Sediment Core

*One core will be sub-sampled completely in the field for eDNA analyses. Only the top, watery sediment will be sub-sampled on the second core to prevent sediments from mixing and to maintain the stratigraphy of the remainder of the core.

1. Set up extruder on level surface, making sure that the rod is straight.
2. Adjust the extruder to the desired extruding interval (¼ cm for the top 10 cm, and every ½ cm thereafter).
3. Siphon off the water overlying the sediment, taking care to not disturb the top layer of sediment.
4. Push sediment up one interval at a time and scrape the sediment into labeled Whirlpak bags. Close the top of the bag, push out extra air, spin around the top of the twist tie, and twist to seal.
   * For the core being sub-sampled for eDNA, collect sediment from the center of the core and place in the eDNA vials. Scrape the remainder of the sediment into the whirlpak bag.
   *Clean the extruder tray and spatula with ethanol between sub-samples to prevent cross-contamination.
5. Label Whirlpak bags (and eDNA vials)
   *Label with core information, depth of sub-sample
6. Place collected sub-samples in a cooler.

After transporting the samples back to USU, place the intact sediment core and associated sub-samples in the refrigerated room, if ready, in the Sediment Lab in BNR. Sub-samples for eDNA analyses will need to be placed in the freezer in the Sediment lab.

Do not operate without consulting the PI to ensure you understand the material presented in this SOP. Before using the instrument or following the protocol, sign below.

Documentation of Training (signature of all users is required)

I have read and understand the content of this SOP:

Name	Signature	Date

Brahney: Laboratory Sampling Methods and Documentation: Initial Core Splitting, Description, and Sampling

EBPL Lab Procedures: Janice Brahney Biotech 3rd floor 435-797-4479 2017

Introduction

Gravity cores are always extruded on site while Piston (Bolivia/Livingstone) and percussion cores are split in half and described prior to initial sampling. The advantage of splitting a core is that a higher precision in sampling interval can occur as the smeareing of sediment and water along the edge of the core tube can be avoided, and, if stratigraphy is present sampling within laminations may be possible. Since the top ~10cm generally have a high water content and are quite 'soupy' these sections are extruded on site before the remainder of the core is capped and returned to the lab. Once the core is split, it is cleaned, photographed, and described as explained below.

The initial core description (ICD) and initial sampling of cores samples is a critical step in any lake sediment study. The ICD consists of photographic and written documentation of cores stratigraphy, structures, biological components, and/or material that may be radiocarbon dated. This step can provide critical information about the changes in depositional conditions that will aid in the interpretation of any proxy. Smear slides may also be taken to describe the mineralogical and biological components.

Important

1. All core sections extruded on site must be weighed immediately as they will lose water content over time. We use Whirl-Pak bags because their weight is less variable than specimen cups. Specimen cups may also be used if avoiding water-loss is deemed more critical.
2. **Prior to any sampling the wet density of the sediments must be determined**. This information becomes critical when attempting to date and determine sedimentation rates for the core. For gravity cores and extruded samples the volume of the sample is determined by the equation for the volume of a cylinder, $V= \pi r^2 h$, where h is the depth of extrusion and r is the radius of the inside diameter of the core barrel. For split cores the wet density is determined by taking a known plug of sample using a syringe as described below.

Safety Information

a. Hazardous chemicals involved: no
b. Potential hazards: Cuts from improper use of the skill saw or from razor blades. Watch fingers and position body out of the line of the cut.

Method: Wet and dry density determination from extruded samples

NOTE: When working with core samples ALWAYS process samples in order e.g. 0-1 cm, 1-2 cm, and so on, AND use sample containers that are also labelled in order. You may think this is an annoying and time-consuming step – but if you mess up this you may lose all critical core data which you spent a lot of time, money, and energy collecting (and or could hinder your thesis). Taking this precaution ensures you can always find your place and you will not lose all (maybe just some) of the data you just collected.

Materials

1. Core samples
2. Empty Whirl-Pak bags
3. Analytical balance
4. Oven/furnace
5. Crucibles
6. Clean Spatula – TFE or plastic if conducting trace element analyses, stainless steel otherwise.
7. Kim wipes
8. Lab book and pencil
9. Powder free nitrile gloves

Procedure

1. Weight three Whirl-Pak bags that are the same size as the bags used for the samples. Determine the average weight.
2. Weight and record each sample baggie.
3. Take note of any distinguishing features, e.g. colour, texture, or the presence of fossils, roots, or vegetation.
4. Determine the wet density (g/cm^3) = (sample weight – avg. bag weight/V)
5. Weigh out crucibles
6. Working from the top sample, mix the sample within the baggie using your fingers to ensure sample is homogenous.
7. Tare the first crucible and use spatula to scoop out ~X g of sample
8. Repeat for all possible samples (until crucibles are used or oven is full)
9. Dry the samples in the oven at 50°C for 24 hours.
10. Re-weight all the crucibles and determined the dry density.
11. For efficiently use these samples for organic matter concentration and inorganic carbon determination (see LOI protocol)

Method: Core Splitting, Cleaning, and ICD

Materials

1. Core to be described
2. Duct tape and sharpies
3. Industrial box of cellophane
4. Razor blades
5. Skill saw and splitter box
6. Core box with camera mount
7. Lab book, pencil, sharpie
8. Nitrile powder free gloves

Procedure

*when doing this for the first/second time as PI Brahney for help

****ALWAYS ENSURE THAT NO BODY PART IS IN THE PATH OF YOUR CUT.**

ALWAYS CUT AWAY FROM ALL BODY PARTS.**

1. Ensure Skill saw is set to only cut most of the way through tube and will not reach sediment
2. Cut on side, cutting through core caps on each end.
3. Wipe the surface clean with a rag and duct tape along the cut edge
4. Flip core over and ensure that second cut will be 180 from the first cut
5. Cut through core and again clean the edge of any tube debris
6. Use a razor blade to cut through the core cap
7. Use the razor blade (or fishing line) to slice through the core itself go slowly and make sure core opens evenly
8. Duct tape end caps on securely to each core half
9. Ensure each tube is labelled so that the top and bottom of the core are discernable
10. Clean each side of the core using the razor blade ensuring that any stratigraphy is clearly visible and the surface is smooth.
11. Wrap archive half in by covering with a long piece of cellophane. Double wrap each end with cellophane and duct tape close. Place in fridge for storage
12. Begin ICD for working half

Note: core units are always described as intervals, e.g. "0-1" cm, never " 0 cm". Work from the top of the core, the sediment water interface, to the bottom starting at 0.

Procedure 1 Initial Core Description

1. Place the working core half in the box with labelling tape.
2. Mark the side of the core tube at 1 cm intervals, use a thicker or distinct mark every 10 cm
3. Move the camera along the track to photograph each section, which later may be stitched together using software
4. Describe the core, working from the top to the bottom as follows
    a. Delineate stratigraphic units (changes in color, lithology, sediment character)
        i. Describe contacts
            1. Sharp/diffuse/gradual
            2. Regular/irregular/disturbed (wavy, ripples, irregular)/inclined
                a. Regular means conformable to the overall distortion from coring
                b. Irregular means unconformable
                c. Disturbed means wavy, could be from coring, or burrowing animals
                d. Loaded – genetic term (from weight of overlying material, from dewatering of pore waters, ball and pillow structures…) also called soft-sediment deformation.
        ii. Determine unit thickness
        iii. Describe unit
        Colour + Bedding + Lithology + Major Modifier + Principal Name + Minor Constituents (e.g. Dark reddish brown, massive, feldspathic clayey silt with carbonaceous debris and trace gastropod fragments.
            1. Colour -> munsell, GSA rock colour chart
            2. Sedimentary structures; includes description of the thickness and repetitiveness of layers and contacts between them. Eg. finely laminated (0.5-1mm) sharp contacts.
                a. Stratification
                    i. lamination < 1cm thick
                    ii. bedding > 1cm think

iii. cross stratification (trough, planar, rippled, hummocky
iv. massive/poorly stratified

3. Lithology (gravel, sand (fine, coarse), silt, clay)

iv. Composition of the sediment (if you can tell)

1. Rock frags, minerals
2. Grain shape, angular rounded
3. Ornamentation (striations)

b. Fossils/ organic content

i. Isolated fossils, in-situ

1. Wood (root, branch) shell (articulated, disarticulated)
2. Concentration of fossils
3. Peat (humic, fibric)

***do not touch fossils with your bare hands, while wearing gloves use forceps to remove vegetation/fossil fragment. Gently clean with DI water and place in fume hood or desiccator to dry. Once dry use forceps to place in labelled vial for later 14C dating.**

Method: Subsampling (general) and subsampling for wet and dry density determination from split cores-subsampling

Subsamples here are placed into crucibles for density determinations and samples may be re-used for efficiently to determine organic content and inorganic carbon content. In general subsamples maybe kept in glass vials, whirl-pak bags (water may be lost over time), or plastic containers. Label each container with lab tape and sharpies.

