I. Project goals:

We are using experimental mesocosms distributed across an elevational gradient to evaluate the effects of changes in growing season length and terrestrial DOM inputs on in-lake planktonic communities. We intend to establish large mesocosms (<1000L) at two locations, the subalpine forest and around treeline in late summer. Ultra violet radiation (UVR) penetration is a strong constrain on primary production (Miller & McKnight 2015), and so placing tanks at varying elevations will help us better account for variation in intensity of UVR. We hypothesize that increases in the ice-free season length, induced by a warming manipulation, will lead to increases in chlorophyll-a, (chl-a) phytoplankton biomass, and zooplankton biomass. Additionally, we expect that there is an interaction between elevation and growing season length, such that the effect size of the warming manipulation will be most pronounced at high elevation.

We will assess UVR stress in zooplankton assemblages by (1) examining Daphnia for photo protective pigments and (2) assessing the prominence of taxonomic groups with high UVR tolerance (e.g., Calanoids and Holopedium) (Kessler et al. 2008). Alternatively, we do not expect differences in zooplankton assemblage’s indicative of differing UVR stress among mesocosms with DOM supplements. Therefore, additions of DOM are expected to amplify the degree of top-down regulation of phytoplankton by zooplankton (Frank et al. 2008; Bunnell et al. 2014). These treatments will help capture major environmental shifts in lakes within the GLV: namely, accelerated ice-off dates and increased inputs of DOM from the upward movement of tundra vegetation and increased phytoplankton production. Although mesocosms cannot capture the full complexity of biological and abiotic interactions unfolding within lakes (Carpenter 1996), we use them here as one of several lines of investigation (alongside long-term data, comparative sampling over an elevation gradient, and ecosystem modeling) to specifically address interactions between phytoplankton and zooplankton.

II. Experimental design:

The experimental design will be 3 x 2 manipulation of tanks where we will test earlier ice-out (yes/no), increased DOM (added yes/no), as well as a combination of earlier ice-out and increased DOM; across two elevation locations (alpine/subalpine). Each site (alpine/subalpine) will include four replicates per treatment (12 tanks total; 2 sites) for 48 total mesocosms (Fig1).

A. Ice-off treatments: 2018-2019 tank albedo trials:
We are currently piloting six 1,200-liter and two 2,400-liter mesocosms at the site Soddie site (40.047874°, -105.571091°, and ~ 3350 meters) (September 2018 – August 2019) to see if tank color via absorption or reflection of solar radiation will change water temperature. Each of the mesocosms has at least one replicate in both high albedo color (beige) or low albedo color (black). To prevent shading each mesocosm has ~1.25 m of space around it and is away from forest shadows. Each mesocosm has also been modified with drain holes covered with mesh 4 cm from the top on both the east and west sides to allow natural flushing. Each mesocosm is instrumented with two thermistors (HOBO Pendant® Temperature/Light 64K Data Logger) that will record both surface and bottom temperature and light intensity in lumens/ft² in 30-minute intervals. These thermistors will provide a more finely resolved record of ice-thaw patterns. As the site is not perfectly flat, and we are unaware of the unique snow drift patterns that could occur in this ribbon forest area, all tanks are supported on the downslope side by T-posts.

Over all this round of piloting would help us determine (1) how well the tanks withstand this location during winter and spring snow patterns (2) how much water accumulates in snow and how much retention occurs throughout the summer (3) the effectiveness of high albedo color as a warming treatment (4) our ability to observe and implement warming and potentially implement a DOC treatment on tanks.

III. Proposed deployment:

A. Earlier ice-out

We will use a passive warming technique of tank color, either dark or light to induce earlier ice-out and overall warming throughout the ice-free period for dark tanks.

B. DOM addition

DOM will be sourced from additions of standard willow “leaf-pack” packs composed of oven dried willow leaves enclosed within Pantyhose mesh (McKnight et al. 1993, Stelzer et al. 2003). Willow leaves can leach up to 40 mg C/g dry leaf mass (McKnight et al. 1993) and would be added in sufficient quantities to raise DOM concentrations in mesocosms to limit UVR penetration to < 1% within the top 10 cm of the tank.
In order to significantly decrease water clarity, we plan to triple current in-lake concentrations of DOC as doubling of DOC in boreal lakes has been associated with a 32% increase in PAR attenuation (Thrane et. al 2014). In regard to the lakes within the Green Lakes Valley (GL4, GL1, and Albion), that implies a minimum DOC concentration of $3.29 \text{ mg/L}$ in treatment mesocosms. Control tanks will contain empty pantyhose bundles to account for any substrate level effects the pack-like objects might have on algal recruitment. In tanks without DOM additions, UVR will be sufficient to limit primary and secondary productivity should be able to penetrate to the bottom of the mesocosm tanks (Sommaruga and Augustin 2006). For the first year of experimentation all mesocosms with be deployed with leaf packs or control packs, prior to filling with winter precipitation and will remain in the tanks for the entire summer.

