Processing and preparation of diatom samples for the purpose of counting and identification (Hasle and Fryxell 1970):

A 10mL subsample of the original periphyton sample is removed and archived in the freezer for future diatom analysis. For analysis, the diatom subsample is thawed in a 1000mL beaker. Using appropriate protective equipment (chemical gloves, goggles, lab coat and apron), at least 20mL of 70% H_2SO_4 is added to the beaker. Saturated aqueous $KMnO_4$ is slowly added to this mixture while swirling until the solution becomes a deep brown color and does not bubble upon addition of additional $KMnO_4$. Saturated aqueous oxalic acid is then slowly added to this mixture while swirling, until the entire solution becomes clear. The beaker is then filled with filtered water and settled for a minimum of 6 hours. After this initial interval, the supernatant is decanted or aspirated to a volume that does not disturb the diatoms, and the beaker is refilled with distilled water. This procedure is repeated until litmus paper tests confirm that the solution is neutral. The beaker is then refilled one last time with distilled water. After 6 hours, the supernatant is decanted or aspirated and the remaining solution containing diatoms is transferred to a 50mL test tube. The diatoms are allowed to settle for another 6 hours before another aspiration is performed and the remaining amount of material is transferred by pipette into a labeled, tared glass vial.

This glass vial is then weighed and recorded before any diatom slide is made. A homogenized volume is removed by pipette; this volume will vary with the density of diatom cells within the solution, and is always recorded. The pipetted subsample is placed on a 22mm coverslip on a slide warmer and allowed to dry. Three drops of Naphrax mounting medium are placed on a clean glass microscope slide; the coverslip with the dried and fixed diatoms is then inverted onto the Naphrax. The slide is heated on a hot plate at the highest setting until the bubbling under the coverslip stops. The slide is then removed from the heat source and placed on a cool surface, and the coverslip is pressed down onto the slide using forceps until the Naphrax has hardened. Excess Naphrax is scraped from the edges of the slide using a blade, and the slide is cleaned with ethanol. Finally, the slide is fixed with a standard label and placed in a slide box for archiving.

Before enumerating diatoms, datasheets are printed that include the type of microscope used, the person counting, the magnification and width of field of view, and the sample ID. Once the slide is placed on the microscope stage, the starting coordinates are randomly chosen and recorded. For diatoms, 250 valves are counted and identified. Once 250 valves are reached, the ending coordinates are recorded and each count is multiplied by 2. Another randomly chosen area on the slide is determined and recorded, and an additional 250 valves are observed; however, only taxa not observed in the first 250 are recorded, and are identified as “rare taxa”. The sum of the two counts is then added and recorded, along with the total distance of all transects traversed on the slide. Digital images of taxa new to the study or of taxa that are morphologically distinct are captured. These images are then added to the diatom image database, along with corresponding ecological, taxonomic and location information. Biovolume estimates of diatoms are also calculated and recorded on the morphometric datasheet using the area, length and width of 20 individuals of each taxon.

Literature Cited:

Hasle, G. R., and Fryxell, G. A. (1970) Diatoms: Cleaning and mounting for light and electron microscopy. *Trans. Am. Microsc. Soc.*, 89: 469-474.