Cores are always lain on the bench or in cradle with the top end to the left. Always avoid the outside layer of core material as it may be contaminated from other core interval smearing during the process of coring. If sampling laminations that are obviously distorted, sample from the middle of the core section.

Sampling may be volumetrically quantitative (as needed for density) or non-volumetric. Ensure that you are using the correct tools for the analytical procedure. Do not use metal spatulas if sampling for trace-element analyses. If sampling for organic compounds – do not use plastic and do not allow samples to come in contact with plastic wrap or skin oils. For magnetic studies, do not use iron-bearing tools**. Be sure to research the needs of your specific analytical interest carefully.**

Subsampling for dating

Radiocarbon sampling is described above. Dating by ^{210}Pb and ^{137}Cs requires 10-15 samples per core. Information on dry and wet density is needed for each interval, whether or not submitted for dating. Weight approximately 100 mg in a centrifuge tube and set aside to send to lab. More details can be found here http://www.mycore.ca/submissions.html

Materials

1. Working half of core
2. Clean syringes (3-5 mL) with top cut off
3. Analytical balance
4. Oven/furnace

5. Crucibles
6. Clean Spatula – TFE or plastic if conducting trace element analyses, stainless steel otherwise.
7. Kim wipes
8. Lab book and pencil
9. Powder free nitrile gloves

Procedure

1. Use ICD and research goals to determine the sampling interval, e.g. every 1 cm
2. Use syringe as a mini piston corer to take a known volume of sediment, 1 cc (cubic centimeter). If unsure how to sample ask PI Brahney.
3. Tare crucible and weight sediment plug, repeat for all samples – use crucibles in numerical order.
12. Determine the wet density (g/cm^3)
13. Dry the samples in the oven at 50°C for 24 hours.
14. Re-weight all the crucibles and determined the dry density.
15. For efficiently use these samples for organic matter concentration and inorganic carbon determination (see LOI protocol)

Brahney: Laboratory Sampling Methods and Documentation: Sediment preparation for Cladocera/Microfossil analysis

EBPL Lab Procedures: Janice Brahney Biotech 3rd floor 435-797-4479 2017

Introduction

The described methods deflocculates and macerates organic material so that microfossils, particularly cladocera carapaces, may be mounted on slides for identification. In addition to cladocera, other chitinous and refractory insect and zooplankton parts may be found – refer to lab guides for identification. See Tracking Environmental Change Using Lake Sediments. Volume 4: Zoological for more details.

Safety Information

c. Hazardous chemicals involved (KOH)
d. Potential hazards
    i. **Very hazardous** in case of skin contact (corrosive, irritant), eye contact, ingestion or inhalation. Tissue damage will depend on length of contact. Eye contact can result in corneal damage and blindness. Skin contact can produce inflammation or blistering. In case of contact immediately flush area and get medical attention immediately. See MSDS for more details
e. Approvals required form PI required: Yes
f. Designated Area: Prepare in fume hood only
g. Handling and storage
    i. Work in fume hood
    ii. Store in cool well-ventilated area. Ensure cap it closed tightly. Do not store above 23°C
h. Personal protective equipment
    i. Gloves
    ii. Eye protection
    iii. Lab coat
    iv. Appropriate lab attire including long pants and shoes
i. Spill and accident procedure
    i. Small Spill: Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary neutralize with a dilute solution of acetic acid.
    ii. Large Spill: Corrosive solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. call EH&S for assistance.
j. Waste disposal
    - Neutralize with a dilute solution of HCL. Dilute slowly as the reaction causes heat ($KOH\ (aq) + HCl\ (aq) \rightarrow H2O\ (l) + KCl\ (aq) + heat$. Check pH is neutral before putting in the drain.

Materials

Apparatus

- Hot Water Bath
- Thermometer
- Slide Warmer

Reagents

- KOH
- DI water
- Glyceran Jelly

Supplies

- 2L bottle
- Several Erlenmeyer flasks (one per sample)
- Stir sticks
- Scupula
- KOH squeeze bottle
- Filter – 38 um mesh
- Squeeze bottle with DI
- 500 mL beak for waste KOH
- Beaker
- Scint vials, one marked at exactly 5mL (prepare by weight)
- Cover slips
- slides

Procedure

Procedure 1 Prepare the 20% KOH Solution

1. Work in the fumehood – **use caution when working with KOH it can cause severe burns and blindness**
2. Weigh ~200g of KOH pellets and place in 2L bottle – do not touch pellets.
3. Fill slowly with distilled water until pellets are dissolved. Solution will get hot, let cool at least 15 minutes before using.
4. Pour into labelled 20% KOH squeeze bottle.
5. Adjust amounts accordingly.

Procedure 2 Prepare Sediment

1. Set up a hot water bath in the fume hood. Fill the metal tray with tap water to about the same level as the 100mL mark on an Erlenmeyer flask. Put tray on heater. Make sure the temperature stays around 80°C, no hotter than 90°C, no cooler than 60 °C. It definitely should not boil!
2. Place empty, *labelled* Erlenmeyer flask on scale and tare. Using the scupula, remove sediment from the bag and place it into the flask (use 3.0g of wet material for each interval). **Make sure you mush the sediment up in the bag and also move all the sediment down from the sides of the bag so that you end up having a homogeneous mixture to scoop from.*
3. Add KOH into the flask using a squeeze bottle so that you can wash the sediment down from the flask sides into the bottom. Swirl the flask to try to break up the sediment. Fill the flask up to the 100mL level with KOH, this time using the 2L bottle (it's faster than the squeeze bottle). **Be careful.**
4. Put the flask into the hot water bath, let sit for 20 minutes.

5. Repeat steps 4 and 5 until all sediments are prepped, making sure to swirl the flasks occasionally to help break up the sediment.
6. Place the filter into a beaker and drain your first prepped flask through it. Rinse out the flask with distilled water (in a squeeze bottle) to make sure you get all of the sediment out (filter all of this).
7. Using the distilled water squeeze bottle, rinse the sediment a few times. Put the waste water into another container in the sink (you need to neutralize the KOH before putting it down the drain).
8. Rinse some more to make sure that the sediment is really free of KOH and try to rinse the sediment down to one side of the filter.
9. Rinse the sediment into a labelled glass vial, trying to use as little water as possible (should try to use only 5mL of water to do this).
10. Place labelled cap on vial and you're done!
11. Repeat steps 7 to 11 until all your sediment is in vials.
12. If any of your vials have more than 5mL of solution in them, you should let them stand overnight so that you can remove the supernatant (ie the top clear fluid) from them. If any of the vials have less than 5mL, top them up to the 5mL level. (An easy way to know how much 5mL is, is to put that amount into a vial – use the scale to weigh out 5mL into the vial).

Procedure 2 Prepare Slides

13. Pre-heat the slide warmer.
14. Put glycerin into a vial and put the vial into a small beaker filled with boiling water. Place this on the slide warmer.
15. Put the labelled slides onto the slide warmer to heat up.
16. Use a pipette calibrated to pipette 100µL to remove your solution from the vial (make sure the solution is well stirred before removing it from the vial). Eject the solution from the pipette onto the appropriate slide.
17. Let the solution on the slide sit for a bit so that some of the water evaporates. After a few minutes use a glass pipette to drop 2 drops of glycerin onto the solution. Mix this into the solution.
18. Let the slide evaporate a bit more and then place the coverslip onto the slide.
19. Repeat this for all of your slides.

Procedure 2 Counting Slides

1. Dried, mounted samples can now be examined under a compound scope using brightfield illumination.
2. All slides should be counted in their entirety to avoid any bias from a non-random distribution of remains under the coverslip.
3. The number of slides required to identify at least 70 individuals (minimum count size; Kurek et al. 2010) will vary depending on the density of remains in the sample.
4. All cladoceran remains (e.g. carapaces, headshields, ephippia and postabdominal claws) should be tabulated separately, but only the most frequent body part for each taxon should be used to estimate species abundance.
5. Badly fragmented remains should only be counted if they contain a clear diagnostic feature.

Identifying specimens

Several keys are available for microfossil identification. These can be found in the EBPL online GoogleDrive and hardcopies are available in the lab. The following keys are available,

- An illustrated guide to the identification of cladoceran subfossils from lake sediments in northeastern North America: Part 1: The Daphniidae, Leptodoridae, Bosminideae, Polyphemidae, Holopedidae, Sididae, and Macrothricidae. Part 2: The Chydoridae (2012)
- Zooplankton microfossil key, Darren Bos.
- Ecology and Classification of North American Freshwater Invertebrates (Thorp & Covich, 3rd Edition, 2010)
- Utah Lake Contemporary Zooplankton Key (Rey and Gaeta)
- Charophytes: An illustrated guide to identification (Kotecki 2014)

Brahney: Laboratory Sampling Methods and Documentation: Methods for removing elements associated with organic and Fe/Mn oxyhydroxides from soils and sediments

EBPL Lab Procedures: Janice Brahney Biotech 3rd floor 435-797-4479 2017

Introduction

Metals found in sediment cores are associated with a variety of sediment fractions including that bound within silicate minerals, metals precipitated with authigenic minerals (calcite, Fe/Mn-oxides, Sulfides), as well as that associated with organic matter. Distinguishing between these fractions can provide information on sediment provenance, lake mixing, and oxygen concentrations at the sediment water interface (Brahney et al. 2008). This protocol outlines methods developed by Brahney (2008).