C. Seeding the tanks: Soil and Plankton

We intend to seed the mesocosms prior to snow precipitation accumulation with sediment as well as leaf packs. Many planktonic taxa have resting egg phases, and so we expect that these communities will emerge when exposed to an aquatic environment in the experimental mesocosm tanks (Alan 1976). Based off of related mesocosm experimentation performed by Matthew Olivier, we will use a posthole digger to collect sediment from GL4 and Lake Albion in liter sized bags to create a ratio of sediment to water of ~0.1% (Strecker et al. 2004).

We will add collected sediments in two events. The first as an inoculation, where we will add quarter of the mixed collected sediment to account for any shock to sediment-based communities when they are transported into the mesocosm tanks. Three days later we will add the remaining mixed sediment into each of the tanks. Sediment will remain refrigerated after collection and prior to tank additions.

After the second addition of sediment, we will add pelagic plankton using a Wisconsin net (80-micron, 20 cm opening and 30cm diameter of inner ring, 90 cm long) from both GL4 and Albion and immediately add the collected plankton to all of the mesocosm tanks. Conservative estimates indicate that each zooplankton 11-meter tow contains about 600 individual adult zooplankton. Ideally, we would like to maintain a zooplankton density of at least 3 individuals per/L, so we will supplement each tank with:

D. Regular sampling protocol

Overview:
We will sample each of the mesocosms four times for nutrients, DOC, chlorophyll-a and zooplankton every other for eight weeks to reflect the regular core sampling of the Green Lake Valley lakes. Before sampling each mesocosm will be stirred 5 times by hand. These samples will be accompanied by weekly water quality measurements from YSI multiprobe meter and Li-Cor Quantum sensor. At each sampling event we will collect chlorophyll-a in a 250 mL amber Nalgene bottle as well as from a tile scrape, nutrients in a 125 mL clear bottle and a DOC sample in a 125 mL burned amber glass bottle, as well as a zooplankton sample from each of the tanks.

Water quality measurements:
Tanks will be monitored weekly immediately after thawing out using a both YSI ProPlus multiprobe meter for water temperature, pH, conductivity, dissolved oxygen, and nitrate. As well as Li-Cor meter with LI-192 Underwater Quantum Sensor for photosynthetically active radiation. Probe measurements will be recorded from 5 centimeters above the bottom of the tank in the same location every time.

**Water samples:**
Samples for nutrients, DOC, chlorophyll-a will be collected in accordance with the limnological protocols used to sample the core lakes within the Green Lakes Valley. All sample bottles (with the exception of the burned DOC bottle) will be rinsed 3 times with mesocosm water prior to the sample collection. All sample bottles will be filled from 5 centimeters above the bottom of the mesocosm. All water samples will be labeled with their tank location, sample type, and collection date and time. All samples will be kept cold and filtered within 24 hours of collection. Chlorophyll-a and DOC samples will be filtered using pre-combusted 47 mm Whatman Glass Fiber Filters pore size 0.7µm. Chlorophyll-a samples will then be frozen and later extracted and analyzed. Nutrients samples will be filtered using a Millipore 0.45µm pore size cellulose membrane filter.

**Chlorophyll-a tile scrapes:**
Each tank will contain 4 small dark-red unglazed clay tiles (~9.5 x 5.7 x 1.1 cm) for benthic algae to colonize throughout the experiment. Prior to sampling, macroinvertebrates will be picked off the tiles and preserved in 80% ethanol. Each tile will be placed in a plastic bag and scrubbed in 100mL in DI water back in the lab. This residue will then be funneled into a 250 mL brown amber bottle and rinsed with an additional 100 mL of DI water. The 200mL of residue will then be filtered using pre-combusted 47 mm Whatman Glass Fiber Filters pore size 0.7µm. Filters will be frozen and later extracted and analyzed (Clifford, Casey, & Saffran, 1992).

**Zooplankton:**
Zooplankton samples will be collected by scooping 10-litres from the mesocosm using a 2-litre beaker and sieving the water through 80µm zooplankton net collection-cup to retain the plankton, and return the filtrate to the mesocosm (Benincà et al., 2008). All captured plankton will then be transferred to a 250 mL glass jar and preserved in an 80% ethanol solution.
References: