2013

Phytoplankton ecology in four high-elevation lakes of the North Cascades, WA

Siana Wong
Western Washington University

Follow this and additional works at: https://cedar.wwu.edu/wwuet

Part of the Environmental Sciences Commons

PHYTOPLANKTON ECOLOGY IN FOUR HIGH-ELEVATION LAKES

OF THE

NORTH CASCADES, WA

By

Siana Wong

Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Robin Matthews

Dr. Leo Bodensteiner

Dr. James Helfield

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Siana Wong
August 19, 2013

PHYTOPLANKTON ECOLOGY IN FOUR HIGH-ELEVATION LAKES

OF THE

NORTH CASCADES, WA

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Siana Wong

June 2013

ABSTRACT

The objective of my project was to describe phytoplankton ecology in high-elevation lakes of the North Cascades, WA. I conducted my field study using a small-scale ecosystem approach encompassing four lakes in the same watershed near Mt. Baker. I used exploratory data analyses to identify spatial and seasonal patterns in phytoplankton and water chemistry. Chlorophyll-*a* levels were less than 2.5 µg/L, and total nitrogen and phosphorus levels were below 198 and 15.9 µg/L, respectively, indicating the low-production and nutrient-poor nature of these lakes. Chlorophyll-*a* was weakly correlated with total nitrogen (Kendall's tau = 0.25, $p < 0.05$) but was not correlated with most other water chemistry variables ($p > 0.05$). In the phytoplankton dataset, 88 unique taxa were found in 44 lake surface samples. The dominant taxa were members of the groups Cyanobacteria and Chlorophyta. All lakes exhibited seasonal phytoplankton succession characteristic of north temperate lakes, despite the short ice-free period. Nonparametric nonmetric cluster analysis (Riffle) resulted in separation of the four lakes into two groups based on water chemistry variables and phytoplankton abundances. The first group (Picture and Highwood Lakes) was characterized by higher water temperatures and total nitrogen levels, and lower nitrate, soluble reactive phosphate, dissolved oxygen, and pH levels compared to the second group (Upper and Lower Bagley Lakes). Higher abundances of Ochrophyta, Chlorophyta, and Cyanobacteria in the first group also contributed to cluster separation. Differences in water chemistry and phytoplankton between cluster groups could be related to differences in the lakes' external physical environments, which in turn may influence the internal chemical and biological properties of the lakes. Results from this study provide baseline information and understanding relevant to larger, longer-term research and monitoring efforts in North Cascade high-elevation lakes.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Background

High-elevation lakes are generally characterized as being small, low-production aquatic systems that are limited by extreme environmental conditions (Larson et al. 1999, Battarbee et al. 2009). Low temperatures and nutrient levels, prolonged snow and ice cover, and rapid transition from snow and ice cover to intense ultraviolet radiation exposure during ice thaw create harsh conditions to which organisms must be well-adapted (Psenner 2003). Consequently, these conditions are often the limiting factors regulating primary productivity and simplifying food web structures in mountain lakes.

In spite of these conditions, a small window exists during the ice-free period during which phytoplankton communities can potentially flourish. These mostly microscopic photosynthetic organisms are ubiquitous across exceptionally diverse aquatic habitats and environmental conditions. They contribute heavily to primary productivity in the open waters and are a major component of aquatic food webs. In the mountain landscape, phytoplankton biomasses tend to be low relative to low-elevation systems, and peak biomasses tend to occur toward the end of the ice-free period shortly before the next freeze cycle (Tolotti et al. 2006). The phytoplankton assemblages encountered are usually not dominated by taxa endemic to the mountain lake environment (Tolotti et al. 2006); nonetheless they must be composed of taxa that can cope well in this environment. The abundance, diversity, and seasonal timings of phytoplankton in mountain lakes depend on many factors related to the physical, chemical, and biological lake characteristics, such as water temperature, nutrient levels, and aquatic organisms, and their ecological interactions. These internal lake properties are further influenced by external features such as lake morphology, hydrologic connectivity, duration of ice and snow cover and

timing of melt, catchment properties, position within the landscape, and regional characteristics (McKnight et al. 1990, Larson et al. 1998, Hinder et al. 1999, Thackeray et al. 2008, Tolotti et al. 2010).

Although high-elevation lakes are fairly protected from intensive agricultural and urban impacts relative to low-elevation lakes, they are not invulnerable to natural and anthropogenic changes. Mountain lakes are rather highly sensitive to small disturbances owing to their remoteness, extreme physical setting, and simplified nature (Skjelkvåle and Wright 1998, Rogora et al. 2003, Battarbee et al. 2009, Eggermont et al. 2010, Williamson et al. 2010). For this reason, mountain lakes have often been regarded as harbingers of environmental change in freshwater systems (Skjelkvåle and Wright 1998). Increasing attention has been focused on mountain lakes across North America and the European Alps concerning projected and documented impacts from climate change, atmospheric nitrogen and organic pollutant deposition, and introduced species (Hauer et al. 1997, Schindler et al. 2001, Rogora et al. 2006, Battarbee et al. 2009). Potential changes in regional hydrology, timberline elevation, ice-out timing of lakes, and lake stratification patterns associated with climate change may alter the biogeochemical processing in mountain lakes and create shifts in aquatic community structures (Hauer et al. 1997). Studies of European Alpine lakes have shown atmospheric nitrogen deposition to be associated with increased acidity in the lakes (Rogora et al. 2006). In addition, atmospheric fallout of organic pollutants has been associated with higher accumulations of PCBs and DDT in Alpine-lake dwelling fish (Psenner 2003). In small mountain lakes of the Sierra Nevada and Canadian Rockies, continued stocking of non-native fish has altered nutrient cycling processes and contributed to increased algal biomasses predominantly through bioturbation of nutrient-enriched sediments (Schindler et al. 2001).

Evidence of substantial glacial retreat (Pelto 2006) and atmospheric nitrogen deposition (Clow and Campbell 2008) in the North Cascades range in Washington State necessitate research and monitoring into how global and regional environmental drivers such as climate change and atmospheric deposition might affect regional freshwater ecosystems. In order to interpret and predict how North Cascade mountain lakes will respond to these environmental drivers, basic understanding of biogeochemical processes and knowledge of the aquatic communities present in these lakes is needed. Previous ecological studies on North Cascade mountain lakes have centered on large landscape-scale approaches (Larson et al. 1998 and Larson et al. 1999). Research and monitoring spanning multiple spatial (local to regional) and temporal (diel, seasonal, and inter-annual) scales allows for improved understanding about the current ranges in variation found among lakes, as well as the possible mechanisms involved (Hauer et al. 1997).

The underlying significance of ecological studies on mountain lakes lies not only in the recognition of mountain lakes as sentinels of change, but also in their social and economic values (Tolotti 2006), for example as places of recreation, as harborers of threatened and endangered fish and wildlife, and as freshwater resources. A greater knowledge about these often understudied remote ecosystems can lead to better comprehension of their ecological and socioeconomic significance. Additionally, a greater knowledge can instill a deeper appreciation for the dynamic nature and biodiversity of these systems and lend support to their conservation and management. With increasing stresses on local and global freshwater resources, these aspects may become more tangible and important.

Objective

The objective of my study was to provide information and add to the ecological understanding about phytoplankton assemblages and water chemistry in high-elevation lakes of the North

Cascades. I used a small-scale ecosystem approach, focusing on four proximal lakes in the same watershed near Mt. Baker during the course of one ice-free season. My main research goals were: (1) to determine the composition and abundance of phytoplankton in these lakes; (2) to determine if phytoplankton communities exhibited seasonal succession; and (3) to investigate whether spatial and seasonal differences in phytoplankton abundance and composition could be related to water chemistry variables. I used the term "water chemistry" to refer collectively to physico-chemical properties, nutrients, and chlorophyll-*a*.

METHODS

Regional Characteristics

The North Cascades range in northwestern Washington State is characterized by extensive rugged and glaciated terrain borne by a dynamic and complex geologic history. Over the past 40 million years, regional uplift, faulting, volcanic activity, and erosion have carved a region of great topological relief with low-lying, broad valleys surrounded by steep, cragged mountains with typical peaks in the 1,800–2,500 m elevation range (Tabor and Haugerud 1999). Throughout the range, bedrock is composed of a folded mosaic of granitic, volcanic, and sedimentary rock (Tabor and Haugerud 1999). Storms originating in the Pacific Ocean and intercepted by the mountains create a temperate maritime climate to the west with up to about 400 cm annual precipitation, and a relatively dry continental climate to the east with up to 25–30 cm annual precipitation (Tabor and Haugerud 1999). About 80% of precipitation in the North Cascades falls as snow from October–April (Pelto 2006). Large tracts of glaciers and snowfields occur at higher elevations, attributable to the high amount of precipitation the region receives.

4

Glacial activity accounts for the formation of many high elevation lakes that dot the North Cascades landscape, including the lakes in this study.

Lake Descriptions

The scope of my study encompasses four small lakes near Mt. Baker within the Mt. Baker-Snoqualmie National Forest—Picture Lake, Highwood Lake, Upper Bagley Lake, and Lower Bagley Lake (Figure 1). The four lakes lie within the upper North Fork Nooksack River watershed, which eventually drains into Puget Sound in the Pacific Ocean. These lakes were selected primarily because of their proximity, ease of access by road or established trail, and opportunistic snow and ice-free conditions during the sampling period. Based on preliminary site reconnaissance, I was also interested in exploring how phytoplankton assemblages in the lakes compare and contrast. Most apparent are similarities and differences among the lakes in elevation, morphology, hydrology, forest and vegetation cover, substrate, and public access (Table 1).

Upper Bagley Lake is a cirque lake nestled between Table Mountain to the west and Mt. Herman to north (Figure 1). Its surface waters are discharged into Lower Bagley Lake via Bagley Creek, a perennial tributary of the North Fork Nooksack River. Lower Bagley Lake, the shallowest of the four lakes, is an elongated reservoir with a small epilimnial dam on the north end of the lake. The dam was built prior to 1969 for hydroelectric power, and has since been discontinued as a source of hydropower. Past the dam, Lower Bagley Lake drains into Bagley Creek. I accessed Upper and Lower Bagley Lakes using an established trail system. Picture and Highwood Lakes are roughly circular kettle lakes whose surface waters do not appear to be hydrologically connected or discharged into a perennial tributary system. The lakes are

surrounded and separated by a nearby road. Picture Lake is encircled by a heavily used interpretive trail, but Highwood Lake is not.

Based on regional classifications, the four lakes lie within the subalpine vegetation zone of the North Cascades, with cover including subalpine herbs, heather, Pacific silver fir, mountain hemlock, rock, and snow (Lomnicky 1995). Picture and Highwood Lakes are situated at about 30–40 m lower elevation than Lower and Upper Bagley Lakes and are surrounded by meadow and forest (Table 1). The littoral zones in these lakes contain substantial macrophytic cover, relative to Upper and Lower Bagley Lakes. Rock and snow cover are greater in Upper and Lower Bagley Lakes than in Picture and Highwood Lakes, with sparse forest and vegetation higher in elevation toward Upper Bagley Lake. The ice-free period for these lakes typically occurs during July–October, although conditions depend on weather and climate phenomena during the given year. All four lakes are known to be historically fishless. Beginning in the 1930s, the four lakes were stocked with Eastern brook trout, coastal cutthroat trout, and then rainbow trout. Stocking was discontinued in the late 1960s in Lower Bagley Lake, late 1980s in Upper Bagley Lake, and early 1990s in Picture and Highwood Lakes (Jeremy Gilman, U.S. Forest Service, personal communication). Currently, Upper and Lower Bagley Lakes have naturally reproducing Eastern brook trout and rainbow trout. The presence of naturally reproducing fish in Picture and Highwood Lakes is uncertain; however, continued angler use of these lakes suggests that fish (rainbow trout) may be present.

Field Sampling

I sampled the four lakes on nine occasions, once per week, during open water conditions from August 15–October 11, 2012. Phytoplankton and water chemistry samples were collected from one fixed site at the approximate center of each lake. I accessed all sites using a float tube and

collected samples at 0.5 m below the water surface using a 3.0 L horizontal Van Dorn sampler.

On September 13, 2012, I sampled two additional sites in the limnetic zone of each lake to assess within-lake variability in phytoplankton and water chemistry. During the first four sampling events in Picture and Highwood Lakes, and on seven occasions in Upper Bagley Lake, I collected additional water chemistry samples at 0.5 m above the lake bottom to compare surface and bottom water chemistry. In total, I collected 62 samples for water chemistry analysis. This included 44 lake surface samples, 16 lake bottom samples, and two samples from Bagley Creek. I collected 44 lake surface samples along with two samples from Bagley Creek for phytoplankton identification and counting.

At each site, water for phytoplankton and chlorophyll-*a* analyses was transferred from the Van Dorn into separate 500-mL amber polyethylene bottles. Phytoplankton samples were preserved with 0.5 mL Lugol's iodine solution immediately after collection. Water for analyses of nitrogen and phosphorus concentrations, pH, and specific conductance was transferred from the Van Dorn into one acid-washed 500-mL clear polyethylene bottle. All samples were stored in a cooler on ice until further processing. I used a calibrated YSI instrument (550A) to measure *in situ* dissolved oxygen concentration and water temperature at 0.5 m below the water surface and at 0.5 m intervals to the lake bottom. I also measured water depth and Secchi depth (a surrogate measurement of lake transparency) at each site using a metered line. Concentrated live phytoplankton samples were collected from the shoreline of each lake using a 20-μm mesh plankton net to aid in phytoplankton identification.

Laboratory Analysis

Water chemistry samples were processed and analyzed at the Institute for Watershed Studies (IWS) at Western Washington University, Bellingham, WA. Water chemistry samples were

analyzed for total phosphorus, soluble reactive phosphate, total nitrogen, nitrate + nitrite (subsequently abbreviated to "nitrate"), and ammonium concentrations, as well as pH, specific conductance, and chlorophyll-*a* concentration. Laboratory procedures followed guidelines in the Standard Methods for the Examination of Water and Wastewater (APHA 2012).

Samples for analysis of dissolved nutrients (soluble reactive phosphate, nitrate, and ammonium) were filtered through 0.45 µm sterilized Fisherbrand® membrane filters on the same day of collection. Soluble reactive phosphate and nitrate samples were stored frozen and analyzed by IWS staff within 30 and 60 days, respectively. Ammonium samples were stored at 4°C and analyzed by IWS staff within 28 days. Filtered samples for dissolved nutrients were analyzed colorimetrically on an automated spectrophotometer. Unfiltered water samples used for total nitrogen and total phosphorus analysis were digested using potassium persulfate, autoclaved, and stored frozen on the same day of collection. Digested samples were analyzed for total nutrients colorimetrically on an automated spectrophotometer within 28 days by IWS staff. Nitrogen and phosphorus concentrations were corrected to and expressed in units of µg-N/L or µg-P/L, respectively. Standard methods and instrument detection levels for nitrogen and phosphorus analyses are shown in Appendix A.

I measured specific conductance and pH from unfiltered water samples on the same day of collection using a calibrated Orion DualStar pH meter and Orion Model 162 conductivity meter. Chlorophyll-*a* samples were filtered within 48 hours using 25 mm Whatman glass fiber filters and stored frozen. Chlorophyll-*a* pigment was extracted in 90% acetone, corrected for phaeophytin, and analyzed on a previously calibrated Turner Design (TD-700) fluorometer within 28 days.

All preserved phytoplankton samples were shipped to and analyzed by Karl Bruun, Nostoca Algae Laboratory, Bainbridge Island, WA. Phytoplankton identification and counts were performed according to Nostoca Algae Laboratory standard operating procedures. Briefly, 50 mL of each sample was settled for at least 48 hours to concentrate the sample. The top 45 mL was then siphoned, and the remaining 5 mL used for analysis. Phytoplankton were subsequently identified and counted under an upright compound light microscope on Sedgewick Rafter or Nanoplankton (Palmer-Maloney) counting chambers at 200X or 400X magnification, respectively. Phytoplankton individuals were identified to species level or to the next highest taxonomic level possible. Biomasses were estimated using cell density and biovolume. Biovolumes were based on the average cell dimensions of 10 individuals of each genus (Hillebrand et al. 1999). Laboratory duplicates were performed on 10% of all samples and assessed using the Bray-Curtis Similarity Index.

Quality Assurance

In order to assess precision during field sample collection, I collected field duplicates for water chemistry analysis at 10% of the total number of samples per event. Laboratory duplicates assess precision of laboratory techniques, and were also performed on 10% of the total number of samples per event for pH, specific conductance, and nitrogen and phosphorus analyses. In the laboratory, I analyzed all chlorophyll-*a* samples in duplicate and reported the mean chlorophyll-*a* as the final sample concentration. I assessed quality assurance results for laboratory and field duplicates following IWS guidelines (Appendix B). Internal quality control measures for nitrogen and phosphorus analyses were performed by IWS staff and included analyses of check standards, blanks, and spike samples.

Data Analysis

I created a list of phytoplankton taxa identified by Nostoca Algae Laboratory, including relative abundances for the entire dataset and presence/absence of individual taxa in each lake. I grouped phytoplankton taxa based on taxonomic classifications accepted by AlgaeBase, a comprehensive up-to-date online database providing information on algae throughout the world (Guiry and Guiry 2013). Some taxa could not be identified to genus and species, but were counted as distinct taxa by Nostoca Algae Laboratory. I included these unidentified taxa in abundance calculations and grouped them as "undetermined."

I used exploratory approaches to look for patterns in water chemistry and phytoplankton data, focusing on my original research goals. Graphical analyses were used to identify seasonal trends in water chemistry and phytoplankton composition and abundance. For each phytoplankton sample, I calculated Pielou's *J* from cell density values as a measure of evenness (Pielou 1975). In order to explore relationships among water chemistry variables and phytoplankton abundances, I applied nonparametric rank-based correlations (Kendall's tau, τ). Using R statistical software, I also performed nonparametric nonmetric clustering analysis (Riffle) to look for cluster patterns in the water chemistry and phytoplankton datasets (Matthews et al. 1991). If cluster patterns were found, the variables contributing most strongly to cluster memberships were those with the highest Proportional Reduction in Error (PRE) scores (Matthews et al. 1991). Association analysis—the combination of Chi-squared (χ^2) test and contingency table—was used to determine if "blind" cluster groups significantly associated with specified lake groups.

RESULTS

Surface Water Chemistry

Among the lakes, water temperatures ranged 4.0–20.7°C, dissolved oxygen concentrations ranged 6.8–13.2 mg/L, pH ranged 6.1–7.3, and specific conductance ranged 9.8–14.9 µS/cm (Table 2, Figures 2–5). Maximum water temperatures in Upper and Lower Bagley Lakes were below 9.0°C and were about 12–14°C lower than in Picture and Highwood Lakes. Maximum water temperatures were observed in mid-September in Upper and Lower Bagley Lakes, and water temperatures overall declined from August to October in Picture and Highwood Lakes (Figure 2). Upper and Lower Bagley Lakes had about 3–4 mg/L higher median dissolved oxygen concentrations and were less acidic than Picture and Highwood Lakes.

Total nitrogen concentrations were lower and nitrate concentrations were higher in Upper and Lower Bagley Lakes than in Picture and Highwood Lakes (Table 2). In Upper and Lower Bagley Lakes, total nitrogen concentrations were below the detection limit of 21.8 µg/L on most sampling dates (Table 2, Figure 6). Nitrate concentrations in Upper and Lower Bagley Lakes were above detection limit on all sampling dates, with values ranging 6.5–28.1 µg/L, and noticeably declined after September 5 (Figure 7). In Picture and Highwood Lakes, total nitrogen concentrations were above detection limit on all sampling dates, with values ranging 67.9–198 µg/L, and nitrate concentrations remained near or below the detection limit of 5.5 µg/L (Table 2, Figures 6 & 7). Ammonium concentrations were below the detection limit of 9.9 µg/L in all four lakes during the sampling period (Table 2). For this reason, I did not further analyze ammonium results. Nitrite was likely negligible because oxygen was consistently present in the lakes, so nitrate probably constituted the majority of dissolved inorganic nitrogen. Dissolved inorganic nitrogen composed less than 9% of total nitrogen in Picture and Highwood Lakes, about 79% in

Upper Bagley Lake, and about 68% in Lower Bagley Lake when averaged over the sampling period.

Total phosphorus concentrations ranged from less than the detection limit of 4.8 µg/L to 15.9 µg/L, and soluble reactive phosphate concentrations ranged from 1.4 to11.4 µg/L among the four lakes (Table 2, Figures 8 & 9). The majority of total phosphorus was in the form of soluble reactive phosphate in Lower and Upper Bagley Lakes (85% and 73%, respectively), when averaged over the sampling period. Soluble reactive phosphate constituted 40% of total phosphorus in Picture Lake and 60% in Highwood Lake.

Maximum chlorophyll-*a* concentrations were less than 2.5 µg/L among the four lakes (Table 2). Chlorophyll-*a* concentrations generally increased from August to October. In Lower Bagley Lake, however, chlorophyll-*a* concentration peaked in mid-September. In Upper and Lower Bagley Lakes, chlorophyll-*a* concentrations were near or below the detection limit of 0.1 µg/L until after September 13 (Figure 10).

Phytoplankton

Summary of Dataset

In the 44 phytoplankton samples, 88 unique taxa representing eight major taxonomic groups were observed, including 79 taxa identified to genus or species (Table 3). The best represented groups by cell density were Cyanobacteria (66% relative abundance) and Chlorophyta (23% relative abundance). Ochrophyta and Bacillariophyceae (diatoms) each contributed over 1% relative cell density, and the remaining groups contributed less than 1% relative cell density.

Unidentified taxa contributed about 5–7% total cell density and biovolume. The contribution of unidentified taxa to relative abundance in each lake was generally more

important in Upper and Lower Bagley Lakes than in Picture and Highwood Lakes, and was less important in terms of biovolume than cell density across all four lakes.

Numerically, *Aphanocapsa* was the most abundant taxon (63% relative cell density), followed by *Botryosphaerella sudetica* (17% relative cell density). Five other taxa contributed greater than 1% relative cell density. The largest contributors by biovolume were *Botryosphaerella sudetica* (16%), *Aphanocapsa* (12%), *Dinobryon sertularia* (11%), and *Oocystis* cf. *marssonii* (9%). Sixteen other taxa contributed greater than 1% relative biovolume.

Total abundances and taxa richness were higher in Picture and Highwood Lakes than in Upper and Lower Bagley Lakes (Figure 11). Twelve taxa were common to all lakes, including five diatoms (*Achnanthes* sp. 1, *Navicula*, *Nitzschia*, *Navicula* cf. *cryptocephala*, and *Aulacoseira*), four unidentified taxa, one dinoflagellate (*Peridinium umbonatum*), one member of Ochrophyta (*Characiopsis curvata*), and a dinoflagellate cyst. Twenty-three rare taxa, defined here as appearing once in 44 samples, were found. This constituted roughly one-fourth of the total number of taxa observed, 1% of total cell density, and 6% of total biovolume. Rare species tended to occur in Picture and Highwood Lakes, with only two rare taxa (both diatoms) found in both Upper and Lower Bagley Lakes.

Composition and Trends in Picture Lake

Sixty-two taxa were observed in Picture Lake, including 11 that were unique to Picture Lake (Figure 11). Evenness (Pielou's *J*) ranged 0.19–0.67, with values generally declining through the sampling period (Figure 12). Cyanobacteria contributed the highest cell density in Picture Lake (71%), whereas Chlorophyta contributed the highest biovolume (45%). *Aphanocapsa* accounted for over 95% of total Cyanobacteria abundance in this lake, emerging in early September and peaking in early October (Figure 13). Four other members of Cyanobacteria occurred in this lake

in low abundances. The dominant member of Chlorophyta was *Botryosphaerella sudetica* (67% Chlorophyta cell density; 37% biovolume), followed by several species of *Oocystis*. Chlorophyta abundance peaked in late August, and declined to steady abundances through the end of the season (Figure 14). Ochrophyta (predominantly *Characiopsis curvata* and *Dinobryon sertularia*) also contributed largely to total biovolume in this lake (23%), peaking in early September and declining thereafter (Figure 15). The diatoms composed less than 1% cell density and about 5% biovolume in Picture Lake, and were most abundant in early August (Figure 16). Members of Myzozoa (dinoflagellates), Charophyta (desmids), and Cryptophyta were observed in low abundances later in the season after mid-September (Figures 17–19).

Composition and Trends in Highwood Lake

The greatest taxa richness was observed in Highwood Lake, with 65 of the 88 taxa represented in this lake. Pielou's *J* ranged 0.25–0.78, with an overall decline from mid-August to October (Figure 12). As in Picture Lake, Cyanobacteria was the dominant group by cell density (56% total cell density), whereas Chlorophyta was the largest contributor by biovolume (28% total biovolume). *Aphanocapsa* was the dominant taxon, emerging in September and peaking in October (Figure 13). *Botryosphaerella sudetica* was the dominant member of Chlorophyta in Highwood Lake (89% relative cell density), peaking in August to mid-September (Figure 14). Similar to Picture Lake, the diatoms made up low relative abundances in this lake (1% cell density; 7% biovolume) and were most abundant early during the sampling period in August (Figure 16). A dinoflagellate cyst contributed largely to total biovolume in mid-August and mid-September (Figure 17). *Characiopsis curvata* and *Dinobryon* were the primary members of Ochrophyta in this lake and were prevalent in low relative abundances through the sampling

14

period. Charophyta was represented by four taxa and was more abundant later in the season beginning in September (Figure 18).

Composition and Trends in Upper Bagley Lake

Taxa richness was lowest in Upper Bagley Lake among the four lakes. Pielou's *J* ranged 0.34– 0.92, with lowest evenness occurring in mid-late August and late September (Figure 12). The major groups, Cyanobacteria, Cryptophyta, Charophyta, and Euglenozoa, were not represented among the phytoplankton in this lake. Diatoms dominated the Upper Bagley assemblage, composing about 60% total biovolume and representing 21 of the 37 taxa observed in the lake. *Achnanthes* sp. 1 (early in the season) and *Diatoma mesodon* (later in the season) were the most abundant diatoms in this lake. The diatoms peaked twice, with the first peak occurring in mid-August, and the second peak of higher magnitude in early October (Figure 16). In terms of cell density, however, unidentified taxa dominated Upper Bagley Lake, contributing about 60% total cell density. The main contribution of the unidentified taxa in Upper Bagley Lake was in late September/early October (Figure 20). Chlorophyta appeared beginning in mid-September and was composed predominantly of two species of C*hlamydomonas* (63% Chlorophyta cell density; 91% biovolume). *Botryosphaerella sudetica* was the only other Chlorophyta member in Upper Bagley Lake, composing 36% Chlorophyta cell density and 8% biovolume; however, it occurred in only one sample from the lake. *Characiopsis curvata* was the only member of Ochrophyta observed in Upper Bagley Lake and appeared twice in September/October in low relative abundances (7% cell density; 1% biovolume). The only identified dinoflagellate, *Peridinium umbonatum*, occurred in one sample from the lake. A dinoflagellate cyst composed over 90% Myzozoa abundance in the lake.

Composition and Trends in Lower Bagley Lake

Twenty-one of the 41 taxa observed in Lower Bagley Lake were diatoms. With the exception of

one sample on September 13, Pielou's *J* remained above 0.60, with lowest evenness occurring in

mid-late September (Figure 12). With the exception of unknowns during the late season, the

diatoms were the dominant group in the lake throughout the sampling period. *Diatoma mesodon*

contributed most to diatom abundance in this lake. Chlorophyta made up less than 5% total

abundance in Lower Bagley Lake but was consistently present in low densities through the

season. *Chlamydomonas* cf. *globosa* was the dominant member of Chlorophyta (41%

Chlorophyta cell density; 67% biovolume). Two species of *Oocystis* were the only other

members of Chlorophyta observed in this lake. *Anabaena, Cryptomonas erosa, and Characiopsis*

curvata composed the only members of Cyanobacteria, Cryptophyta, and Ochrophyta,

respectively, and were observed in low relative abundances in this lake. A dinoflagellate cyst

composed the majority of Myzozoa abundance. *Peridinium umbonatum* was the other member of

Myzozoa observed in this lake, although it occurred in only one sample.

Correlation of Water Chemistry Variables

Significant correlation was found between most water chemistry variable pairs ($p < 0.05$; Table

4). The strongest correlation was between water temperature and dissolved oxygen ($\tau = -0.62$, p

$= 1.5 \times 10^{-9}$), followed by water temperature and nitrate ($\tau = -0.61$, $p = 2.70 \times 10^{-9}$). Chlorophyll-*a*

positively correlated with total nitrogen ($\tau = 0.25$, $p = 4.70 \times 10^{-3}$), and negatively correlated with

nitrate ($\tau = -0.33$, $p = 1.60 \times 10^{-4}$) and soluble reactive phosphate ($\tau = -0.26$, $p = 3.60 \times 10^{-3}$).

Correlations between chlorophyll-*a* and all other water chemistry variables were non-significant

($p > 0.05$). Chlorophyll-*a* also significantly correlated with total phytoplankton biovolume ($\tau =$

0.45, $p = 1.10 \times 10^{-5}$) and cell density ($\tau = 0.52$, $p = 3.10 \times 10^{-7}$).

Nonmetric Clustering

Nonmetric clustering resulted in separation of water chemistry variables into two clusters, as is shown in a plot of two water chemistry variables with the highest PRE scores (Figure 21, Table 5). Significant association was found between the two cluster groups and specified site groups, defined here as the Picture and Highwood Lakes system and the Bagley system (Upper and Lower Bagley Lakes and Bagley Creek) ($\chi^2 = 46$, p = $2.50\text{x}10^{-9}$). No misclassifications of samples were generated using association analysis on the two groups (Table 5). Three trials of nonmetric clustering on the water chemistry data produced identical results, indicating the stability of cluster results. Five variables—water temperature, dissolved oxygen, pH, total nitrogen, and soluble reactive phosphate—tied for the top PRE score (0.96), and were the main contributors to cluster separation (Table 5). Total phosphorus and chlorophyll-*a* produced the lowest PRE scores (0.33 and 0.36, respectively) and contributed least to cluster separation.

Nonmetric clustering of major phytoplankton groups also showed separation into two clusters, with significant association between cluster groups and the same specified lake groups (Table 5, Figure 21). Association analysis performed on phytoplankton biovolumes yielded one misclassification of a sample from Highwood Lake. Two misclassifications, one from Highwood Lake and the other from Picture Lake, were generated with association analysis performed on cell densities. Three phytoplankton groups—Ochrophyta, Chlorophyta, and Cyanobacteria— produced high PRE scores on both biovolume and cell density data (Table 5), thus contributing most to cluster separation. Three trials of nonmetric clustering on the phytoplankton data generated identical results, thus indicating cluster stability.

I performed association analysis on the water chemistry cluster results and phytoplankton cluster results to determine the goodness of fit between the two sets. Significant association was

found between the water chemistry and phytoplankton biovolume cluster groups (χ^2 = 38.4, p = 5.78x10^{-10}) and water chemistry and phytoplankton cell density cluster groups (χ^2 = 35.0, p = 3.31x10^{-9}), indicating that the two datasets clustered well into the same groups (Table 6). One misclassification was produced between water chemistry and biovolume cluster groups, and two misclassifications were produced between water chemistry and cell density cluster groups.

Complementary Sampling

Spatial Variability in Water Chemistry and Phytoplankton within Lakes

Surface water chemistry was fairly consistent among the three limnetic sites within each lake (Figures 2–10). The exception was Lower Bagley Lake, in which total nitrogen, soluble reactive phosphate, and chlorophyll-*a* concentrations were noticeably higher at one site relative to the other two. More noticeable variation in phytoplankton abundance and composition occurred among sites within each lake (Figures 13–20). For example, Cyanobacteria cell densities ranged 400–15,000 cells/mL among the three sites in Picture Lake, and of the five phytoplankton groups in Lower Bagley Lake, only the diatoms were represented at all three sites.

Depth Profiles of Water Chemistry Variables

Surface and bottom nutrient concentrations within each lake were generally similar. In addition, weekly depth profiles of water temperature and dissolved oxygen concentration in the four lakes were uniform, or well-mixed, throughout the water column. On several dates, however, surface and bottom water temperature differences were observed in Highwood and Upper Bagley Lakes. For example, temperature differences of about 10°C and 6°C were observed in Highwood Lake on August 15 and 30, respectively, and temperature differences of about 1°C and 1.5°C were observed in Upper Bagley Lake on September 5 and 27 (Figure 22).

Water Chemistry and Phytoplankton in Bagley Creek

Water chemistry in Bagley Creek was slightly higher in chlorophyll-*a* concentration, pH, and soluble reactive phosphate than in Upper and Lower Bagley Lakes (Figure 23). Gradients in total nitrogen and nitrate concentrations were apparent from Upper Bagley Lake, downstream through Bagley Creek, to Lower Bagley Lake. In total, 10 phytoplankton taxa were observed in Bagley Creek, of which six were diatoms. All taxa were shared with either Upper or Lower Bagley Lake, or both lakes.

DISCUSSION

Regional Comparisons

With low concentrations of total nitrogen (< 21.8–198 µg/L), total phosphorus (< 4.8–47.9 µg/L), and chlorophyll-*a* (< 0.1–2.5 µg/L), Picture, Highwood, Upper Bagley, and Lower Bagley Lakes fall within the oligotrophic classification of freshwater lakes, corresponding to systems receiving low nutrient inputs (Nürnberg 1996). Oligotrophy is characteristic of mountain lakes high up in the watershed where nutrient input from agricultural and urban runoff is minimal compared to low-elevation lakes (Dodson 2005). As comparison to other regional oligotrophic lakes, maximum total nitrogen, phosphorus, and chlorophyll-*a* concentrations in my study were in the upper limits or above the range of values observed in two previous studies of North Cascade high-elevation lakes. For example, total nitrogen concentrations ranged 16–149 µg/L and total phosphorus concentrations ranged 1–30 µg/L in a landscape-scale study of 58 mountain lakes in the North Cascades (Larson et al. 1999). Additionally, total nitrogen and phosphorus concentrations ranged 50–270 µg/L and 2–7 µg/L, respectively, and chlorophyll-*a* concentrations

ranged 0.23–1.5 µg/L in a study of four North Cascades high-elevation lakes situated above 1300 m (Sheibley et al. 2012).

In spite of the low production and watershed scale of my study, the total phytoplankton taxa richness observed in the four lakes (88 taxa) was similar to those of larger regional studies in North Cascade mountain lakes. For example, 93 taxa were found in 55 samples from 51 North Cascade mountain lakes (Larson et al. 1998) and, in another study, 97 taxa in 58 lakes (Lomnicky 1995) distributed throughout the North Cascades range. As in Larson et al. (1998) and Lomnicky (1995), Cyanobacteria was highest in relative abundance in my study, and similar to Larson et al. (1998), *Aphanocapsa* was the most abundant Cyanobacteria in my study. However, the relative proportion of phytoplankton groups and species composition in my study did not always compare well to those observed in previous studies. Although the contributions of diatoms and Chlorophyta were similar to those observed in Larson et al. (1998), I found a higher proportion of Cyanobacteria (66% cell density) and lower proportion of Ochrophyta (4% cell density). This contrasts with the 37% Cyanobacteria and 20% Chrysophyta (i.e. Ochrophyta) observed in Larson et al. (1998). Additionally, although *Botryophaerella sudetica* was high in relative abundance in my study (17% total cell density), it was not observed in Larson et al. (1998).

Unstable Stratification

The lack of stable thermal stratification, in which vertical temperature and density gradients are persistent, is characteristic of shallow lakes (Padisák and Reynolds 2003). Because the lakes in my study were fairly shallow (< 6.5 m), the temperature differences observed in Highwood and Upper Bagley Lakes on several occasions were probably indicative of unstable stratification, a condition similar to the formation of thermal discontinuity layers in the epilimnion (surface layer)

of some stratifying temperate lakes (Figure 22; Wetzel 1983). During this process, secondary

metalimnia may form in the usually well-mixed epilimnion of thermally stratified lakes during

intense calm and sunny weather conditions. Unlike the primary metalimnion (layer of rapid

vertical temperature change), the secondary metalimnia can be disrupted more readily by wind or

cool night-time temperatures (Wetzel 1983). During my study, sunny weather and calm lake

surface waters on most sampling dates, as was documented in field notes, provided conditions

suitable for the development of unstable stratification.

Nutrient Limitation

In an assessment of nutrient limitation in Japanese freshwater lakes, Sakamoto (1966) concluded

that summer algal biomass was limited by nitrogen when total nitrogen/phosphorus ratios were

less than 10 and by phosphorus when ratios were greater than 17 by weight. Based on these

interpretations, Upper and Lower Bagley Lakes were nitrogen-limited with total

nitrogen/phosphorus < 7, except on one occasion in Upper Bagley Lake. Picture and Highwood

Lakes were phosphorus-limited (total nitrogen/phosphorus > 17) or limited by either nitrogen or

phosphorus (total nitrogen/phosphorus between 10 and 17) depending on the sampling date.

Using the dissolved inorganic forms of nitrogen and phosphorus, all four lakes would be

considered nitrogen-limited (dissolved inorganic nitrogen/phosphorus < 5). Positive correlation

between chlorophyll-*a* and total nitrogen also suggests nitrogen limitation in these lakes ($\tau = 0.25$,

$p = 4.72 \times 10^{-3}$; Table 4). Although phosphorus limitation is the generally accepted paradigm in

freshwater lakes (Hecky and Kilham 1988), it is not uncommon to find nitrogen as a limiting or

co-liming factor with phosphorus, especially in less-productive small mountain lakes (Morris and

Lewis 1988, Tolotti et al. 2012).

The nutrient limitation concept provides useful ecological assessment of aquatic systems; however, correlations between chlorophyll-*a* and nutrient constituents were non-significant (total phosphorus, $\tau = 0.10$, p = 0.25) or weak (total nitrogen, $\tau = 0.25$, p = 4.72×10^{-3}; soluble reactive phosphate, $\tau = -0.26$, p = 3.58×10^{-3}; nitrate, $\tau = -0.33$, p = 1.62×10^{-4}). The lack of strong correlations between chlorophyll-*a* and measured water chemistry variables is perhaps more interesting than the significant correlations found among the majority of water chemistry variables (Table 4). It suggests that factors other than those measured may be more important regulators to overall phytoplankton biomass in the four lakes. These could include external physical factors such as the short ice-free period and high exposure to ultraviolet radiation during the open water period, or internal factors such as zooplankton grazing pressures and low concentrations of other essential nutrients (Carpenter et al. 1985, Larson et al. 1998, Williamson et al. 2010).

Clustering of Lakes

Distinct patterns in water chemistry and phytoplankton were observed spatially among the four lakes. Readily apparent is the clustering of the four lakes into two groups—the Picture and Highwood Lakes system and the Bagley system (Figure 21). Picture and Highwood Lakes were associated with higher water temperatures and total nitrogen concentrations; lower dissolved oxygen, soluble reactive phosphate, and pH; and higher abundances of Ochrophyta, Chlorophyta, and Cyanobacteria relative to Upper and Lower Bagley Lakes.

Conceptually, separation of the two systems could be related to differences in external physical environment, which have interrelated effects on the ranges and seasonal timings in water chemistry variables such as water temperature, dissolved oxygen concentration, and nutrient concentrations (Larson et al. 1999). For example, Upper Bagley Lake was substantially

ice-covered until after mid to late August, whereas the other three lakes were ice-free throughout the sampling period (Appendix C). Prolonged ice cover on the lake can in turn contribute to water temperature and light-limiting conditions for phytoplankton.