Safety Information

k. Hazardous chemicals involved
   i. **Sodium Pyrophosphate:** See SDS for more details - Sodium pyrophosphate is hazardous in case of skin contact (Irritant), Harmful if swallowed – harmful if inhaled – risk of serious damage to eyes. If contact with skin or eyes – rinse thoroughly for several minutes, remove contact lenses. If irritation persists – call for medical attention. If swallowed – immediately call poison center or physician if you feel unwell. In case of spill – neutralize with acetic acid. Spread water on surface and dispose according to lab requirements.
   ii. **Sodium Citrate Safety Information:** See SDS for more details – sodium citrate may cause irritation to skin, eyes, and respiratory tract. Always work in a fume hood wearing a lab coat, eye protection, and gloves. In case of spill – remove all sources of ignition and ventilate area.
   iii. **Sodium Hydrosulfite Dithionite:** See SDS for more details – dithionite is slightly hazardous in case of skin contact and hazardous in case of eye contact or ingestion. Always work in a fume hood wearing a lab coat, eye protection, and gloves. In case of spill – remove all sources of ignition and ventilate area.
   iv. **Hydrogen Peroxide (30%): is a strong oxidizer and very hazardous** in case of skin contact, eye contact (irritant). May produce burns and tissue damage, inhalation may lead to coughing and irritation of respiratory tract. The substance is toxic to mucous membranes and lungs. In case of contact, immediately flush area with cold water for 15 minutes and get medical attention immediate. See MSDS for more details.
l. Potential hazards: **See MSDS for above reagents**
m. Approvals required form PI : **YES**
n. Designated Area **Fume Hood**
o. Handling and storage: **Dry chemicals are stored in the dry chemical cabinet behind glass**
p. Personal protective equipment: **Nitrile gloves, eye protection, lab coat, long pants and shoes**
q. Ventilation controls: **Work in Fume Hood**
   i. Large Spill: Call EH&S for assistance.
r. Waste disposal:
s. Decontamination
t.

Materials

1. Analytical Balance
2. Shaker
3. Aspiration set up
4. 2 L flask
5. 1 L flask
6. Stir plate
7. Centrifuge
8. Centrifuge tubes 50 mL
9. *If keeping digest solution, filtration apparatus and Whatman GF/F filters or disposable pipettes for transferring liquids

Reagents

1. Sodium pyrophosphate ($NaP_2O_7 \cdot 10H_2O$)
2. Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)
3. Sodium hydrosulfite (dithionite) ($Na_2S_2O_4$)

Procedure

Procedure 1 Prepare Reagents

While working in a fume hood, prepare the following reagents as described and modify volumes as required.

Sodium pyrophosphate solution (0.1 M)

- 89.2 g made up to 2L

Sodium citrate solution 0.68 M

- 200 g made up to 1L

*Procedure 2 Remove humic and fulvic acid complexed metals using **Sodium Pyrophosphate***

1. weight centrifuge bottle with cap
2. weigh and record ~0.3 g of sample in centrifuge tube
3. In a fume hood, prepare sodium pyrophosphate as described above
4. add 30 mL of 0.1 sodium pyrophosphate solution
5. Tighten cap on tubes and shake 16 hours and 200 rpm
6. Centrifuge 20 minutes at 18000 rpm
7. *note the colour of the solution and keep via filtration if interested in organic complexed metals
8. wash and aspirate at least three times, and centrifuge for 10 minutes between each wash
    a. Leave as little water in the remaining sample as possible WITHOUT aspirating out any material

**Note for washes you will have metals come out in this fraction, so wash with a known volume of water, which you add to your sample.

*Procedure 3 Use the reduction agent **Sodium-Citrate Dithionite** to dissolve non-silicate Fe in the sediment. *note changes in colour of the solution.*

1. In a fume hood prepare sodium citrate solution as above
2. Add 25 mL of sodium citrate

3. Add 0.4 g dithionite
4. Tighten cap on tubes and shake 16 hours at 200 rpm
5. Centrifuge 20 minutes at 18000 rpm
6. *note the colour of the solution and keep via filtration if interested in organic complexed metals
7. wash and aspirate at least three times, and centrifuge for 10 minutes between each wash

Procedure 4 If additional organic matter needs to be removed and or reduced minerals oxidized use 30% ***Hydrogen Peroxide or Ash and use a HCL extraction.***

If sulfides are present reaction may be strong so add peroxide slowly – e.g. few mL at a time and wait

1. Place centrifuge tubes in a water bath and heat at (80-90 ˚C) (don't boil)
2. Add a 10 mL of 30% H_2O_2 and let react for 1-2 hours
3. Let cool and leave for 48 hours, agitate every now and again to see if reaction continues
4. Dilute and rinse as above.

Or

1. Ash at 500°C for 4 hours
2. Extract with 10% HCL

The residual fraction will be dominated by allogenic minerals. The former is useful for distinguishing mineral source, if reduced minerals are not removed with H_2O_2 these can be used to infer conditions in the hypolimnion. Analyzing the pyrophosphate and sodium-citrate dithionite solutions can provide further information on conditions in the water column.

Do not operate without consulting the PI to ensure you understand the material presented in this SOP. Before using the instrument or following the protocol, sign below.

Documentation of Training (signature of all users is required)

I have read and understand the content of this SOP:

Name	Signature	Date

Brahney: Laboratory Sampling Methods and Documentation: Extraction of phosphorus fractions from sediments and soils

EBPL Lab Procedures: Janice Brahney Biotech 3rd floor 435-797-4479 2017

Introduction

Total phosphorus within a sample includes phosphorus found in different fractions, which control the bioavailability and mobility of phosphorus on the landscape. This protocol is modified form the standard extraction protocols (SEDEX, Bray-Curtis, Hedley) and describes the extractions for the following P fractions, Loosely sorbed, Authigenic carbonate fluorapatite + biogenic apatite + $CaCO_3$ associated P, Fe and Al exchangeable, Detrital apatite P, Ferric Iron bound, refractory organic P, and bioavailable P. The different chemical forms are operationally defined by the reagents used as the extractant. Bioavailable P is considered the P extracted by the rainwater solution (Salt-P) and the sodium bicarbonate - sodium hydroxide solution (NaCO3-P). The sequence of extractions will depend on the specific research objectives, clear each method with PI Brahney prior to extraction.

Safety Information

a) Hazardous chemicals involved

2. **Acetic Acid:** Causes severe eye and skin burns. Causes severe digestive and respiratory tract burns. **Flammable liquid and vapor.** May be harmful if absorbed through the skin.
    a. **Eyes:** In case of contact, immediately flush eyes with plenty of water for a t least 15 minutes. Get medical aid immediately.
    b. **Skin:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse.
    c. **Ingestion:** If swallowed, do NOT induce vomiting. Get medical aid immediately. If victim is fully conscious, give a cupful of water. Never give anything by mouth to an unconscious person.
    d. **Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
3. **Ammonium Nitrate: Danger!** Strong oxidizer. Contact with other material may cause a fire. Causes eye, skin, and respiratory tract irritation. May cause methemoglobinemia. Ammonium nitrate when contaminated with oil, charcoal, or other organic materials should be considered an explosive capable of detonation by combustion or by explosion of adjacent explosive materials.
    a. **Eyes:** Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.
    b. **Skin:** Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
    c. **Ingestion:** If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
4. **Calcium Oxide: Danger!** Can cause severe skin burns and eye damage
    a. **Inhalation**: Remove victim to fresh air. Supply oxygen if breathing is difficult. Seek immediate medical attention.
    b. **Ingestion**: Do not induce vomiting. Seek immediate medical attention.

c. **Skin**: Wash affected area with mild soap and water. Remove any contaminated clothing. Seek immediate medical attention.
d. **Eyes**: Flush eyes with water, blinking often for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. Seek immediate medical attention.
e. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

5. **Magnesium Chloride:** Caution! May cause eye irritation
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids until no evidence of chemical remains. Get medical aid
    b. **Skin:** Immediately flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid.
    c. **Ingestion:** Do not induce vomiting. If victim is conscious, give 2-4 glasses of water or milk. Get medical aid at once.
    d. **Inhalation:** Give artificial respiration if necessary. Move victim to fresh air. Keep victim warm and at rest. Get medical aid at once. Do not use mouth-to-mouth resuscitation.
6. **Magnesium Sulfate: Caution!** May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed, inhaled, or absorbed through the skin.
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
7. **Potassium Chloride:** Warning, skin and eye irritant.
    a. I**nhalation**: Remove from exposure. If breathing is difficult or has stopped, administer artificial respiration or oxygen as indicated. Immediately seek medical aid.
    b. **Skin Contact**: Wash thoroughly with soap and water. Seed medical aid.
    c. **Eye Contact**: Flush immediately with large amounts of water, lifting the lower and upper lids occasionally. Seek medical help.
    d. **Ingestion:** Give 1 -2 large glasses of water or milk. Induce vomiting. Immediately seek medical aid. Never give liquids to an unconscious person.
8. **Sodium Acetate: Caution!** May cause eye, skin, and respiratory tract irritation.
    a. **Eyes:** Get medical aid. Immediately flush eyes with plenty of water for at least 15 minutes.
    b. **Skin:** Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.
    c. **Ingestion:** Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
9. **Sodium Bicarbonate:** Causes eye and skin irritation. May cause respiratory tract irritation.
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.
    b. **Skin:** Get medical aid. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
    c. **Ingestion:** Do not induce vomiting. Get medical aid.

d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.

