**SAMPLING ON LAKE 222 AND 221 –BACKGROUND SAMPLES FOR LENS**

*Dried filters have either been run (data in LENS folder), packed (trays labeled and sample IDs in LENS folder), or are in the cardboard filter box that I showed Andrew before I left. Liquid samples are either frozen (in walk in freezer) or in the walk in fridge. These samples do not include those from graduate or undergraduate student projects – they were responsible for their own samples. I made copies of all of the relevant pages from my lab notebook and put them in the Whole Lakes binder. Field data is in the yellow books. – beth*

**Sampling in 2012**

* Pelagic samples were taken from the epilithon at center buoy on the following dates: 30 May 2012, 19 June 12, 09 July 2012, 30 July 2012, 14 Aug 2012, 17 Oct 2012. There was also a sampling trip in Feb 2013. Samples include depth profiles (temp, light, conductivity, DO, secchi depth) as well as dissolved nutrient concentrations (TDN, TDP, DOC, NH4, NO3), seston C, N, P, chlorophyll a, bacterioplankton C, N, P, chlorophyll a, algal counts, and pigments. DNA samples were taken for the later sampling dates. CTC and leucine uptake assays were also conducted on most sampling days. The hypolimnion was sampled on 30 May 12, 14 Aug 12, and 17 Oct 12. Rotifer samples (abundance, CNP) were taken for the 1st sampling date, but zooplankton only for the rest (lost the rotifer net). The metalimnion was sampled on 14 Aug 12 for dissolved nutrients only.
* Background Ag samples (TAg, DAg, seston Ag, BP-Ag, 1.2-0.2Ag, 35-1.2Ag, and zooplankton Ag) were taken on 19 June 12, 30 July 12, and 17 Oct 12.
* Samples for natural abundance 15N and 13C were taken from the epilimnion and hypolimnion on 14 Aug 12. Samples include seston, bacterioplankton, water, and zooplankton. Water samples were taken from the epilimnion only. The 15N water samples are frozen and the 13C water samples are in the fridge.
* A single sediment trap with three collection cups was placed near center buoy in each lake on 29 May 12 (check this date in the yellow book). The traps were changed on 29 June 12, 03 Aug 12, 24 Aug 12, and 12 Oct 12. Each cup was treated as a rep (A, B, C) and sampled for: C, N,P, and Ag. Note: the filtering volumes are in the whole lake binder…copied from Beth’s notebook.
* Data loggers were installed in each lake at the beginning of the summer (check yellow book for dates). HOBO temperature loggers were hung from center buoy at 1 m depth. Pressure transducers were installed in the littoral to record depth. The locations of these pressure loggers should be on the bathy maps in the whole lakes binder. But in case they are not, for L222, the logger was placed about 10 m along the shore to the right of the dock (facing the lake) and for L221, the logger was placed about 25-30 meters along the shore to the left of the dock. HOBOs and level loggers were removed on 26 Aug 12.
* Conducted an uptake and nitrification assay in both L221 and L222 using 1L bioassays. Spiked whole water (no dilution) with 15NH4. Some bags clear, others darkened. Allowed to incubate in situ overnight. Filtered for seston uptake (2 GFF filters), bacterioplankton uptake (2 GFF filters), and 1.2 um filter. These samples are dried, but not processed. Water samples were frozen but never processed. Spiking information and filtering volumes are with Beth.
* Littoral macroinvertebrate samples were taken according to the OBBN protocol on 21 Aug 12 for L221 and 22 Aug 12 for L222. See yellow book for sampling times. Most locations are flagged in the field and all are marked on the bathy maps in the whole lake binder. Several individuals from representative groups were frozen for isotope natural abundance.
* Organic matter decomposition was measured in 8 lakes, including 222 and 221. Measured the rates of decomposition for 3 organic matter types: sugar maple leaves, dried macrophytes collected from L239, and tongue depressors as a proxy for woody debris. Pre-weighed packs of organic matter were installed in the littoral areas of each lake in one location, in direct contact with the bethos. Packs were made from 1 mm window screen. Packs were installed on 07 June 12 and collected on 28 June 12, 19 July 12, 09 Aug 12, and 26 Aug 12. Sugar maple packs were also collected on 30 Oct 12. Beth has raw data.
* Macrophyte samples were collected from both lakes on 15 Aug 12. Three transects per lake were selected and are labeled on the bathy maps. Transects were selected to have a high macrophyte biomass. 5 quadrats of 25 cm x 25 cm were randomly selected within each transect. All above sediment biomass was removed and dried. Samples are in paper bags in the computer room.
* Periphyton samples were collected from both 221 on 15 Aug 12 by Paul Frost. Three samples were collected by removing periphyton from a known area of rock. See Paul for details. Samples were processed for CN, P, chlorophyll a, Ag, and 15N/13C natural abundance. Dan Braun was responsible for the filtering data. Beth only has the data from L221 (which should also be in the binder). No idea where the L222 data are.
* Excretion rates were measured for yellow perch in L222 on 07 Aug 12. There are no perch in L221, so L239 was used as a reference. Fish were caught via seining, weighed and measured, and placed in bags of filtered lake water for ~30 min. Weight and length data are in the binder (copied from Beth’s notebook).
* Sediment cores were taken from both lakes on 16 Aug 12. 5 cores were taken from around the lake, the 1st 9 cm were dried. An Ekman grab was also taken from center bouy from each lake on 22 Aug 12. Samples were preserved in ethanol and are in mason jars.
* Several graduate and undergraduate projects were conducted in L221 or L222.
  + Bioassay conducted by Graham Blakelock in several lakes, including L222. Used 1 L bioassays to investigate the interactions between P and Ag on bacterioplankton growth and stoichiometry. Graham has data and study included in his masters thesis.
  + Bioassay conducted by Dan Braun in several lakes, including L222. Used nutrient diffusing substrates to investigate the interactions between P and Ag on periphyton growth and stoichiometry. Beth and Paul have the data.