In addition to lake ice cover, snowmelt from adjacent mountain slopes can affect physical and chemical attributes including water temperatures and nitrate concentrations in the receiving lake. In Upper Bagley Lake, for example, 75% of nitrate concentration was previously attributed to snowpack source from adjacent slopes (Loranger and Brakke 1988). Prolonged snow conditions in Upper Bagley Lake could have contributed to higher nitrate concentrations in Upper and Lower Bagley Lakes relative to Picture and Highwood Lakes. Additionally, seasonal declines in nitrate concentration concurrent with increasing chlorophyll-*a* concentrations in Upper and Lower Bagley Lakes would suggest nutrient uptake by phytoplankton as temperatures warmed and the lakes thawed (Figures 7 & 10). Although it cannot be determined from my study, a similar seasonal pattern may have occurred before the sampling period in Picture and Highwood Lakes, which thawed at least one month earlier than Upper and Lower Bagley Lakes. If this is the case, then different seasonal timings could have contributed to lower nitrate concentrations in Picture and Highwood Lakes relative to Upper and Lower Bagley Lakes.

Higher total nitrogen concentrations and lower pH in Picture and Highwood Lakes relative to Upper and Lower Bagley Lakes could be related to a combination of factors. Different than Upper and Lower Bagley Lakes, total nitrogen in these lakes was comprised by 91% organic nitrogen. Higher values and higher organic proportions of total nitrogen in Picture and Highwood Lakes might be attributed to dissolved organic nitrogen inputs from extensive littoral macrophytes (Wetzel 1983), external nitrogen inputs owing to greater public access, or contributions by nitrogen-fixing Cyanobacteria. Greater organic composition of lake substrates,

higher rates of organic matter decomposition owing to higher temperatures, or leaching from acidic soils dominated by heather could have contributed to higher acidity in Picture and Highwood Lakes relative to Upper and Lower Bagley Lakes (Iason and Hester 1993, Härdtle et al. 2007).

Differences in water chemistry could in turn be driving differences in phytoplankton abundance and composition between the Picture and Highwood Lakes and Bagley systems. Chlorophyta and Cyanobacteria are known to have high temperature requirements ($> 15°C$) for photosynthesis relative to other major phytoplankton groups such as the diatoms ($> 5°C$; Wetzel 1983). Cyanobacteria in particular tend to have optimal growth rates at elevated temperatures of 25–35°C (Reynolds 2006). Furthermore, one winter diatom in Lake Baikal, *Aulacoseira baicalensis*, experienced optimal growth rates at 2–3°C, whereas the cyanobacterium, *Synechocystis limnetica*, experienced sustained high growth rates well above 8°C (Richardson et al. 2000). Low seasonal maximum water temperatures ($< 9°C$) in my study may perhaps be the prevailing factor limiting Chlorophyta and Cyanobacteria abundances in Upper and Lower Bagley Lakes. The ability of diatoms to grow optimally under wider ranges in temperature may explain the prevalence of this group in both the Bagley and Picture and Highwood Lakes systems, and consequently why this group did not contribute significantly to cluster separation despite their dominance in the Upper and Lower Bagley Lake phytoplankton assemblages (Tables 3 & 5).

In addition to temperature tolerances and preferences of different phytoplankton groups, prolonged ice cover conditions in Upper and Lower Bagley Lakes could be limiting to slower growing taxa and seasonally late arrivers, which in effect do not have enough time to grow and develop before the next freeze cycle. Included in this slow-growing and late-arriving assemblage are members of Charophyta and Myzozoa that have complex cell wall structures and slow cell

division cycles (Brook 1981, Butterwick et al. 2005, Reynolds 2006). This could partially explain the lack of desmids found in Upper and Lower Bagley Lakes relative to Picture and Highwood Lakes. It might also explain why the cyst form of a dinoflagellate was commonly observed in Upper and Lower Bagley Lakes, whereas the vegetative form of this slow-grower was rare in both lakes (Table 3). In contrast, vegetative dinoflagellates (mainly *Peridinium umbonatum*) were commonly observed in Picture and Highwood Lakes.

Seasonal Succession of Phytoplankton

Variations in phytoplankton assemblages over time in the four lakes indicated seasonal succession patterns typical of those observed in north temperate lakes, albeit squeezed into a short-lived growing season (Figures 13–20). The north temperate pattern includes a burst of spring diatoms, followed by the development of Chlorophyta in the early summer, and a late summer/early autumn increase in Cyanobacteria (Wetzel 1983, Salmaso 2000; Figure 24). The presence of large-sized members of Ochrophyta (e.g. *Dinobryon*), Charophyta, and Myzozoa (e.g. *Peridinium umbonatum*) is also common in oligotrophic waters, with increased abundances during the mid to late season characteristic of these larger, slower growing organisms. (Brook 1981, Reynolds 2006, Bellinger and Sigee 2010). The north temperate seasonal succession pattern fit well with the Picture and Highwood Lakes system, but a slightly different pattern emerged in Upper and Lower Bagley Lakes where the diatoms dominated the assemblages throughout the season. In Upper Bagley Lake, a small peak in diatoms coincided with ice-melt on the lake and was followed by a second burst of diatoms late in the season—a pattern commonly observed in less-productive north temperate lakes (Figure 16; Wetzel 1983). In Lower Bagley Lake, diatom abundance was relatively uniform through the season. One reason for this could be the shallow water depth (~1.0 m) contributing to greater benthic influences in this lake.

The heavy siliceous cell wall and non-motility of diatoms make them more susceptible to sinking, thus potentially contributing to high relative phytoplankton biomass near the benthos (Reynolds 2006). Other reasons might be related to hydrology. For example, surface outflow or controlled releases at the Lower Bagley Lake dam could hamper seasonal variations in diatom abundance. If diatom growth rates do not keep pace with flushing rates, seasonal patterns may not be apparent.

A pattern common to all four lakes was a burst of unknown phytoplankton, collectively grouped as "undetermined", late in the sampling period (Figure 20). The tiny sizes (range 6–1767 μm^3 per cell; median 95 μm^3), morphological traits (mostly unicellular flagellates), and seasonally late emergence of this group suggest that most of the unknowns may be members of the family, Chrysophyceae, in the phylum, Ochrophyta (Karl Bruun, Nostoca Algae Laboratory, personal communication). The prevalence of tiny unicellular forms is common in oligotrophic mountain lakes, where being tiny and motile provides advantage by allowing efficient nutrient uptake (high cell surface area/volume ratio) and mortality avoidance by grazers or sedimentation (Wetzel 1983, Naselli-Flores et al. 2007).

Questions for Future Study

I propose some questions that were not investigated in my study, but that would lead to better understanding of the ecology of North Cascade high-elevation lakes. At the scale of my study, one question would be to determine the role of hydrology in contributing to similarities and differences in water chemistry and phytoplankton among the four lakes. Considering that the lakes clustered best into two groups—one group in which the lakes were hydrologically connected by surface water, the other group in which the lakes were not—it would interesting to determine the extent to which surface hydrological connectivity contributed to similarities in

water chemistry and phytoplankton between Upper and Lower Bagley Lakes, and the extent to which the lack of surface hydrological connectivity contributed to differences, if any, between Picture and Highwood Lakes. Further investigation might also include determining the effects of the Lower Bagley Lake dam on water chemistry and phytoplankton in the Bagley system.

A second question concerns the role of top-down controls on phytoplankton composition and abundance as well as nutrient cycling, and conversely, the role of bottom-up controls on higher trophic levels in the four study lakes. In high-elevation lakes of the Beartooth Mountains in Montana, for example, the presence of zooplankton, in conjunction with cold temperatures and high ultraviolet radiation, effectively suppressed phytoplankton biomasses (Williamson et al. 2010). In addition, the presence of fish can alter phytoplankton biomasses indirectly by means of a trophic cascade, in which planktivorous fish suppress grazing zooplankton (Carpenter et al. 1985), or through bioturbation, in which nutrients may be recycled from the bottom sediments into the water column (Schindler et al. 2001). Furthermore, the relative importance of top-down vs. bottom-up controls may be dependent on various morphological factors (Moss et al. 1994). The role of top-down vs. bottom-up controls in aquatic systems has been a topic of interest and debate (Carpenter et al. 1985, DeMelo et al. 1992, Carpenter and Kitchell 1992). In the lakes of my study, an investigation into trophic dynamics would add an important piece of understanding to the ecology of these high-elevation systems.

A third question would be to compare and contrast limnetic versus littoral phytoplankton assemblages. Phytoplankton assemblages are known to be patchy in aquatic systems owing to differences in microhabitat (e.g. water depth, substrate, macrophytes) or to external factors such as wind and circulation patterns (Brook 1981, Bellinger and Sigee 2010). In my study,

phytoplankton patchiness in the limnetic zones of the lakes was evident (Figures 13–20), and laboratory notes indicated qualitative differences between littoral and limnetic phytoplankton.

Because it is difficult to make sweeping generalizations about patterns after one season of field study, a fourth question would be to determine how basic patterns observed in my study compare and contrast inter-annually. For example, my study occurred after a prolonged La Niña winter and during a two-month period of no recorded precipitation (National Atmospheric Deposition Program 1984). Weather conditions on the majority of sampling dates were calm and sunny and contributed to unstable stratification patterns in two lakes. The presence of unstable stratification may affect the distribution of organisms in the water column (Pernica and Wells 2012), and thus influence which organisms were encountered in surface water samples. Additionally, ice-out timing in Upper and Lower Bagley Lakes was at least four weeks later than in Picture and Highwood Lakes. In some previous years, Upper Bagley Lake was substantially ice-covered through the summer. It would be interesting to see if different patterns emerge under different climate and meteorological conditions.

Results from my study indicate that physical forces (e.g. ice-cover duration and snowpack influence) may be important regulators in phytoplankton composition and abundance in North Cascade high-elevation lakes. A final question concerns how climate change will affect the magnitude and timing of snowpack melt and lake thaw conditions over the long term, and in turn how these will affect the aquatic communities in North Cascade lakes. Projected physical changes to lakes in the Pacific coastal region of North America include reductions in snowpack and lake-ice thickness and duration, increases in mean annual water temperatures, and prolonged periods of thermal stratification (Dibike et al. 2011). These factors may influence the phenological timing of phytoplankton (Meis et al. 2009) as well as Cyanobacteria dominance in

lakes (Wagner and Adrien 2009). In the lakes of my study, one hypothesis is that, over the long term, regional climate change might contribute to higher total biomasses in the four lakes, and higher abundances of Cyanobacteria, Chlorophyta, and some slower growing taxa in Upper and Lower Bagley Lakes. With increasing attention focused on projecting climate change impacts to freshwater systems, perhaps this is the underlying issue at the broader scale.

SUMMARY AND CONCLUSIONS

The overarching objective of my study was to provide basic information and understanding of phytoplankton assemblages in a small set of high-elevation lakes of the North Cascades. I established and focused on three research questions: determining phytoplankton composition and abundances in the lakes; identifying seasonal succession patterns in phytoplankton; and relating trends in phytoplankton to water chemistry variables.

Water chemistry results indicated a nutrient-poor, low-production, cold temperature environment in these lakes. In spite of the small scale of my study, I observed a phytoplankton assemblage comparable in richness to larger regional studies of North Cascade mountain lakes. Temporal variations in phytoplankton generally reflected seasonal succession patterns common in north temperate lakes, although compressed into a much smaller window of opportunity for the phytoplankton to grow and develop.

Spatial variations also differentiated phytoplankton assemblages, most distinctively by the clustering of the four lakes into two groups—the Picture and Highwood Lakes system and Bagley system. These two groups further associated with the clustering of water chemistry variables into the same two groups. Differences in water chemistry between the two lake systems could be explained conceptually by differences in their external physical environments, where

the Bagley system was influenced by a longer period of lake ice cover and snowpack melt from adjacent mountain slopes. At the coarse scale, this may have been the distinguishing feature effecting differences in the abundance, composition, and timing of phytoplankton between the two systems.

This study provides a small piece of understanding to the ecology of high-elevation lakes in the North Cascades. Further investigations into the ecology of North Cascade mountain lakes would be useful, especially in the larger context of interpreting natural variations and projecting impacts of environmental change on the freshwaters of this region.

LITERATURE CITED

APHA. 2012. Standard Methods for the Examination of Water and Wastewater, 22nd Edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Battarbee, R.W., M. Kernan, and N. Rose. 2009. Threatened and stressed mountain lakes of Europe: assessment and progress. Aquatic Ecosystem Health and Management 12:18–128.

Bellinger, E.G. and D.C. Sigee. 2010. Freshwater Algae: Identification and Use as Bioindicators. John Wiley & Sons, Ltd, Chichester, UK.

Brook, A.J. 1981. The Biology of Desmids. University of California Press, Berkeley and Los Angeles.

Butterwick, C., S.I. Heaney, and J.F. Talling. 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. Freshwater Biology 50:291–300.

Carpenter, S.R., J.F. Kitchell, and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience 35:634–639.

Carpenter, S.R. and J.F. Kitchell. 1992. Trophic cascade and biomanipulation: Interface of research and management—a reply to to the comment by DeMelo et al. Limnology and Oceanography 37:208–213.

Clow, D.W. and D.H. Campbell. 2008. Atmospheric deposition and surface-water chemistry in Mount Rainier and North Cascades National Parks, U.S.A., water years 2000 and 2005– 2006. U.S. Geological Survey Scientific Investigations Report 2008–5152.

DeMelo, R., R. France, and D.J. McQueen. 1992. Biomanipulation: Hit or myth? Limnology and Oceanography 37:192–207.

Dibike, Y., T. Prowse, T. Saloranta, and R. Ahmed. 2011. Response of Northern Hemisphere lake-ice cover and lake-water thermal structure patterns to a changing climate. Hydrological Processes 25:2942–2953.

Dodson, S. 2005. Introduction to Limnology. McGraw-Hill, New York.