10. **Sodium Chloride: Caution!** May cause eye, skin, and respiratory tract irritation.
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
11. **Sodium Hydroxide: Danger!** Causes eye and skin burns. Causes digestive and respiratory tract burns.
    a. **Eyes:** In case of contact, immediately flush eyes with plenty of water for a t least 15 minutes. Get medical aid immediately.
    b. **Skin:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse.
    c. **Ingestion:** If swallowed, do NOT induce vomiting. Get medical aid immediately. If victim is fully conscious, give a cupful of water. Never give anything by mouth to an unconscious person.
    d. **Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
12. **Sodium Pyrophosphate:** Sodium pyrophosphate is hazardous in case of skin contact (Irritant), Harmful if swallowed – harmful if inhaled – risk of serious damage to eyes.
    a. **Eyes:** In case of contact – rinse thoroughly for several minutes, remove contact lenses. If irritation persists – call for medical attention.
    b. **Skin:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. f irritation persists – call for medical attention.
    c. **Ingestion:** immediately call poison center or physician if you feel unwell.
    d. **Inhalation:** If inhaled, remove to fresh air.
    e. In case of spill – neutralize with acetic acid. Spread water on surface and dispose according to lab requirements.
13. **Sodium Citrate Safety Information: s**odium citrate may cause irritation to skin, eyes, and respiratory tract. Always work in a fume hood wearing a lab coat, eye protection, and gloves. In case of spill – remove all sources of ignition and ventilate area.

    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of soap water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.

14. **Sodium Hydrosulfite Dithionite:** dithionite is slightly hazardous in case of skin contact and hazardous in case of eye contact or ingestion. Always work in a fume hood wearing a lab coat, eye protection, and gloves. In case of spill – remove all sources of ignition and ventilate area.
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of soap water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
15. **Hydrogen Peroxide (30%): is a strong oxidizer and very hazardous** in case of skin contact, eye contact (irritant). May produce burns and tissue damage, inhalation may lead to coughing and irritation of respiratory tract. The substance is toxic to mucous membranes and lungs. In case of contact, immediately flush area with cold water for 15 minutes and get medical attention immediate. See MSDS for more details.
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of soap water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
16. **Oxalic acid: Serious eye danger.**
    a. **Eyes:** In case of contact – rinse thoroughly for several minutes, remove contact lenses. If irritation persists – call for medical attention.
    b. **Skin:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. f irritation persists – call for medical attention.
    c. **Ingestion:** Rinse mouth thoroughly. Do not induce vomiting. Drink sips of water. Seek medical attention if discomfort persists. Never give anything by mouth to an unconscious person.
    d. **Inhalation:** If inhaled, remove to fresh air.
17. **NH_4 oxalate:** Irritant
    a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
    b. **Skin:** Immediately flush skin with plenty of soap water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
    c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
    d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
18. **Hydrochloric acid: Danger, corrosive and an irritatn**

a. **Eyes:** Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.
b. **Skin:** Immediately flush skin with plenty of soap water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.
c. **Ingestion:** Do not induce vomiting. Get medical aid if irritation or symptoms occur.
d. **Inhalation:** Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.

e. Potential hazards: **See MSDS for above reagents**
f. Approvals required form PI : **YES**
g. Designated Area **Fume Hood**
h. Handling and storage: **Dry chemicals are stored in the dry chemical cabinet behind glass**
i. Personal protective equipment: **Nitrile gloves, eye protection, lab coat, long pants and shoes**
j. Ventilation controls: **Work in Fume Hood**
    i. <u>Large Spill:</u> Call EH&S for assistance.

Materials

- Volumetric flasks
- Scale
- pH meter
- centrifuge tubes, 50 mL
- centrifuge
- orbital shaker
- TP vials (clean, see acid-washing SOP)
- 10-25mL pipette and tips
- Phosphate plate reader
- Freeze-dryer
- filters

Reagents

While working in a fume hood, prepare the following reagents as described and modify volumes as required.

Sodium Bicarbonate – Sodium Hydroxide solution

Sodium hydroxide, 1M (40 g/L)
Sodium bicarbonate, 0.5 M (42 g/L)

- Weigh 1.11 g of calcium chloride in a volumetric flask and dilute to 1 L using DI water
- Create a 0.5 M sodium bicarbonate solution by weigh out 42g of sodium bicarbonate in a volumetric flask and dilute with DI water to 1 liter
- Adjust the pH of the solution to 8.5 with 1M sodium hydroxide. Use pH meter to measure pH value of reagent (calibrate using buffer solutions)

Acetate buffer solution – (from Chen et al. 2006)

Sodium Acetate CH3COO-Na 1M (82g/L)
Acetate acid, concentrated

- Weigh 82 g of Sodium Acetate in a volumetric flask and dilute to 1 L using DI water
- Combine 1 M Sodium Acetate CH_3COONa (82g/L)
- Buffer solution to pH 4 using acetic acid

Magnesium Chloride (Mg_2Cl) Rinse solution (pH 8)

- Magnesium Chloride $MgCl_2$, 1M (95.211 g/L)

Acid oxalate

- 0.1M oxalic acid
- 0.175M NH4-oxalate

1N Hydrochloric Acid

- 41.32 ml of 12.1 N HCl to 500 ml with DI

Sodium pyrophosphate solution (0.1 M)

- 89.2 g made up to 2L

Sodium citrate solution 0.68 M

- 200 g made up to 1L

Procedure

Sediment preparation

1. Freeze dry samples (see SOP for freeze-drying)
2. Weigh 0.5 g of dry sediment into 50 mL centrifuge tubes

Loosely sorbed: removal of exchangeable or loosely sorbed P by formation of $MgPO_4^-$ complex and or mass action displacement by Cl^-

1. Add 50mL 1M $MgCl_2$ (pH8), shake 2h, 25 C
2. Centrifuge for 10 minutes at 3900xg, filter supernatant through 0.45 um filter or use a transfer pipette and aspiration system, **discard first 2 ml**
3. Repeat wash of 50mL $MgCl_2$ (pH8), shake 2h, 25 C
4. Centrifuge as above.
5. Wash **TWICE** with H_2O, shake 2h, 25C
6. Centrifuge as above.

Authigenic carbonate fluroapatite + biogenic apatite + CaCO3 associated P: Authigenic carbonate fluorapatite + Biogenic hydroxyapatite +$CaCO_3^-$ fractions are determined by acid dissolution at

moderately low pH and (or) chelation of Ca^{2+} by acetate. This step is shifted to before the NaOH extraction to separate authigenic P from any P liberated in subsequent extractions that could potential combine with Ca. Note that any weak acid also has the potential to remove acid lysable organic matter.

1. Add 50 mL of 1 M Na acetate buffer to pH 4 with acetic acid shake 6h, 25 C
2. Centrifuge as above
3. Wash **TWICE** with 50mL $MgCL_2$ (ph8), shake 2h, 25 C
4. Centrifuge as above.
5. Wash with H_2O, shake 2h, 25C
6. Centrifuge as above.

Amorphous Al and Fe extractable P

1. Add 20 mL of Ammonium oxalate extraction, agitate in the dark for 2 hours
2. Filter using Whatman No 20 filter paper
3. Wash with H_2O, shake 2h, 25°C
4. Centrifuge as above.
   this extraction cannot be measured by molybdate blue, use ICP-AES or ICP-MS

Detrital apatite P Detrital apatite by acid dissolution. **This step would remove acid soluble organics**

1. Add 50 mL of 1 M HCL, shake 16h, 25 C
2. Centrifuge as above
3. Wash H2O

Fe/Mn oxides Easily reducible or reactive ferric Fe-bound by reduction of Fe^{3+} by dithionite and subsequent chelating by citrate. This step is done later in the sequence then Ruttenburg because of its potential effects on chelated P to organics and after the HCL because citrate is a chelator of Ca (e.g Pettersson 1998).

1. Add 1.125 g of Na-dithionite ($Na_2S_2O_4$) to 45 mL of citrate bicarbonate (0.3M Na_3-citreate, 1.0M $NaHCO_3$ pH 7.6)
2. Add to sediment residue, shake for 8 h at 25 C
3. Centrifuge as above.
4. Wash with 50mL $MgCL_2$ (pH8), shake 2h, 25 C
5. Centrifuge as above.
6. Wash with H_2O, shake 2h, 25C
7. Centrifuge as above.