**Sampling in 2013**

* Mike Rennie and Beth proposed a drip site for L222 and a pseudo drip site in L221. Sites are marked on bathy maps, but are pretty much opposite the outflows.
* Pelagic samples were taken from the epilithon at center buoy on the following dates: 03 June 13, end of June 13 (check yellow book for date), 02 Aug 13, end of Aug 13 (check yellow book for date), and end of Oct 13 (check yellow book for date). Samples include depth profiles (temp, light, conductivity, DO, secchi depth) as well as dissolved nutrient concentrations (TDN, TDP, DOC, NH4, NO3), seston C, N, P, chlorophyll a, bacterioplankton C, N, P, chlorophyll a, algal counts, pigments and DNA samples. CTC and leucine uptake assays were also conducted on most sampling days. The hypolimnion was sampled on the same dates. Rotifer and zooplankton samples collected on each date.
* Background Ag samples (TAg, DAg, seston Ag, BP-Ag, 1.2-0.2Ag, 35-1.2Ag, and zooplankton Ag) were taken on June sampling dates. TAg only was sampled on Aug dates.
* Sediment traps with three collection cups each were placed near center buoy in each lake on 30 May 13. Two additional traps were installed in each lake on 02 Aug 13. The traps were placed in a transect extending from the proposed drip site to the outflow. There was one smaller trap designed for a more shallow depth and two larger traps (same size as those installed in 2012). The traps are labeled as follows: DRIP (the small trap, closest to the drip site), CB (center buoy), and OUT (closest to the outflow). The traps were changed on 27 June 13, 31 July 13, 27 Aug 13, and 18 Oct 13. Each cup was treated as a rep (A,B, C) and sampled for: C, N,P, Ag, and AFDM. The OUT trap from L222 sunk sometime after 27 Aug sampling. So no 18 Oct samples for this trap. Note: the filtering volumes are in the whole lake binder…copied from Beth’s notebook.
* HOBO temperature loggers were hung from center buoy at 1 m depth on 03 June 13 (check date in yellow book). Pressure transducers were installed on 13 June 13 in the same locations as 2012. Loggers were removed in October 13.
* Organic matter decomposition was measured in 8 lakes, including 222 and 221. Measured the rates of decomposition for 3 organic matter types: sugar maple leaves (same as 2012), dried macrophytes collected from L239 (same species as 2012), and tongue depressors as a proxy for woody debris. Pre-weighed packs of organic matter were installed in the littoral areas of each lake in one location, in direct contact with the bethos. Packs were made from 1 mm window screen. Packs were installed on 04 June 13 and collected on 05 June 13 (+24h collection for SM and MAC only), 26 June 13, 01 Aug 13, 26 Aug 13, and 22 Oct 13. Beth has raw data.
* Periphyton was sampled using ceramic tiles placed in 5 locations around each lake. Groups of 10 tiles were attached to a larger tile with silicon and weighed to the benthos with rocks on 02 June 13. Tile locations are marked on the bathy maps (same location as the rock bags). 3 tiles were randomly selected and sampled in August (check binder for date and tile number) and 3 tiles were sampled on 23 Oct 13. The remaining tiles were used by Kat to sample littoral zooplankton. Tiles were sampled for C, N, P, chlorophyll a, and Ag.
* Littoral macroinvertebrate samples were taken according to the OBBN protocol on 19 Aug 13 for L221 and 18 Aug 13 for L222. See yellow book for sampling times. Most locations are flagged in the field and all are marked on the bathy maps in the whole lake binder. Several individuals from representative groups were frozen for isotope natural abundance.
* Macroinvertebrates were also sampled using rock bags. Rock bags were constructed from fish nets with ~2-3 cm mesh. Rocks were collected from the sand pit and washed with tap water. 15 bags were installed in each lake in 5 different locations (3 bags per site, marked on bathy map). Bags were collected from 221 on 14 Aug 13 and from 222 on ??? (check the labels) by hooking the bag and quickly placing a kick net under to reduce macroinvertebrate loss. Samples were washed and animals were preserved in ethanol.
* Nutrient diffusing substrates (+N, +P, +N+P, and control) were placed in each lake on 28 Aug 13 to determine the nutrient limiting periphyton growth before the Ag addition. NDS were placed near the proposed drip site, about 0.5 m depth. NDS were removed on 23 Oct 13. Pots were sampled for C, N, P, and chlorophyll a. Beth has details for pot construction.
* I feel like we took sediment samples in 2013, but I am not seeing any notes in my book. Maybe check the yellow book. If we did, the samples would be preserved in ethanol.
* Greenhouse gas samples were taken along the drip to outflow transect in both lakes. Keunyea Song has these details.
* Several invertebrates and fish samples were collected and dried or frozen for background 15N and 13C signatures.
* Emergence traps were placed in each lake along the drip to outflow transect. These traps did not work well. Many fell apart in the field and several sank. Individuals collected from the traps were frozen, but these samples should NOT be used to calculate an emergence rate due to the difficulty in sampling and the loss of many individuals.
* Several graduate student projects were conducted in 222 in 2013:
  + A bioassay was used to investigate the effects of interactions between zooplankton density and Ag. This bioassay was done in L222 by Kat Cetenic.
  + Many littoral zooplankton samples were taken in both L222 and L221 by Kat Cetenic
  + A bioassay was used to investigate the effects of interactions between Ag and cypro on plankton. This assay was run by Dan Rearick.
  + Pelagic samples were taken along the drip to outflow transect. These data were collected as part of Dan Rearick’s dissertation research but the filtering volumes are in the binder.