Eggermont, H., D. Verschuren, L. Audenaert, L. Lens, J. Russell, G. Klaassen, and O. Heiri. 2010. Limnological and ecological sensitivity of Rwenzori mountain lakes to climate warming. Hydrobiologia 648:123–142.

Guiry, M.D. and G.M. Guiry 2013. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org.

Härdtle, W., G. von Oheimb, M. Niemeyer, T. Niemeyer, T. Assmann, and H. Meyer. 2007. Nutrient leaching in dry heathland ecosystems: effects of atmospheric deposition and management. Biogeochemistry 86:201–215.

Hauer, F.R., J.S. Baron, D.H. Campbell, K.D. Fausch, S.W. Hostetler, G.H. Leavesley, P.R. Leavitt, D.M. McKnight, and J.A. Stanford. 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, USA and Canada. Hydrological Processes 11:903–924.

Hecky, R.E. and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnology and Oceanography 33:796–822.

Hillebrand, H., C.D., Durselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35:403–424.

Hinder, B., M. Gabathuler, B. Steiner, K. Hanselmann, and H.R. Preisig. 1999. Seasonal dynamics and phytoplankton diversity in high mountain lakes (Jöri lakes, Swiss Alps). Journal of Limnology 58:152–161.

Iason, G.R. and A.J. Hester. 1993. The response of heather (*Calluna vulgaris*) to shade and nutrients—predictions of the carbon-nutrient balance hypothesis. Journal of Ecology 81:75–80.

Larson, G.L., C.D. McIntire, R.E. Truitt, W.J. Liss, R.L. Hoffman, E. Deimling, and G.A. Lomnicky. 1998. Phytoplankton assemblages in high-elevation lakes in the northern Cascade Mountains, Washington State, USA. Archiv fur Hydrobiologie 142:71–93.

Larson, G.L., G. Lomnicky, R. Hoffman, W.J. Liss, and E. Deimling. 1999. Integrating physical and chemical characteristics of lakes into the glacially influenced landscape of the Northern Cascade mountains, Washington State, USA. Environmental Management 24:219–228.

Lomnicky, G.A. 1995. Lake classification in the glacially influenced landscape of the North

    Cascade mountains, Washington, U.S.A. Ph.D. Dissertation, Oregon State University,

    Corvallis.

Loranger, T.J. and D.F. Brakke. 1988. The extent of snowpack influence on water chemistry in a

    North Cascades Lake, Water Resources Research 24:723–726.

Matthews, R.A., G.B. Matthews, and W.J. Ehinger. 1991. Classification and ordination of

    limnological data—comparison of analytical tools. Ecological Modelling 53:167–87.

McKnight, D.M., R.L. Smith, J.P. Bradbury, J.S. Baron, and S. Spaulding. 1990. Phytoplankton

    dynamics in three Rocky Mountain lakes, Colorado, U.S.A. USGS Staff—Published

    Research. Paper 258. http://digitalcommons.unl.edu/usgsstaffpub/258.

Meis, S., S.J. Thackeray, and I.D. Jones. 2009. Effects of recent climate change on

    phytoplankton phenology in a temperate lake. Freshwater Biology 54:1888–1898.

Morris, D.P., and W.M. Lewis. 1988. Phytoplankton nutrient limitation in Colorado mountain

    lakes. Freshwater Biology 20:315–27.

Naselli-Flores, L., J. Padisák, and M. Albay. 2007. Shape and size in phytoplankton ecology: do

    they matter? Hydrobiologia 578:157–161.

National Atmospheric Deposition Program. 1984. NADP/NTN Monitoring Location WA19.

    Available online at http://nadp.sws.uiuc.edu/sites/siteinfo.asp?net=NTN&id=WA19

    (accessed 11 April 2013).

Nürnberg, G.K. 1996. Trophic state of clear and colored, soft- and hardwater lakes with special

    consideration of nutrients, anoxia, phytoplankton and fish. Lake and Reservoir

    Management 12:432–447.

Padisák, J. and C.S. Reynolds. 2003. Shallow lakes: the absolute, the relative, the functional and the pragmatic. Hydrobiologia 506–509:1–11.

Pelto, M.S. 2006. The current disequilibrium of North Cascade glaciers. Hydrological Processes 20:769–779.

Pernica, P. and M. Wells. 2012. Frequency of episodic stratification in the near surface of Lake Opeongo and other small lakes. Water Quality Research Journal of Canada 47: 227–237.

Pielou, E.C. 1975. Ecological Diversity. John Wiley & Sons, New York.

Psenner, R. 2003. Alpine lakes: extreme ecosystems under the pressure of global change, EAWAG News 55:12–14.

Reynolds, C.S. 2006. The Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Richardson, T.L., C.E. Gibson, and S.I. Heaney. 2000. Temperature, growth and seasonal succession of phytoplankton in Lake Baikal, Siberia. Freshwater Biology 44:431–440.

Rogora, M. R. Mosello, and S. Arisci. 2003. The effect of climate warming on the hydrochemistry of alpine lakes. Water, Air, and Soil Pollution 148:347–361.

Rogora, M., R. Mosello, S. Arisci, M.C. Brizzio, A. Barbieri, R. Balestrini, P. Waldner, M. Schmitt, M. Stähli, A. Thimonier, M. Kalina, H. Puxbaum, U. Nickus, E. Ulrich, and A. Probst. 2006. An overview of atmospheric deposition chemistry over the Alps: present status and long-term trends. Hydrobiologia 562:17–40.

Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Archiv fur Hydrobiologie 62:1– 28.

Salmaso, N. 2000. Factors affecting the seasonality and distribution of cyanobacteria and chlorophytes: a case study from the large lakes south of the Alps, with special reference to Lake Garda. Hydrobiologia 438:43–63.

Schindler, D.W., R.A. Knapp, and P.R. Leavitt. 2001. Alteration of nutrient cycles and algal

      production resulting from fish introductions into mountain lakes. Ecosystems 4:308–321.

Sheibley, R.W., J.R. Foreman, P.W Moran, and P.W. Swarzenski. 2012. Atmospheric

      deposition, water-quality, and sediment data for selected lakes in Mount Rainer, North

      Cascades, and Olympic National Parks, Washington, 2008–10: U.S. Geological Survey

      Data Series 721.

Skjelkvåle, B.L. and R.F. Wright. 1998. Mountain lakes: sensitivity to acid deposition and global

      climate change. Ambio 27:280–286.

Tabor, R.W and R.A. Haugerud. 1999. Geology of the North Cascades: A Mountain Mosaic. The

      Mountaineer Books, Seattle.

Thackeray, S.J., I.D. Jones and S.C. Maberly. 2008. Long-term change in the phenology of

      spring phytoplankton: species-specific responses to nutrient enrichment and climatic

      change. Journal of Ecology 96:523–35.

Tolotti, M., M. Manca, N. Angeli, G. Morabito, B. Thaler, E. Rott, and E. Stuchlik. 2006.

      Phytoplankton and zooplankton associations in a set of alpine high altitude lakes:

      geographic distribution and ecology. Hydrobiologia 562:99–122.

Tolotti, M., A. Boscaini, and N. Salmaso. 2010. Comparative analysis of phytoplankton patterns

      in two modified lakes with contrasting hydrological features. Aquatic Sciences 72:213–

      226.

Tolotti, M., H. Thies, U. Nickus, and R. Psenner. 2012. Temperature modulated effects of

      nutrients on phytoplankton changes in a mountain lake. Hydrobiologia 698:61–75.

Wagner, C. and R. Adrian. 2009. Cyanobacteria dominance: quantifying the effects of climate

      change. Limnology and Oceanography 54:2460–2468.

Wetzel, R.G. 1983. Limnology. Second Edition. Saunders College Publishing, Harcourt Brace

    College Publishers, Fort Worth.

Williamson, C.E., C. Salm, S.L. Cooke, and J.E. Saros. 2010. How do UV radiation, temperature,

    and zooplankton influence the dynamics of alpine phytoplankton

    communities? Hydrobiologia 648:73–81.