Analyze using ICP-AES or ICP-MS (citrate interferes with molybdate blue)

*Refractory organic P: D*ry oxidation at 550°C and 1M HCL extraction of residue. Ashing will oxidize all remaining recalcitrant organics, which are subsequently solubilized in HCL for analyes.

1. Weight dry sediment into crucible and ash at 550°C for 4 hours
2. Once cool, add 2 ml of 1 N HCL and 10 mL of DI
3. Cover and place in oven at 104°C for 2 hours

4. After cool, and 2.5 mL of mixed molybdate reagent (See Molybdate Blue method for SpectroMax Microplate)
5. Allow 30 minutes for reaction to occur and measure at 885 nm on the SpectroMax

Bioavailable P

Sodium Bicarbonate – Sodium Hydroxide extraction: this method removes labile alkaline soluble organics (including poly-P), as well as Fe- ,Mn-, and Al- exchangeable, and can partially dissolve Si diatom valves releasing intracellular P. Several studies have demonstrated this extraction is correlated with the bioavailable fraction.

1. Add 30mL the sodium bicarbonate-sodium hydroxide solution to dust residue
2. Shake solution for 16 hours
3. Spin on centrifuge for 5-7 minutes until supernatant is separated from the solid
4. Decant liquid in container labeled with sample name and "NaCO3-P"
5. Rinse sample with 30 mL of 1M $MgCl_2$, add supernatant to NaCO3-P sample
6. Rinse sample with 30 mL of DI water, add supernatant to NaCO3-P sample
7. Analyze sample solution within 24 hours or freeze for later analysis
8. Allow dust residue to dry 1-2 days in a fumehood

Acetate solution extraction

1. Add 30mL of acetate buffer solution to dust residue
2. Shake solution for 16 hours
3. Spin on centrifuge for 5-7 minutes until supernatant is separated from the solid
4. Decant liquid in container labeled "Acet-P"
5. Rinse sample with 30 mL of 1M $MgCl_2$, add supernatant to Acet-P sample
6. Rinse sample with 30 mL of DI water, add supernatant to Acet-P sample
7. Analyze sample solution within 24 hours or freeze for later analysis

- NaCO3-P & Acet-P solutions must be analyzed with the ICP
- Create matrix solutions:
    - Matrix solution for analysis – NaCO3-P
        - 30 mL sodium bicarbonate-sodium hydroxide solution
        - 30 mL $MgCl_2$
        - 30 mL DI water
    - Matrix solution for analysis – Acet-P
        - 30 mL acetate buffer solution
        - 30 mL $MgCl_2$
        - 30 mL DI water

Total P analysis: Determined either through a complete digestion of bulk sediment followed by ICP-MS analyses, or, a summation of the extractants plus the digested residue analyzed for P using ICP-MS.

The exchangeable fraction can be measured on the Lachat QuikChem Analzyer. When possible, other fractions can be measured on the SpectroMax M2E microplate and cuvette reader. When extractants interfere with absorbance or molybdate reactions they must be measured by ICP-AES or ICP-MS.

Determining P concentration using molybdenum blue method (see Phosphate Ascorbic Acid Method)

- Calibrate spectrophotometer using blanks and known concentrations of P
- Add each filter sample to labeled flasks.
- To each flask add .2 mL of molybdate reagent and 0.5mL of ascorbic acid reagent
- Measure absorbance using spectrophotometer

Digesting Samples for TDP analysis –(see Lachat Total Phosphorus Method)

- measure out at least 6mL of each sample into separate triple rinses and acid washed TP vials
- add 500 uL sulfuric acid (5.3M) /24 mL (adjust according to sample volume) to each sample
- replace cap on TP vial and shake the samples
- add 500 uL of persulfate /24 mL (adjust according to sample volume) to each sample
- replace cap on TP vial and shake the samples
- loosen the caps of all TP vials prior to placing samples in the Autoclave
- Cycle samples through the Autoclave (follow preset instructions), do not remove samples until they have cooled

Do not operate without consulting the PI to ensure you understand the material presented in this SOP. Before using the instrument or following the protocol, sign below.

Documentation of Training (signature of all users is required)

I have read and understand the content of this SOP:

Name	Signature	Date

Brothers: Field Sampling Methods and Documentation

Recommended Standard Operating Procedure for Near-Shore Sediment Core Retrieval and Macrophyte Identification, Soren Brothers, Utah State University

Scope and Applicability

This standard operating procedure (SOP) has been compiled specifically for work to be carried out on Utah Lake, within the project entitled "Paleolimnology and Paleoecology of Utah Lake" (principal investigator, Dr. Janice Brahney, Utah State University). This SOP specifically pertains to a portion of this project being directed by Dr. Soren Brothers (USU), investigating the historical presence and extent of near-shore submerged macrophyte communities in the lake. **Prior to any sample collection**, contact Dr. Brothers (BNR 269, 435-797-4152) to ensure that all appropriate field and laboratory preparations have been carried out.

Summary of Methods

The primary purpose of this sampling is to determine if, and where, submerged macrophyte species occurred in Utah Lake (UT). Submerged macrophytes live fully underwater, and thus require ample light transmission through the water column to support their growth. As water clarity is closely linked with water quality in many lakes, the historical presence of submerged macrophytes can thus be used to provide evidence of the history of Utah Lake's water quality. While submerged macrophytes have rarely been observed in Utah Lake in recent decades (J. Gaeta, pers. comm.), their presence has been historically described (Brotherson 1981; Miller and Crowl 2006). However, knowing the extent of their historic range may provide valuable information for lake restoration targets: wind-driven resuspension is widely considered to be a major driver of the high turbidity of Utah Lake (contemporary Secchi Depths are typically < 0.3 m), though benthic algae and submerged macrophytes can also reduce such resuspension events (James et al. 2004; Vignaga 2012). Understanding whether historic macrophyte communities existed solely within wind-protected areas of Utah Lake (such as Provo Bay), or in the main body of the lake, may thus have far-reaching implications for the historical ecological state of Utah Lake, as well as the potential for success in various recovery options.

Here, we focus on the submerged macrophyte species *Chara aspera*, which historically lived in Utah Lake (Brotherson 1981). *Chara spp*. macrophytes produce distinctive macrofossil oospore remains which can be preserved in the sediment records of lakes, and are considered good indicators of historical clear-water conditions in lakes (Lambert-Servien et al. 2006). Being relatively heavy, macrophyte oospores are typically buried at the site of the macrophyte communities (pers. comm., S. Hilt). Assuming that Utah Lake's *C. aspera* communities were most likely concentrated in shallow, near-shore areas, near-shore cores are thus necessary for identifying the preliminary spatial extent of this species in Utah Lake. Unfortunately, near-shore conditions (including wave action and emergent macrophyte effects on sediments) are not ideal for sediment core dating. This SOP thus focuses solely on the retrieval of near-shore sediment cores in Utah Lake, and the top-bottom laboratory analysis of those cores for macrophyte macrofossil oospores.

Health and Safety Warnings

Field sampling, even under near-shore conditions (i.e. without involving the use of a boat) can be hazardous. Special precautions must therefore be taken by each member of a field campaign to ensure the

safety of all field participants. Prior to entering the field, Dr. Brothers will share with any field assistant(s) a field safety plan describing potential hazards associated with field work, including emergency contact information for all participants and advance knowledge of the nearest medical services, if necessary. In accordance with Utah State University (USU) and the Department of Watershed Sciences policy, an emergency first-aid kit will be taken to the field. As some of the proposed sampling sites on Utah Lake are likely to be more than 30 minutes from the nearest medical services or out of cell phone range, an emergency inReach (Garmin) GPS device will be brought along to call in case of emergency. The primary safety concerns associated with near-shore sampling will likely be related to the weather; upon any indication of a storm and/or lightning, all field researchers should immediately leave the water and return to the safety of a vehicle. Harmful algal blooms also frequently occur on Utah Lake. Immediately prior to departure for collecting field samples, the online water quality monitoring advisory (https://deq.utah.gov/water-quality/utah-lake-algal-bloom-monitoring-2019) must be checked, and its guidelines followed. If no harmful algal bloom has been reported and field work proceeds as planned, precautions should regardless be taken to properly wash off any part of the body which comes in contact with Utah Lake, especially before eating.

