**Methods for 2018 Macro Invert Survey**

This data was collected by Samuel Yevak and Kelly Loria as part of 2018 REU project and later incorporated into an honors thesis through the Ecology and Evolutionary Biology Department at the University of Colorado Boulder (Yevak 2019). This dataset includes three data entities one for observations of water and site quality, one for taxa abundance and one for taxa traits.

During the summer of 2018, alpine lakes and streams were sampled for benthic macroinvertebrates. Each lake was sampled approximately every two weeks for eight weeks (53 samples total) alongside the ongoing limnological monitoring. Within each lake there were six sample sites: inlet, outlet, two shoreline sites on the east side of the inlet-outlet orientation, and two shoreline sites on the west side of the inlet-outlet orientation. The exact amount of times each location was sampled was based on physical accessibility and time constraints.

At each sampling location 5 to 3 fully submerged, similarly sized (2000-4000 cubic centimeters each) rocks were selected all within one meter of the shore. Each rock was removed from the water and placed into a four-gallon bucket with a known quantity of water to estimate rock volume via measurement of displaced water (liters). Once in the bucket the rock was thoroughly scrubbed to remove all benthic macroinvertebrates (Huryn and Wallace 1987; Hannaford and Resh 1995; Barbour et al. 1999). Once removed the sediment where the rock had previously been was agitated for 20 seconds followed by three dip-net sweeps to catch any macroinvertebrates living in the sediment under the rock (Lenat 1988; Barbour et al. 1999). Once scrubbed the rock was returned to its original position and the water in the bucket was poured through a 500 μm Mesh D-frame dip-net to concentrate all macroinvertebrates (Huryn and Wallace 1987; Hannaford and Resh 1995; Barbour et al. 1999). The dip-net was then flipped inside out, and the contents of the net were placed back into the bucket in a small amount of corresponding water. The contents of the bucket were then poured into a 500 mL Nalgene bottle using a funnel and preserved with an 80% ethanol solution. The bottle was then labeled with: date collected, time collected, sample location, site location, and stored until microscope analysis.

Certain environmental parameters were also quantified at each site. Using visual observation, the dominant on shore ground cover was characterized within three meters of each site. For example, if a site contained more than 50% vegetation then it was considered vegetated. A Garmin eTrex 10 Global Positioning System (GPS) was used to measure the site coordinates at each site and a YSI 556 multi-probe meter was used to measure on shore water quality (i.e. temperature in degrees Celsius, dissolved oxygen saturation and concentration in milligrams per liter, pH, and nitrate in milligrams per liter). Water quality measurements were made 0.1 m under the surface and 0.5 m off shore. All YSI probe measurements were recorded at every site before benthic macroinvertebrates were sampled.

All preserved samples of benthic macroinvertebrates were taxonomically identified and the first 50 individuals when present measured using an Olympus SZ51 Stereomicroscope (Olympus Corporation of the Americas) using a magnification range of 8-40x. The full contents of each sample (Nalgene bottle) were analyzed to the lowest taxonomic resolution using taxonomic guides (Ward et al. 2002; Iowa State University Department of Entomology 2019). The taxonomic identifications of all benthic macroinvertebrates were cross referenced with previous taxonomic information from the Green Lakes Valley (invertebrate surveys in 1960 and early 1980’s) (Bushnell et al. 1987). All benthic macroinvertebrates were taxonomically identified to at least family. In most cases Trichoptera (caddisflies), Ephemeroptera (mayflies), and Plecoptera (stoneflies) were taxonomically identified to genus level and in few cases species level.

**Citations:**

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish (Vol. 339). Washington, DC: US Environmental Protection Agency, Office of Water.

Bushnell, J. H., Foster, S. Q., & Wahle, B. M. (1987). Annotated inventory of invertebrate populations of an alpine lake and stream chain in Colorado, 1-13.

Hannaford, M. J., & Resh, V. H. (1995). Variability in macroinvertebrate rapid-bioassessment surveys and habitat assessments in a northern california stream. Journal of the North American Benthological Society, 14(3), 430–439.

Huryn, A. D., & Wallace, J. B. (1987). Local Geomorphology as a determinant of macrofaunal production in a mountain stream. Ecology, 68(6), 1932–1942.

Iowa State University Department of Entomology. (2019). Bugs.net “Class Insecta - Insects.” Retrieved from https://bugguide.net/node/view/52/tree.

Yevak, S. (2019). Benthic Macroinvertebrates in the Alpine Lakes of Green Lakes Valley.

Ward, J. V., Kondratieff, B. C., Züllig, R. E., & Ward, J. V. (1992). An illustrated guide to the mountain stream insects of Colorado. Niwot, CO: University Press of Colorado.