FIGURES
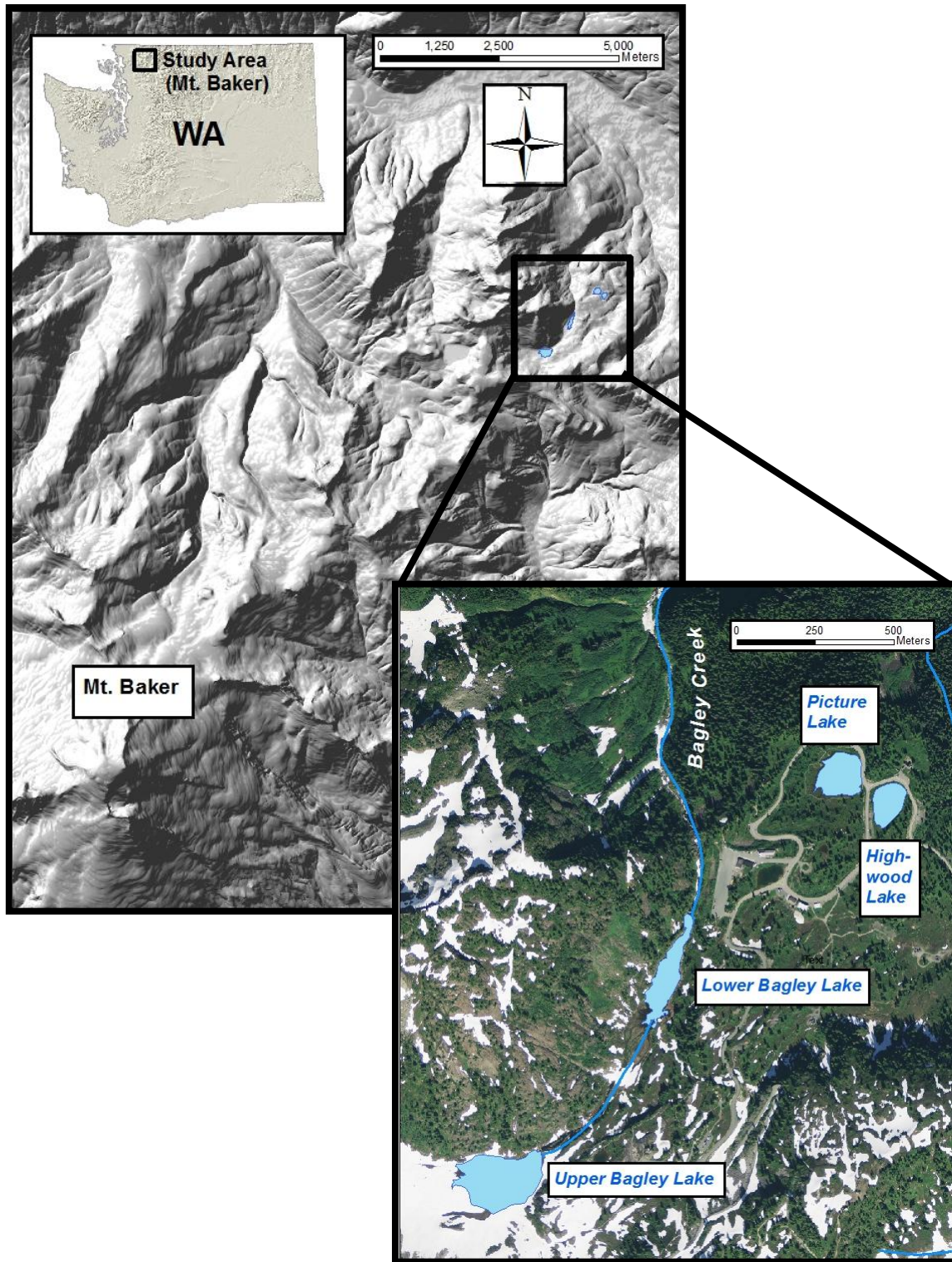
Figure 1. Map showing locations of the four study lakes near Mt. Baker, WA.
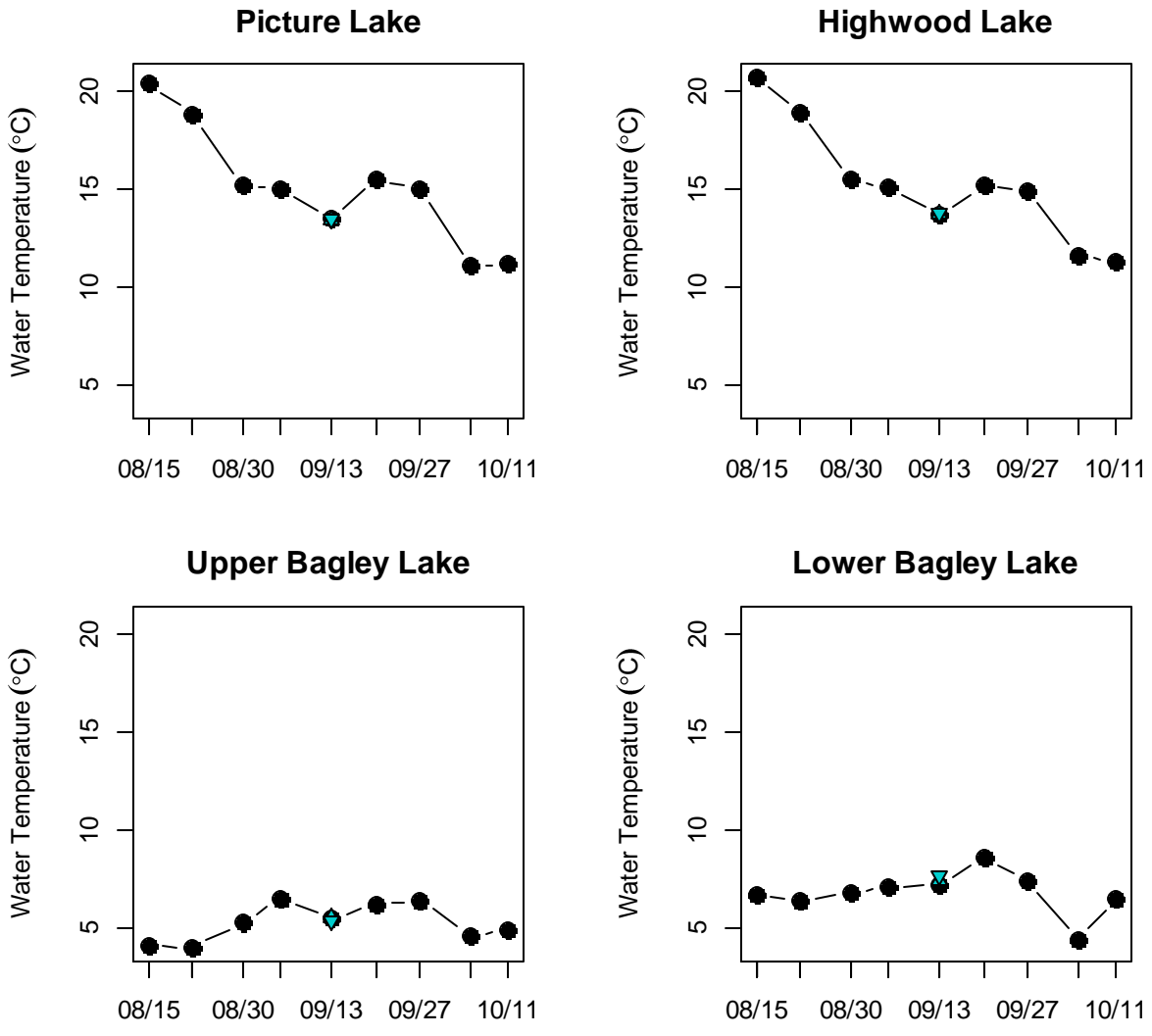
Figure 2. Water temperatures in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
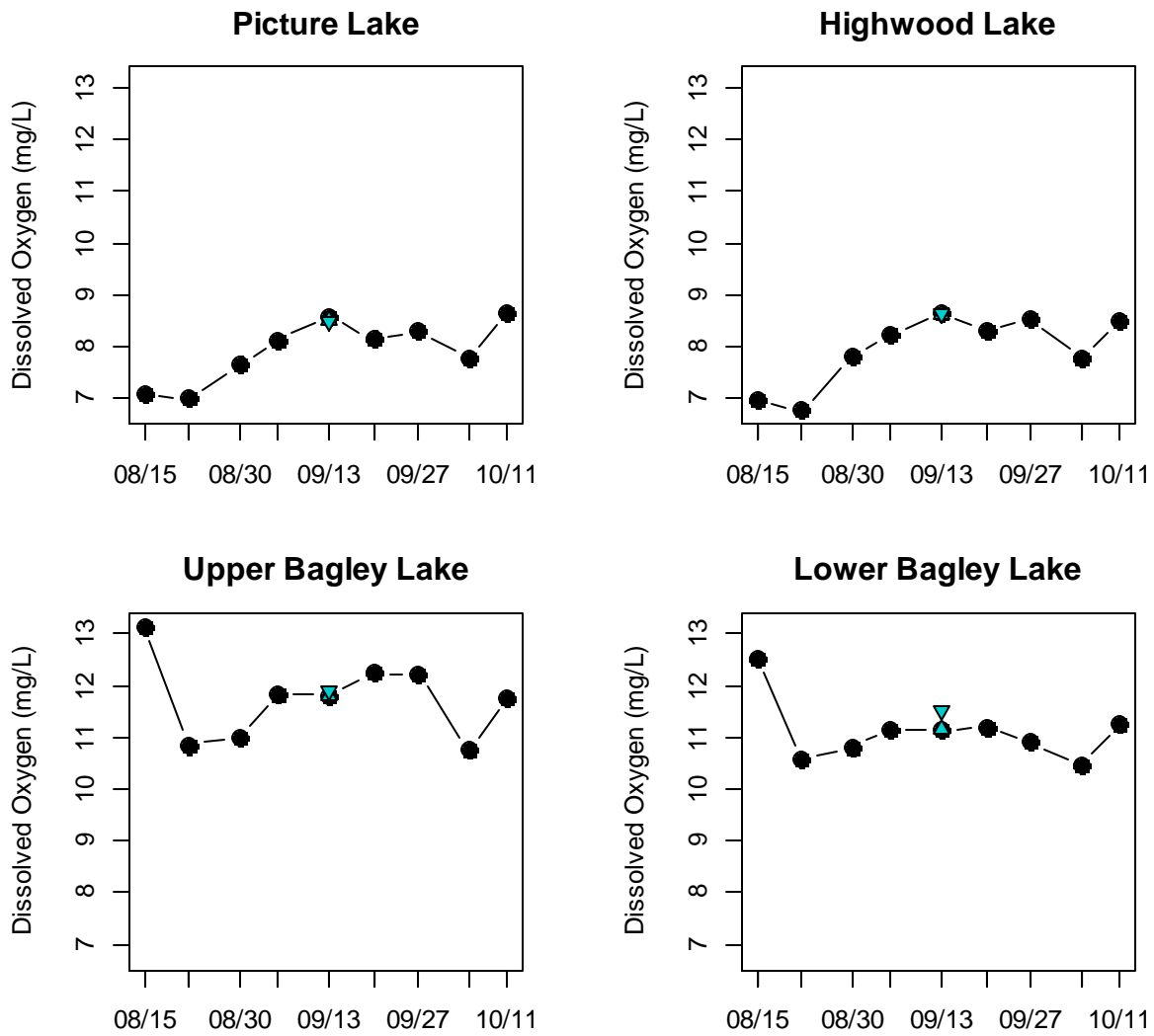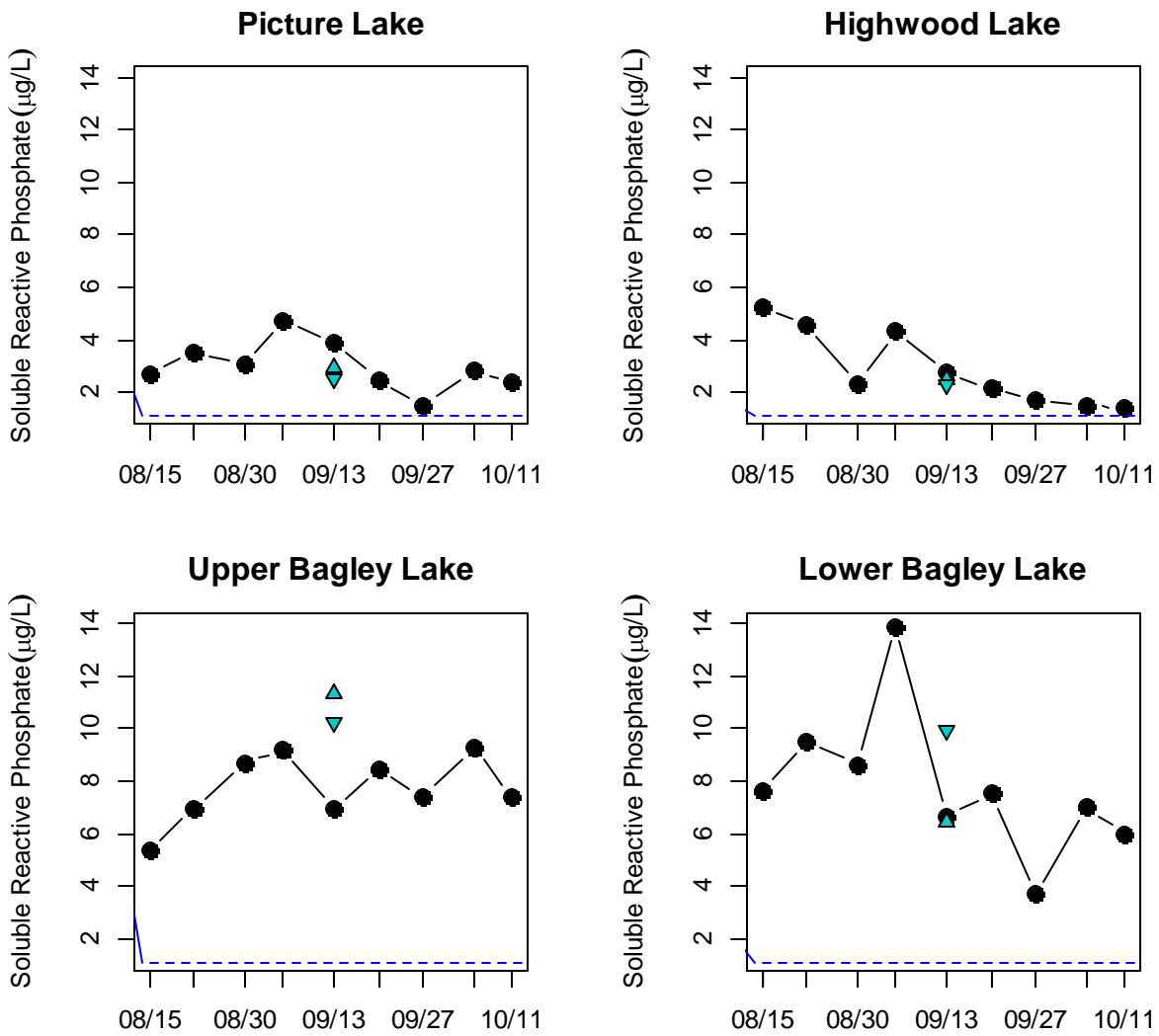
Figure 3. Dissolved oxygen concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
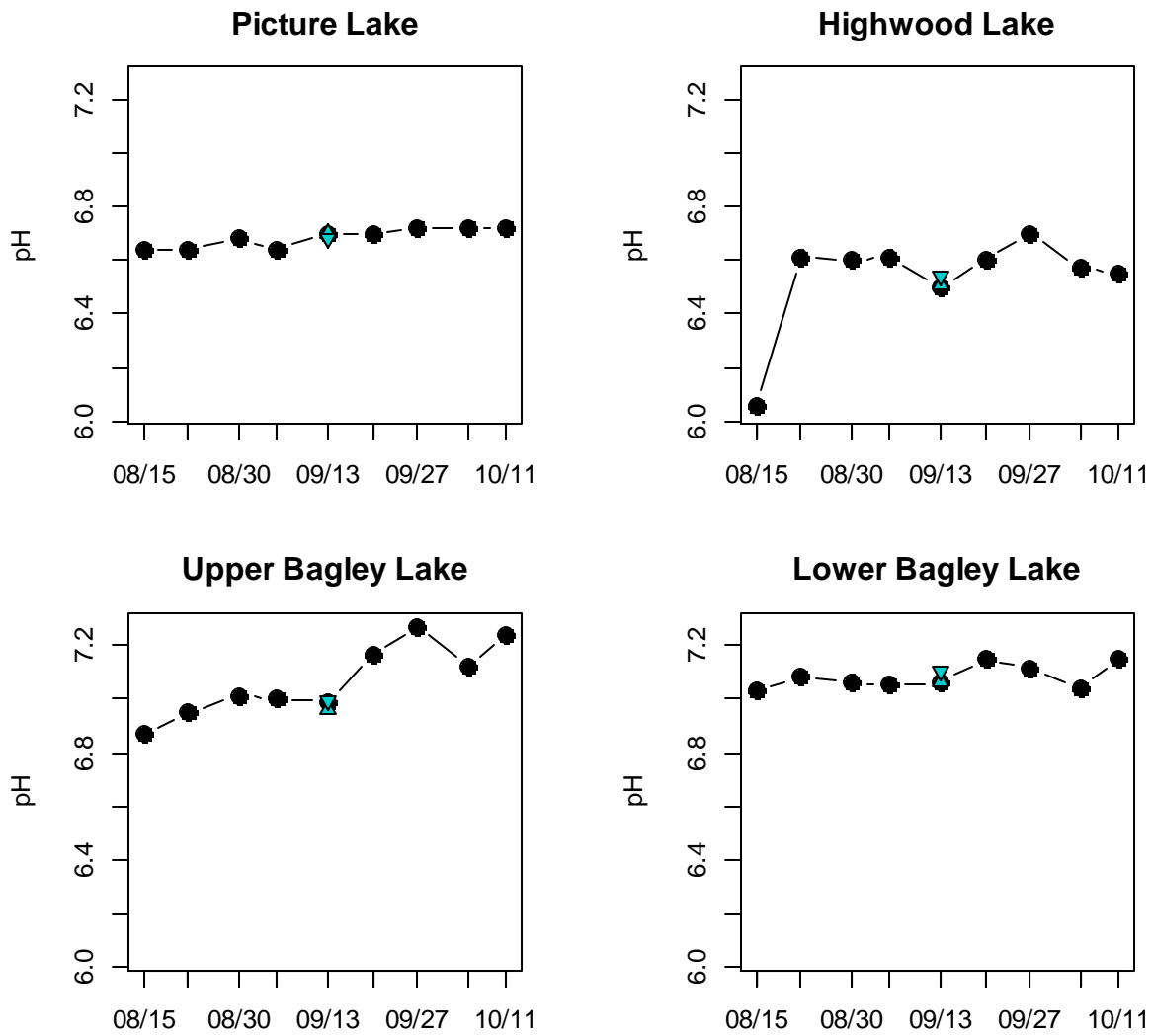
Figure 4. pH in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
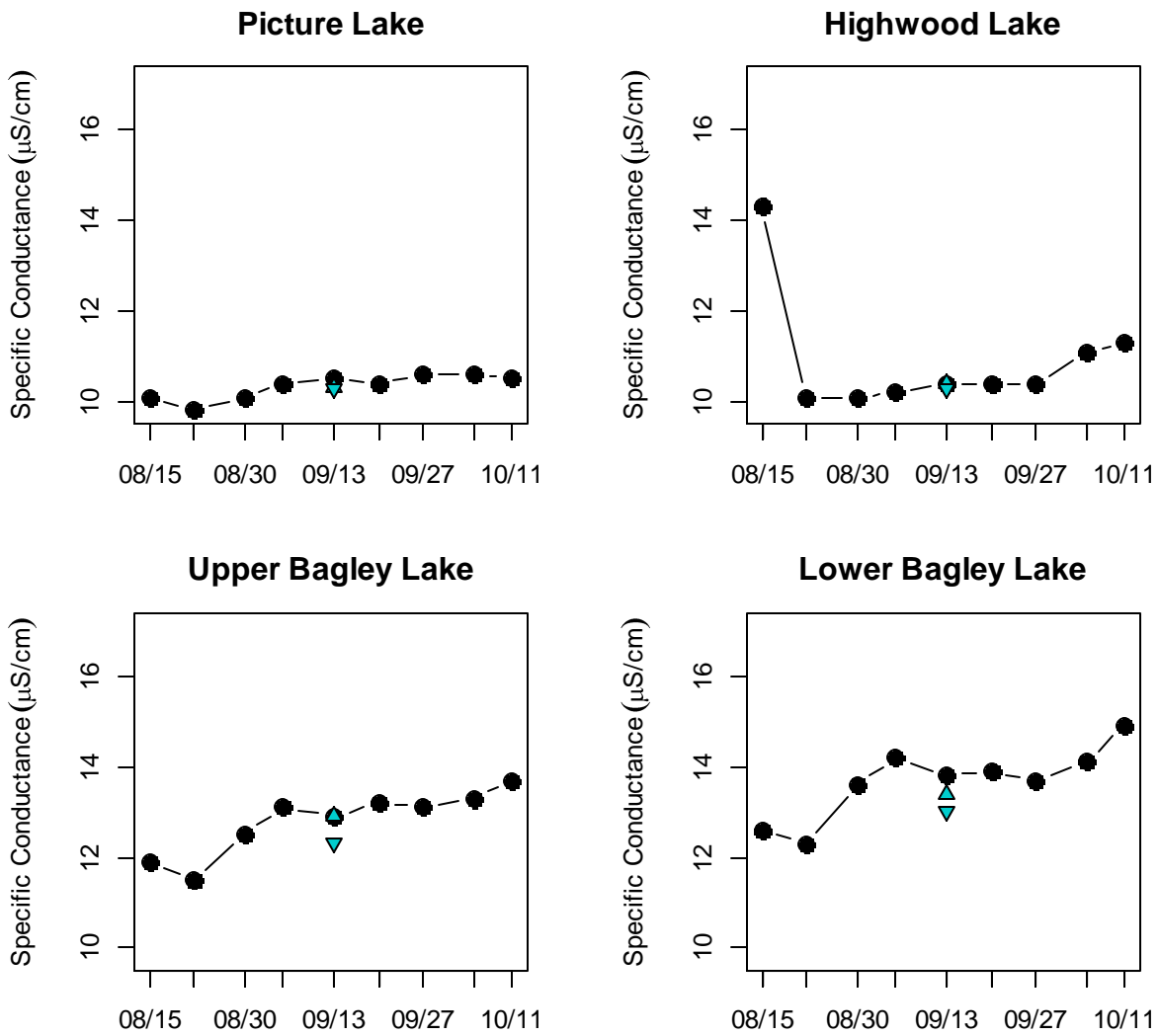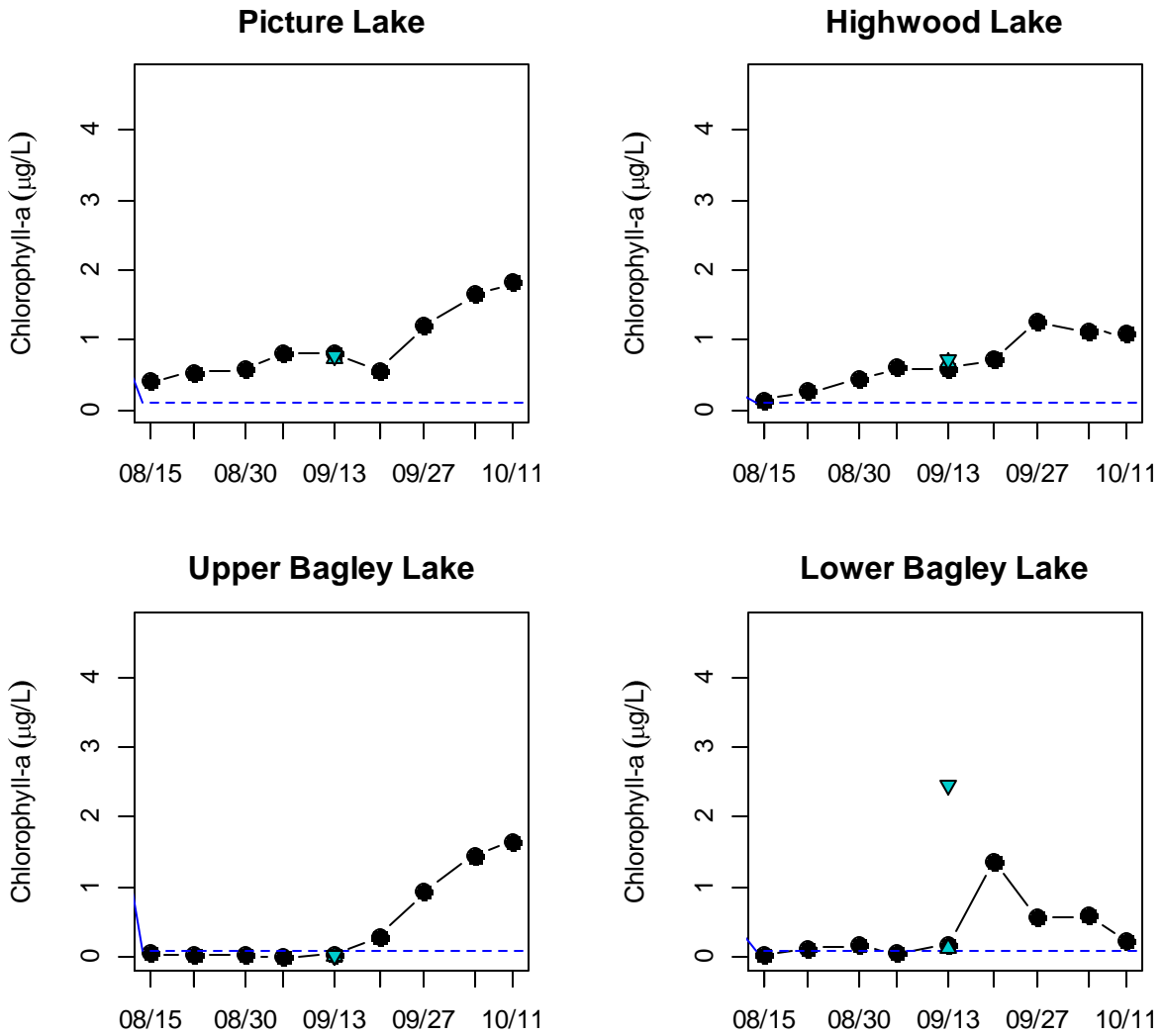