Equipment and supplies

Field Campaign

__ Copy of this SOP

__ Chest waders (2)

__ Plastic coring tubes (3)

__ Top/bottom caps/plugs fitted to tubes (6)

__ Fitted core extruder (1)

__ GPS Device (1)

__ Camera (1)

__ Measuring tape/ruler (1)

__ Duct tape (1)

__ “Rite in Rain” Field Notebook + pen (1)

__ Battery-powered scale (1)

__ Cooler + ice packs (1)

__ Core slicer/paddle (1)

__ Russian peat corer (1)

__ Heavy duty work gloves (2 pairs)

__ Whirl-Pak bags + marker (30)

__ Squeeze bottle w. distilled water (1)

Laboratory Analysis

__ Dissecting microscope (1)

__ Glass/plastic sampling/Petri dish (1)

__ Spoon (1)

__ 100 μm mesh strainer (1)

__ 400 μm mesh strainer (1)

__ Whirl-Pak bags + marker (30)

__ Squeeze bottle w. distilled water (1)

__ Notebook + pen (1)

__ Scale (1)

Procedure

Core retrieval

General sampling locations around Utah Lake have been selected for 1) road accessibility and 2) representation of multiple lake bays and regions. However, upon arrival at each location, a detailed assessment should be made to ensure that it is appropriate for the task. Ideally, a location should be identified where direct bioturbation (from boat traffic and/or recreational swimmers, if present) is minimal, ***and*** there are no/minimal emergent macrophytes (Phragmites, etc.). Prepare a site on shore for sample processing (i.e. cooler, Whirl Pak bags, spoon) before entering the water. Field campaign members who are tasked with retrieving the cores must wear hip or chest waders, and take care to minimize disturbance of the sediment surface with their steps. Locate two sites (they do not necessarily need to be side by side) where an appropriate sediment surface is 30-50cm below the water surface, record the GPS location of each site, and photograph each, including a field location indicator (i.e. notebook with field station number, etc.) in the frame of the photograph so that it can be properly identified later. As the sediments may be thick with roots and difficult to core, work gloves can be helpful in forcefully pushing the cores into the sediments. If 50cm cores cannot be retrieved due to sediment resistance, a Russian peat corer can be used, which is designed to slice through rhizomatous sediments. Carefully return each core to the lake shore sample processing site. Before processing a core, photograph it and measure the depth of the sediment column retrieved (which should be in the vicinity of 50cm).

Core processing

From each retrieved core, the top and bottom 2-cm intervals should be transferred to clean Whirl-Pak bags, each ***pre-labeled*** with detailed sample ID information (Sampling Date, Sample Location ID, Core ID, Top/Bottom). Carefully place the core on the extruder/stand, and lower the core gradually, allowing any overlying water to spill over until the surface of the sediment-water interface is at the top of the core. At this point fit the core slicer/cup to the top of the core, and extrude the top 2cm of the core,

capturing the sediments and transferring them into the appropriate pre-labeled Whirl-Pak bag. Weigh the bag on the field scale, and if it weighs less than 150g, add 1-cm sediment intervals until it weighs approximately 200g. Seal the Whirl-Pak bag, record its weight and the number of core cm contained in it, and place it in the cooler. Thoroughly rinse off the core slicing/cup apparatus with distilled water to avoid contamination of deeper sediment layers. For the bottom sediment interval, continue to extrude the remainder of the sediment core until 2-cm before the plug at the bottom of the core. At this point fit the core slicer/cup to the top of the core, and extrude the bottom 2cm of the core, capturing the sediments and transferring them into the appropriate pre-labeled Whirl-Pak bag. Record the weight of this Whirl-Pak bag + sediments, seal the bag, and place it in the cooler. Upon return to USU, store all samples in a fridge in the Brothers Laboratory (BNR 048).

Laboratory sample processing

The goal of this process is to identify *Chara spp*. oospore macrofossils in the retrieved sediments. Once an appropriate and available dissecting microscope has been secured, prepare a lab notebook, sampling/petri dish, and work area for the samples to be analyzed on that date. Ensure that the sampling/petri dish contains a grid pattern (or create one, using a marker) so that a systematic analysis can be carried out. At a sink, nest together the 400 (top) and 100 (bottom) μm sieves. Transfer 1-2 spoonful of sediments to the top (400 μm) sieve, and run under a low-flow tap. Gently spread the materials around under the flowing water until only the 400 μm size fraction remains. Set aside that sieve, and carry out the same procedure on the 100 μm size fraction. Transfer the 100 μm size fraction of the sediments with a clean spoon into fresh ***pre-labeled*** Whirl-Pak bags with detailed sample ID information (Sampling Date, Sample Location ID, Core ID, Top/Bottom, 100 μm fraction)- these will be stored for potential future analyses of smaller size-fraction macrofossils. Transfer the 400 μm size fraction of the sediments with a clean spoon into the sample/petri dish, and systematically examine each grid of the dish for macrofossil remains. Identification of these remains will be initially trained by Dr. Brothers. Using a scale to determine how much of the sample is being processed (and recording that in your notebook), repeat the above procedures until approximately half (~100g) of each sediment sample has been analyzed, recording how many oospores are present in each sample. Upon completion, record the new weight of each sample + Whirl Pak bag, and return all samples to cold storage for potential later analyses.

Data and records management

Students working will be required to keep detailed lab notes of the progress of their work, and all data generated by these analyses will be stored and backed up on multiple computers and/or hard drives, and will be available upon request. Upon completion of the project, the results of these objectives will be included in the official report to the Division of Water Quality.

References

Brotherson, J. D. 1981. Aquatic and Semiaquatic Vegetation of Utah Lake and Its Bays. Gt. Basin Nat. Mem. **5**: 68–84. doi:10.2307/23376547

James, W. F., E. P. Best, and J. W. Barko. 2004. Sediment resuspension and light attenuation in Peoria Lake: Can macrophytes improve water quality in this shallow system? Hydrobiologia **515**: 193–201. doi:10.1023/B:HYDR.0000027328.00153.b2

Lambert-Servien, E., G. Clemenceau, O. Gabory, E. Douillard, and J. Haury. 2006. Stonewords (Characeae) and associated macrophyte species as indicators of water quality and human activities in

the Pays-de-la-Loire region, France. Hydrobiologia **570**: 107–115. doi:10.1007/978-1-4020-5390-0

Miller, S. A., and T. A. Crowl. 2006. Effects of common carp (Cyprinus carpio) on macrophytes and invertebrate communities in a shallow lake. Freshw. Biol. **51**: 85–94. doi:10.1007/s00198-005-1915-3

Vignaga, E. 2012. The effect of biofilm colonization on the stability of non-cohesive sediments. PhD Thesis 281.

Brothers: Laboratory Sampling Methods and Documentation

Sediment samples will be analyzed at USU. Sediments will be analyzed using a top/bottom investigation for macrophyte macrofossil remains. A small amount (1 teaspoon) of material will be sieved and washed through 400 μm and 100 μm mesh sizes, with materials being retained from both size fractions. Most macrophyte macrofossil remains are expected to be found in the 400 μm size fraction (S. Hilt, pers. comm). A dissecting microscope with a white background will be used to scan for macrophyte remains. Judging from Utah Lake literature (Brotherson 1981, Miller and Crowl 2006), the lake's historic submerged macrophyte community included *Ceratophyllum spp*., *Chara spp*., and *Potamogeton spp*., among some others which we expect to be less common. Although any apparent identified macrofossil remains will be reported, particular attention will be paid to *Chara spp*., as it has been used elsewhere as a key macrophyte for indicating clear-water conditions in lakes (Lambert-Servien et al. 2006). For each analyzed sediment subsample, the frequency of macrofossil remains will be documented (i.e. counts of each species macrofossil remains identified per gram of material analyzed), and any unanalyzed sediments will be stored for future possible analyses. Cores from which top-bottom analyses show no macrophyte remains will be assumed to be taken either from locations which did not contain the relevant submerged macrophytes, coring depths were insufficient to reach a historic macrophyte layer, or wave action may have destroyed macrofossil remains. Cores from which top-bottom analyses show macrophyte remains in both top and bottom samples (for submerged macrophyte species which are not known to occur in the lake today) will be assumed to have mixed from sediment resuspension/turbation.

Brothers: Historical production modeling

The resolution of Objective 1, determining the historical presence or absence of submerged macrophytes will be accomplished following the above-described procedures. Simply identifying the historic presence of submerged macrophytes in lake sediments will be a key output of that work, though we also plan to glean from that data (including the macrophyte species identified, the depth and location of sediment cores, etc.) information on potential historical water clarity. For Objective 2, phytoplankton productivity will be calculated using measured values and historic estimates (partially derived from Obj. 1) of water clarity, Chlorophyll *a* concentrations, and phytoplankton photosynthesis-irradiance (P-I) curve characteristics (taken from either literature or laboratory measurements) following established phytoplankton productivity models (derived from Fee 1973), and using the R package "Phytotools")(Silsbe and Malkin 2015). Benthic (periphyton) primary production rates are typically only limited by light, and not nutrients. These rates will therefore be calculated using light-based models applied to the bathymetry, seasonal water clarity, and water depth of Utah Lake, again following established models (Vadeboncoeur et al. 2008, Brothers et al. 2016, 2017), applying benthic algal P-I curve values from the literature and/or laboratory measurements. Students working will be required to keep detailed lab notes of the progress of their work, and all data generated by these analyses will be stored and backed up on multiple computers and/or hard drives, and will be available upon request. Pairing Objectives 1 and 2, we anticipate a more detailed output of the historic water quality condition of Utah Lake, with potential analytical outcomes including, for instance, non-linear responses of benthic primary productivity (and thus a positive water-quality feedback) with increasing modeled water clarity, providing potential guidelines of use for lake management agencies.