Figure 5. Specific conductance in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
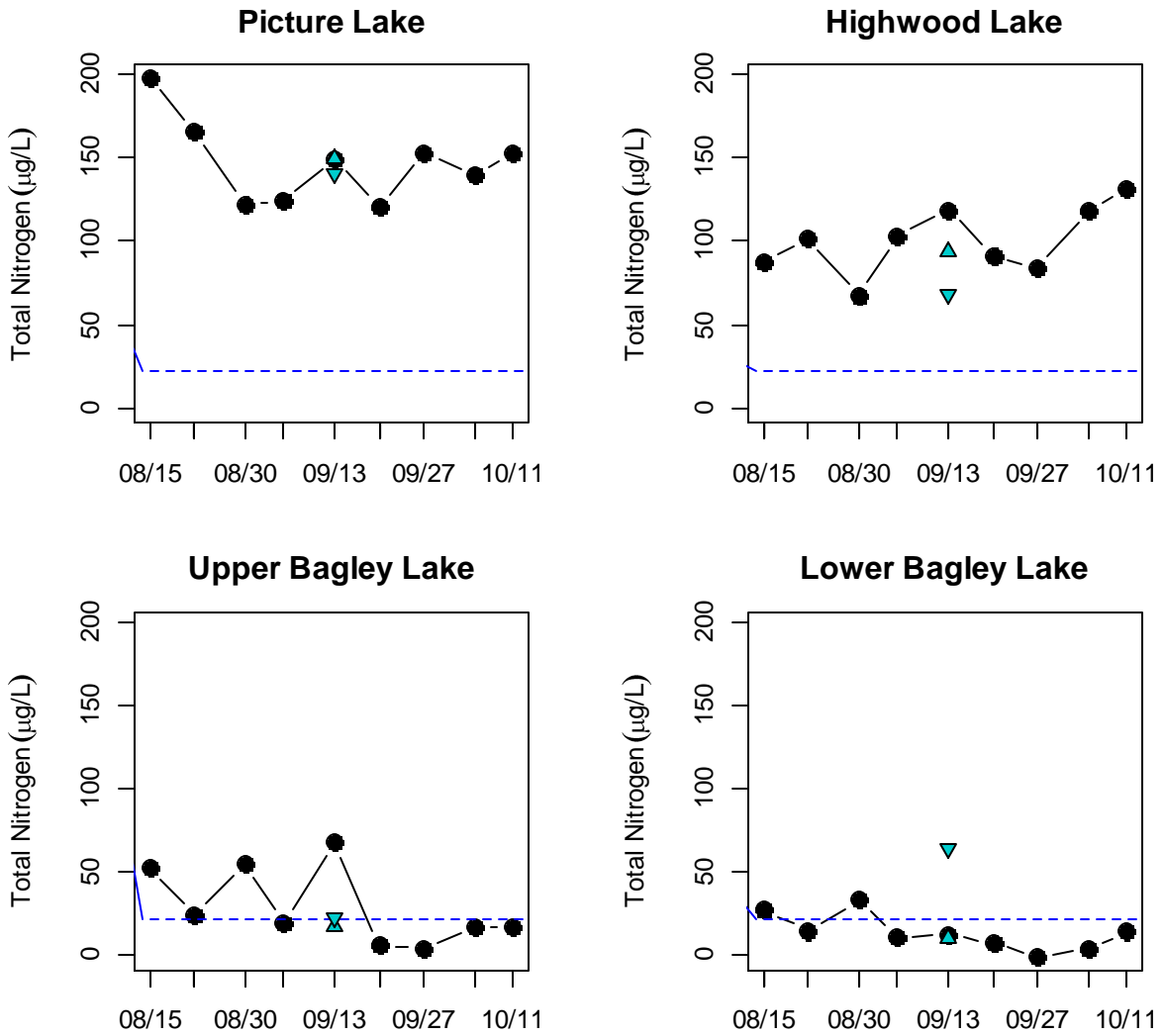
Figure 6. Total nitrogen concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles). Detection limit is shown (dashed line).
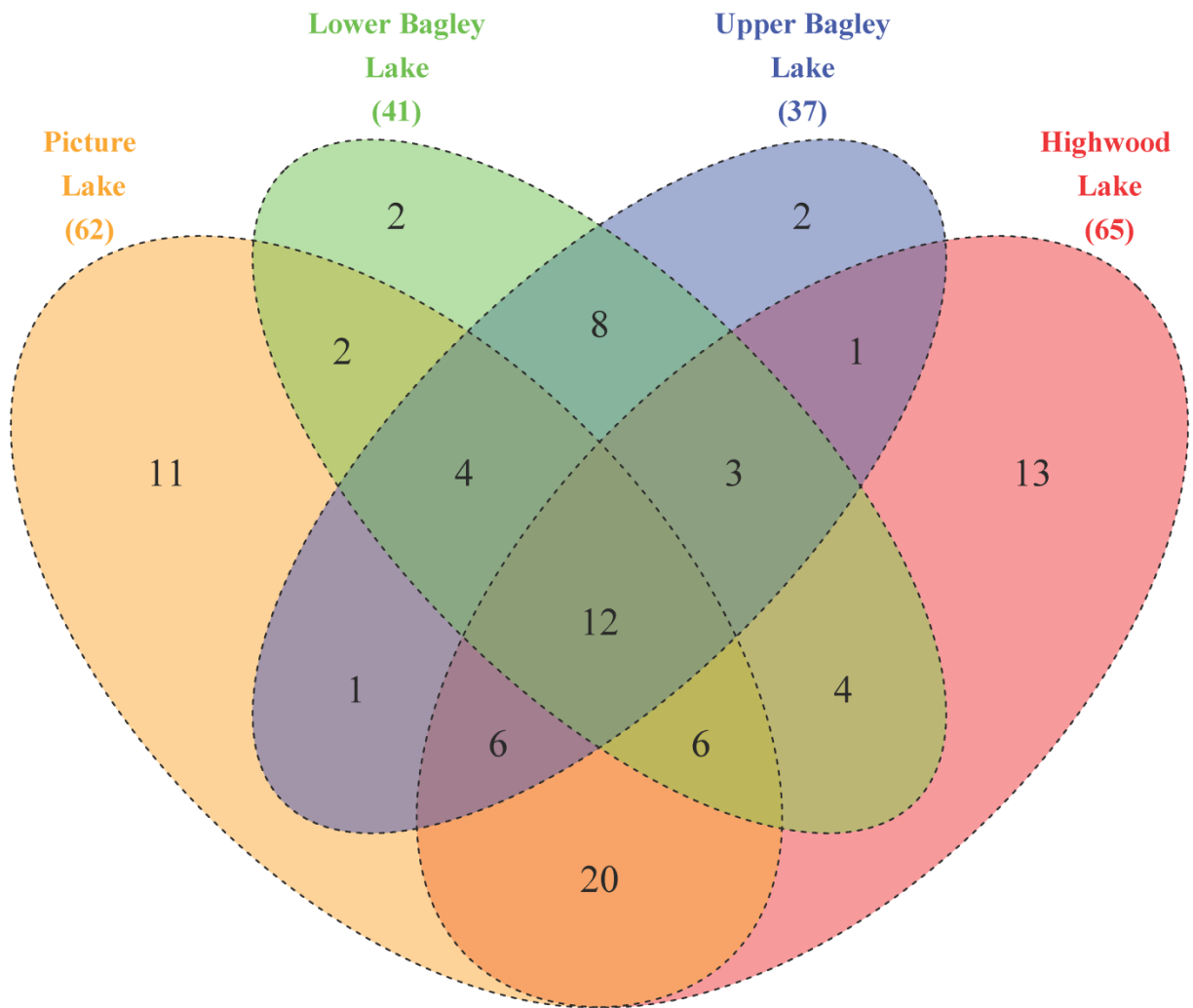
42
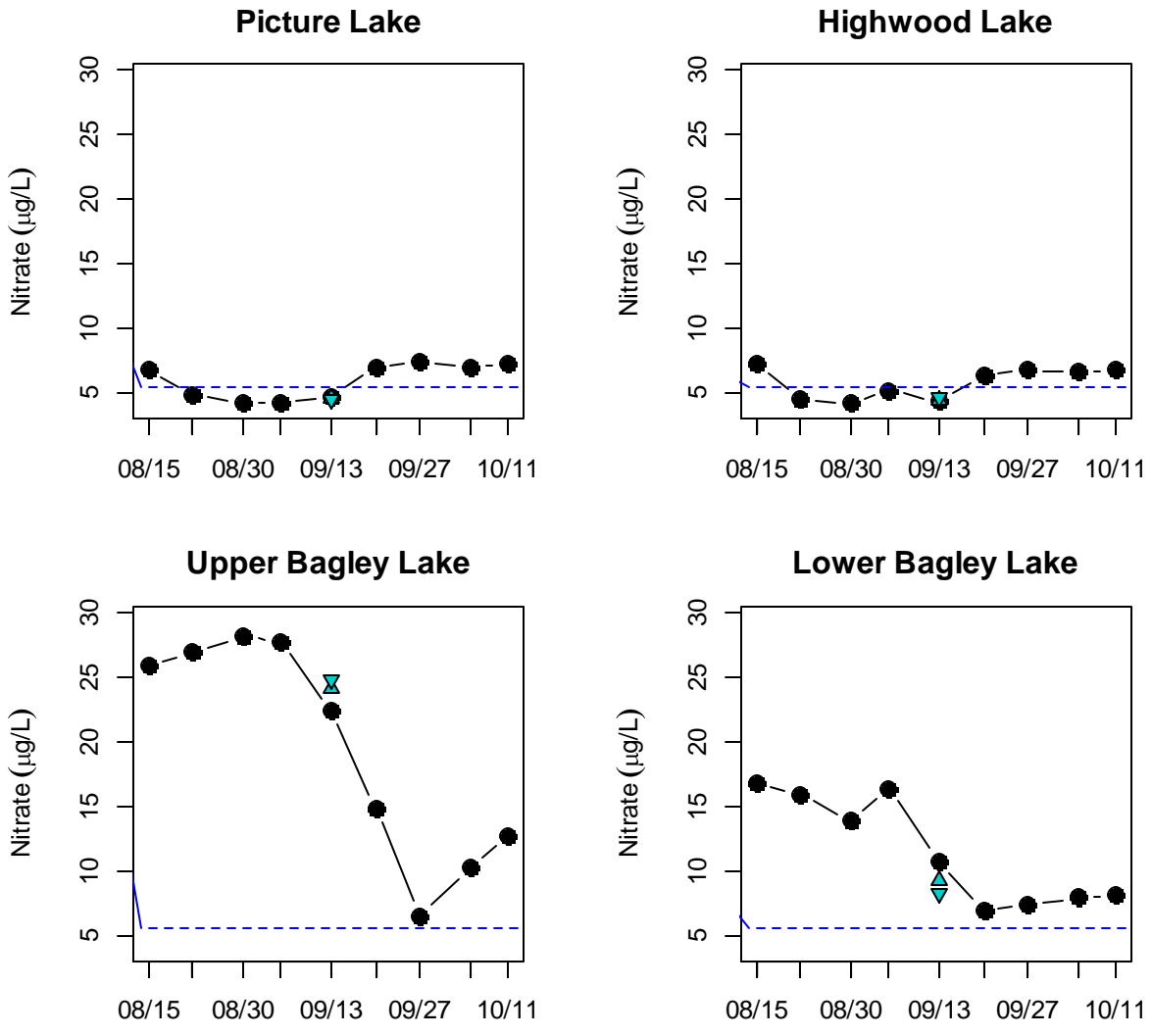
Figure 7. Nitrate concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles). Detection limit is shown (dashed line).
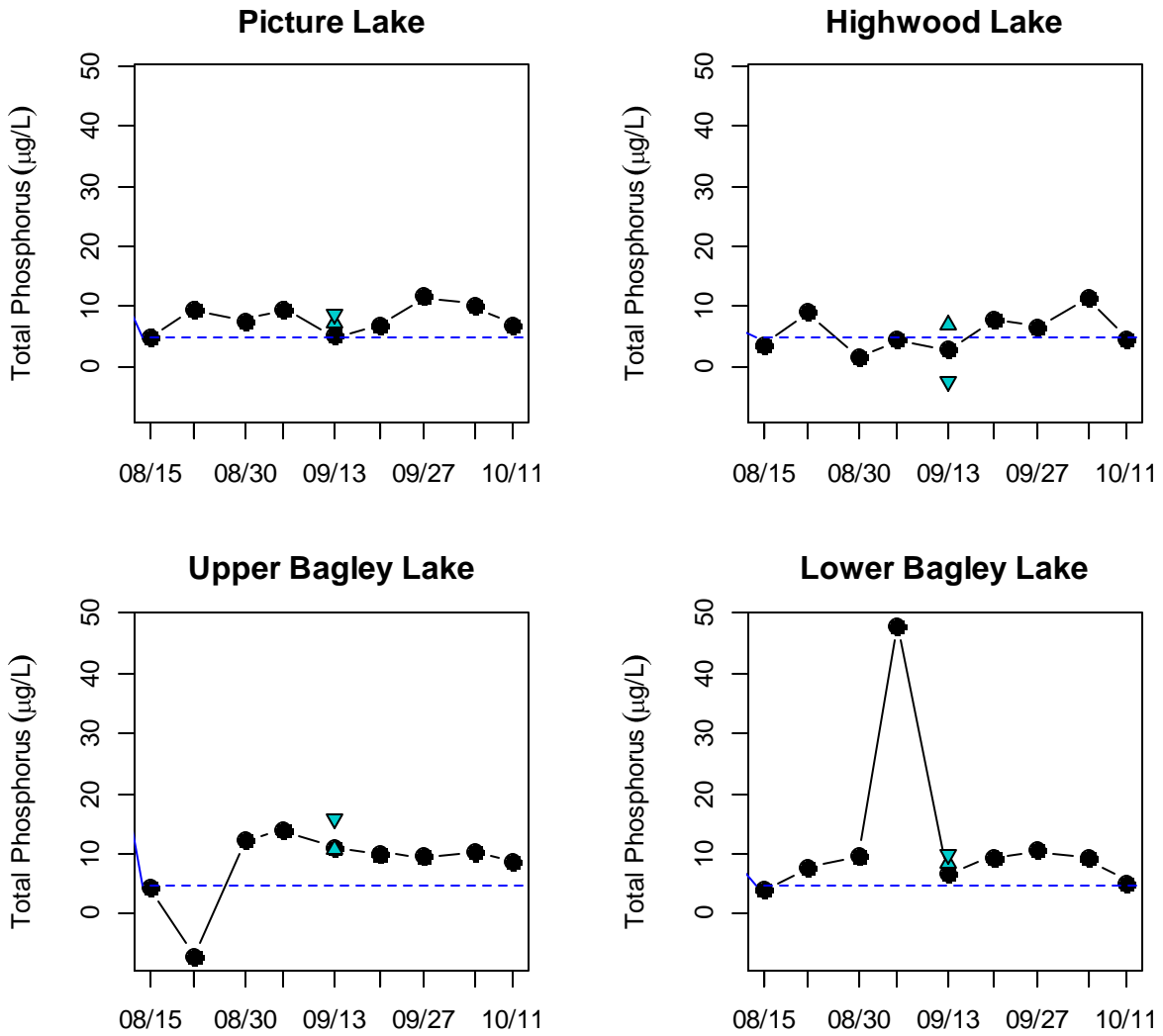
Figure 8. Total phosphorus concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles). Detection limit is shown (dashed line).