Power: Field Sampling Methods and Documentation

Power Paleoecology Lab Procedures: Mitchell Power NHMU 4th floor 801-581-6520

The frozen sediment core will be extracted using dry ice and 90% Ethanol to create a super-freezing reaction, allowing near-surface sediments (the upper ~50 cm) to adhere to the outside of the stainless steel freeze core device.

Safety Information

Safety is critical during field and laboratory research. The safety protocols followed by the Power Paleoecology lab are similar to those details listed above by Dr. Brahney above. All participants will receive a safety orientation prior to participation on the project and are required to sign a University of Utah waiver prior to participating in any fieldwork activities. All partipants will wear Type I and Type II US Coast Guard approved life jackets at all times during fieldwork on or around Utah Lake. Daily and hourly forecast will determine the suitability of conditions for fieldwork.

Field Materials

- Frozen Sediment Corer
    - 50-80 lbs of Dry Ice
    - 7-8 liters of 95% ethanol
    - 20-30 meters of plastic coated cable
    - Double insulated cooler with addition 20-30lbs of dry ice for transporting samples
- Flat edge scraper for removing core from stainless steel plates
- Aluminum foil for wrapping and labeling frozen core
- Leather and rubber gloves for handling frozen core and dry ice
- 30 m tape and digital fish finder to measure and record water depth.
- Electrical and masking tape
- Spatula and chisel for detaching frozen core from device
- Rags and cloths for cleaning up after extraction
- Whirl-paks for samples
- Field notebook
- GPS Unit

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*The Power paleoecology lab also uses a livingstone/piston core-sediment extraction system and employs similar field- and lab- based methods as outlined above by Brahney.

Field Methods

As stated by co-PIs Braney and Brothers, prior to departing for fieldwork, safety protocols include reviewing weather forecast and lake conditions. Target sediment coring locations identified from satellite imagery prior to fieldwork, will be located with GPS devices and coring extraction site will be recorded.

Collecting the Frozen Sediment Core

Approximately 15-20 lbs of dry ice is first pulverized into a powder form and then poured into the top of the freeze core device. A “super-freezing” reaction is accelerated by adding 1 liter of 95% ethanol to the powdered dry ice inside the freeze core. As the ethanol and dry ice mix, CO_2 gas is released. The freeze core device has a CO_2 spring-activated release valve that allows pressure to leave the freeze core once the lid has

been closed on the device. The freeze core is then lowered into the lake on a steel cable and plunged into the bottom sediments. Depending on sediment type and compaction, a 30-50 cm penetration depth is expected, where the freeze core remains stationary for 25 minutes. After 25 minutes, the frozen core is extracted from the bottom and loose, unfrozen sediment is scraped from the surface and edges of the device, and the remaining frozen sediment "slabs" are carefully removed from the stainless steel plates on the outside of the freeze core. These are measured for depth, color changes and any other notable features, labeled top and bottom, wrapped in aluminum foil and stored in the cooler with dry ice to keep them frozen for transport back to the lab at NHMU.

Power: Laboratory Sampling Methods and Documentation

Before using the Power Paleoecology lab, all participants are required to be trained by Power prior to beginning laboratory analysis. The Power paleoecology lab is equipped with an emergency eyewash station, face shields, heavy-duty rubber gloves and rubberized bibs, as well as laboratory coats, nitrile gloves, and safety glasses. The lab is equipped with a state-of-the-art perchloric acid fume hood that is inspected quarterly by University of Utah maintenance for airflow and exhaust performance.

Fume Hood Operations

First, check to be sure the fume hood monitoring system was recently calibrated before beginning. The Power Paleoecology Lab uses a relatively new (2013 model) PVC-lined Perchloric Acid Fume Hood with an airflow monitor. The PVC inner shell allows for ease of maintenance and cleaning and eliminates the opportunity for acid salt to build-up. The PVC liner is susceptible to heat damage and all heat emitting processes, including the hot plate, are required to be a minimum of 6" away from the interior fume hood walls. The inside of the hood has a safety orange tapeline indicating 6" buffer from the walls.

The fume hood has preset heights that lock the sliding window at the required height to optimize are flow through the system. The monitor has a safety alarm on the hood that will sound if the airflow is not sufficient. The fume hood is also equipped with an emergency purge button that will also sound an alarm. The monitor light may appear in three colors; green = everything is fine, yellow= use caution and inspect airflow, red= danger and discontinue use. An emergency alarm will sound when the red light is on.

Perchloric Acid Fume Hood Check list

- Turn the lights and the blower on, raise the sash, check for any obstructions and let the blower run for 5 minutes.
- Load your work materials into the hood, do not obstruct the air baffles, let everything sit for a minute after the hood is loaded to let the system purge.
- Keep work materials at least 6" inside the hood, they recommend segregating your clean and contaminated operations.
- When done, let the blower run for a few minutes to purge contaminants before shutting the blower off
- Unload the work materials and clean the hood.
- Wash the hood down after every use and a thorough wash down for 10 minutes at least once a day.
- Turn everything off and close the sash.
- Post the sign on the lab door "Pollen Processing - Do Not Disturb" and avoid going in the out of the room when the hood is in use.

- The use of hydrofluoric acid in this hood, requires due diligence and careful cleanup (e.g. wiping all surface) following sample processing.

Sub-sampling the Sediment Core

Once back at the lab, one of the frozen sediment cores will be sub-sampled for pollen and charcoal analysis. Sampling resolution will be adjusted based on preliminary chronology (see Brahney above for protocols) and knowledge of sediment accumulation rates (cm/yr). Sediment subsampling will range from ~1 mm-thick sample intervals to 0.5 cm – thick sample intervals, depending on type of proxy (e.g. charcoal versus pollen) and preliminary age-depth models.

Pollen Processing Protocols

- Fill out the pollen processing form (put a copy in white notebook in wet lab when processing complete).
- Label vials (use small white labels, fine point sharpie, put a piece of scotch tape over label) and place a few drops of silicon oil in each.
- Fume hood should be on; turn on light inside fume hood.
- Set up WASTE BEAKER - large beaker with a scoop of baking soda in the bottom.
- Place dishtub in sink with ~ 2 tbsp. baking soda, a few drops of dish detergent, and water.
- Place pan with water and tube rack in it on hot plate; turn hot plate on, set to 10.

STEPS 1-25 completed only by trained personnel. These methods are modified from Faegri and Iverson (Faegri et al. 1989).

1. Add 10% HCl to 2 ml to lycopodium tablet and your 1cc sediment sample. After it is done reacting, fill to 10 ml with HCl. If your sediments react strongly to first contact with HCl wait until the reaction subsides and add more HCl in small increments. If the sample threatens to overflow, add a few drops of ETOH to slow the reaction.
2. C & D (1800 rpm for 2 minutes) HCl into WASTE BEAKER with baking soda to neutralize.
3. Add distilled water to 2 ml, vortex, fill to 10 ml with distilled water, C & D into WASTE BEAKER as above.
4. Add 10% KOH to 2 ml, vortex, fill to 10 ml with KOH, place in hot water bath for 10 minutes. Stir GENTLY with wooden stir rod.
5. Wash samples with distilled water through 150 micron screen into a numbered beaker that matches your numbered tubes. This step and step number 6 may be done on the countertop. Save or discard material on top of screen.
6. Transfer solution from numbered beaker to numbered 50 ml tube using distilled water, C & D (1800 rpm for 2 minutes) until transfer from beakers is complete (you can decant down the drain at this point, it is a very dilute solution). **IMPORTANT:** Fill 50 ml tubes to no more than 40 ml; use large sleeves and hub in the centrifuge and CENTRIFUGE ONLY 6 AT A TIME USING THE OUTSIDE SLEEVES ONLY. Vortex between each C & D; CONTINUE DISTILLED RINSE WITH C&D until the decanted liquid is the color of very weak tea; record number of rinses on processing sheet.
7. When in 50 ml tubes, vortex tube to loosen plug. Add a small amount of HCl, vortex, transfer to 15 ml tubes with HCl, top off to 10 ml with HCl, C & D into WASTE BEAKER to neutralize, then down the drain.

8. Add a few drops HF, stir with wooden stir rod or vortex, add a few more drops. Fill all tubes to same level with HF (5-8 ml). If you use wooden stir rods, neutralize with baking soda before placing in garbage.
9. Place tubes in hot water bath for 60 minutes. Remove and let cool for 10 minutes. Add distilled water to 10 ml, then C & D into PLASTIC WASTE CONTAINER.
10. Place TBA large bottle and small bottle close to the hotplate to liquefy the chemical. Don't melt the bottles!
11. After decanting the HF, add distilled water to 2 ml. Vortex, fill to 10 ml with distilled water and C & D into PLASTIC WASTE CONTAINER.
12. Add glacial acetic acid to 2 ml. Vortex, fill to 10 ml with glacial acetic, C & D into GLASS WASTE BOTTLE.
13. Place the small tube rack in the pan and turn the hot plate to 10 for acetolysis. Pour about 8 ml of concentrated sulfuric acid into a small beaker; keep the beaker covered with a watch glass. Have your pipette and pipette pump ready for step 15. Time is of the essence for acetolysis - perform steps 14-17 quickly and efficiently.
14. Add 5 ml of acetic anhydride to your first 6 samples using the repeating pipette (glass bottle with funny neck). NOTE: Hold the tubes in your hand, do not attempt to use repeating pipette on tubes in a rack.
15. Using a pipette with the pipette pump, add **0.55** ml of concentrated sulfuric acid to each sample.
16. Stir each sample with its own clean, DRY, GLASS STIR ROD and put all 6 samples into the hot water bath at the same time for 2.0-2.5 minutes. NO LONGER THAN THAT OR THEY'LL GET COOKED! Note: Leave the glass stir rods in the test tubes for use in step 17.
17. Take all samples out of the hot water bath in the same order you put them in, immediately add a generous squirt of glacial acetic acid to each to stop the reaction. Fill to 10 ml with glacial acetic acid, stir gently with the glass stir rod, C & D into GLASS WASTE BOTTLE.
18. Repeat steps 14, 15, 16 & 17 with remaining 6 samples.
19. Add glacial acetic acid to 2 ml in all samples, vortex, fill to 10 ml, C & D into GLASS WASTE BOTTLE.
20. Place 2 to 4 drops of stain in each sample, depending on how much sample is left. Add distilled water to 2 ml, vortex, fill to 10 ml, C & D into WASTE BEAKER.
21. Add 95-100% ETOH to 2 ml in all samples, vortex, fill to 10 ml, C & D into GLASS WASTE BOTTLE. ****IF YOU NEED TO NITEX, STOP HERE AND GO TO NITEXING INSTRUCTIONS ****
22. Add TBA to 2 ml in all samples, vortex, fill to 10 ml with TBA, C & D into GLASS WASTE BOTTLE.
23. Add TBA to 2 ml in all samples, vortex, C & D into GLASS WASTE BOTTLE.
24. Use disposable pipettes and a small amount of TBA to transfer samples to labeled vials (be sure they contain a few drops of silicon oil). Set samples inside hood and lower sash to allow air draw to evaporate off the TBA, or place them in the dessicator. Be sure your samples are not drying out (if they start to "cake" there is not enough silicon oil, add a few more drops – but not too much!).
25. Clean up:
    - Wash all equipment in the soapy/baking soda water. Rinse with tap water - ANY EQUIPMENT THAT COMES IN CONTACT WITH SAMPLES MUST BE RINSED AGAIN WITH DISTILLED WATER (tubes, beakers, glass stir rods, 150 micron screen, etc.). Be sure to wash and rinse all centrifuge rotors, metal sleeves, brackets and plugs. Put dry equipment away the next day.
    - Re-fill all reagent bottles in the hood. (The 10% HCl is in a small carboy under the sink.) If any reagents under the hood are getting low, notify Andrea! Make sure there is enough room

in the waste bottles for the next person processing. (Save all empty glass reagent bottles for use as waste.)

- Wipe out the hood and centrifuge.
- Wipe off countertop, sweep and mop the floor (use baking soda and a few drops dishsoap in the water).
- Wipe apron with dishwashing sponge. Wash lab coat at home with mild detergent and a little baking soda – DO NOT USE BLEACH. Please return clean lab coat to lab within 2 days.

Sedimentary Charcoal sample preparation using 5% KOH

Begin by preparing volumetric subsamples from the sediment core (e.g. 0.25 to 0.5 ml volume) by placing subsamples into a 15ml plastic test tube.

1. Remove 12 – 15 ml test tubes from the rack (check for numbers 1-12) and rinse them with distilled water
2. Remove 12 – 250 ml beakers from cabinet and rinse with distilled water
3. Fill the two glass beakers in fume hood 2/3 full of water and turn hotplate to 4
4. Check if sample weights have been recorded
5. Transfer sample from whirlpak to 50(15) ml test tube with KOH, give the whirlpak a final rinse with distilled water (be careful not to use more than ~ 45 ml in the transfer)
6. ******Label the whirlpak bag with the corresponding number on the test tube***
7. Place test tube samples in hot water bath for 10 to 15 minutes, stirring occasionally with a wooden stirring stick
8. Remove the samples from hot water and pour directly into the 125 micron sieve with bottom pan attached (*NOTE: carefully rinse the 125 micron sieve and bottom pan with distilled water between each sample)
9. Transfer entire contents of 15 ml test tube with distilled water into 125 micron sieve. Begin washing sediment through sieve using smaller distilled water bottle for greatest water pressure
10. As the bottom pan fills with water, transfer the contents into a plastic 250 ml beaker with the corresponding number as the test tube until sieve is mostly clean
11. Using distilled water, rinse the contents of the 125 micron sieve (now mostly charcoal and macrofossils) into the original sample bag
12. Pour the contents of the 250 ml beaker back into the 50 (or 15) ml test tube and place in centrifuge for four minutes. Continue centrifuging and decanting (decant in white waste bucket in fume hood after adding a small amount of baking soda to bucket, this may be dumped into a waste disposal container, stored in the locked cabinets below the fume hood). University of Utah Health and Safety personel will be notified to remove the waste containers once they are full.
13. Create new whirlpaks (preferably the smaller ones) with same labeling information as original, but add “KOH Wash” to label.
14. Transfer the sediment plug (using as little distilled water as possible) into the newly labeled whirlpak with distilled water
15. Upon completion, wash all test tubes and beakers and wipe down all counter tops. Leave the workspace cleaner than before.

References

BOLLAND, R. F. 1974. Paleoecological interpretation of diatom succession in recent sediments of Utah Lake. Unpublished dissertation, Univ. of Utah.

BRAHNEY, J., J. J. CLAGUE, B. MENOUNOS, AND T. W. D. EDWARDS. 2008. Geochemical reconstruction of late Holocene drainage and mixing in Kluane Lake, Yukon Territory. Journal of Paleolimnology 40:489–505.

BROTHERS, S., G. KAZANJIAN, J. KÖHLER, U. SCHARFENBERGER, AND S. HILT. 2017. Convective mixing and high littoral production established systematic errors in the diel oxygen curves of a shallow, eutrophic lake. Limnology and Oceanography: Methods 15:429–435.

BROTHERS, S., Y. VADEBONCOEUR, AND P. SIBLEY. 2016. Benthic algae compensate for phytoplankton losses in large aquatic ecosystems. Global change biology 22:3865–3873.

BROTHERSON, J. D. 1981. Aquatic and semiaquatic vegetation of Utah Lake and its bays. Great Basin Naturalist Memoirs:68–84.

BUSHMAN, J. R. 1980. The Rate of Sedimentation in Utah Lake and the use of Pollen as an Indicator of Time in the Sediments. Brigham Young University.

FAEGRI, K., P. E. KALAND, AND K. KRZYWINSKI. 1989. Textbook of pollen analysis. John Wiley & Sons Ltd.

FEE, E. J. 1973. A numerical model for determining integral primary production and its application to Lake Michigan. Journal of the Fisheries Board of Canada 30:1447–1468.

JAVAKUL, A., J. A. GRIMES, AND S. R. RUSHFORTH. 1983. Diatoms in sediment cores in Utah Lake, Utah, USA. Hydrobiologia 98:159–170.

LAMBERT-SERVIEN, E., G. CLEMENCEAU, O. GABORY, E. DOUILLARD, AND J. HAURY. 2006. Stoneworts (Characeae) and associated macrophyte species as indicators of water quality and human activities in the Pays-de-la-Loire region, France. Pages 107–115 Macrophytes in Aquatic Ecosystems: From Biology to Management. Springer.

LEAVITT, P. R., AND D. A. HODGSON. 2002. Sedimentary pigments. Pages 295–325 Tracking environmental change using lake sediments. Springer.

MILLER, S. A., AND T. A. CROWL. 2006. Effects of common carp (Cyprinus carpio) on macrophytes and invertebrate communities in a shallow lake. Freshwater Biology 51:85–94.

MUSTAPHI, C. J. C., J. BRAHNEY, M. A. AQUINO-LÓPEZ, S. GORING, K. ORTON, A. NORONHA, J. CZAPLEWSKI, Q. ASENA, S. C. PATON, AND J. P. BRUSHWORTH. 2019. Guidelines for reporting and archiving 210Pb sediment chronologies to improve fidelity and extend data lifecycle. Quaternary Geochronology.

SILSBE, G. M., AND S. Y. MALKIN. 2015. phytotools: Phytoplankton Production Tools. An R package available on CRAN: https://cran. r-project. org/web/packages/phytotools/index. html.

VADEBONCOEUR, Y., G. PETERSON, M. J. VANDER ZANDEN, AND J. KALFF. 2008. Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89:2542–2552.