Figure 9. Soluble reactive phosphorus concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles). Detection limit is shown (dashed line).

Figure 10. Chlorophyll-*a* concentrations in Picture, Highwood, Upper Bagley, and Lower Bagley Lakes from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).

Figure 11. Venn diagram showing number of unique and shared taxa in the four study lakes. Taxa richness of each lake is given in parentheses.
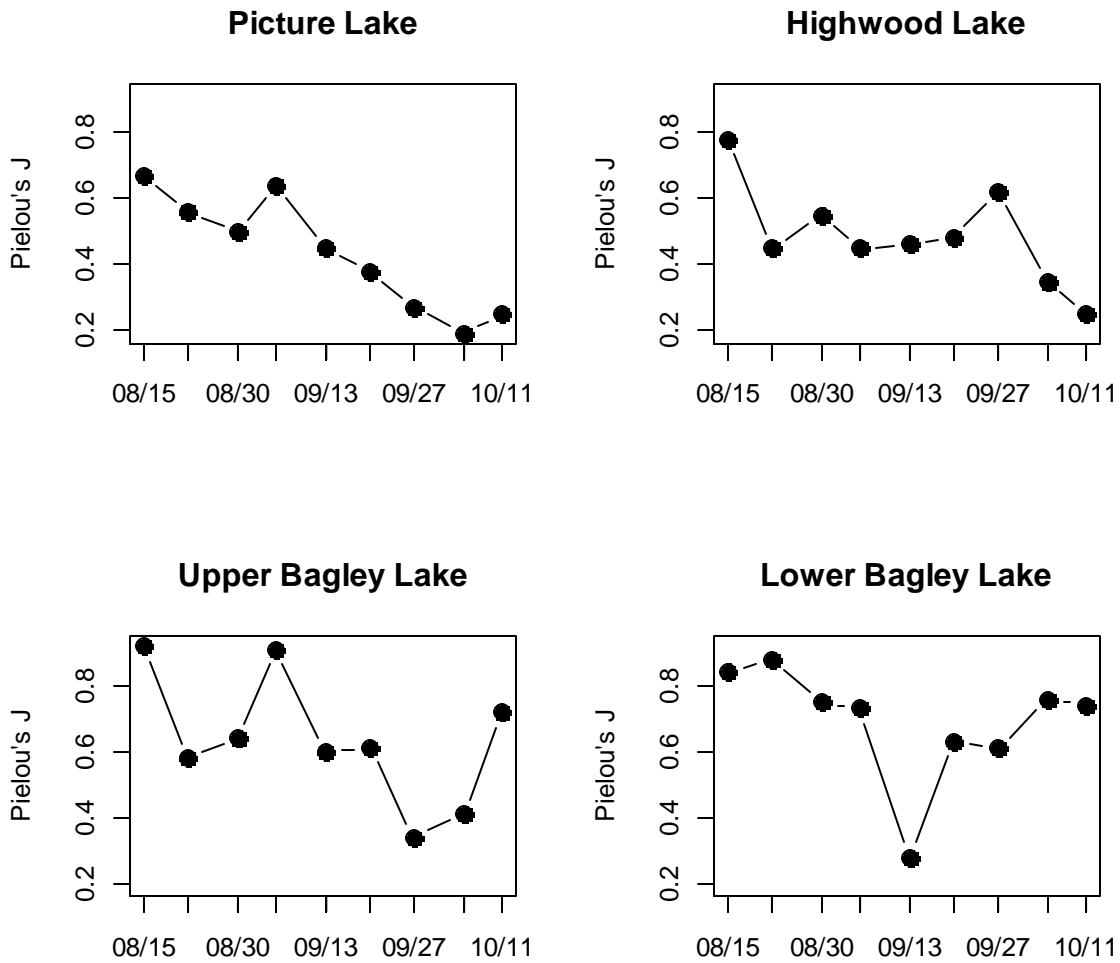
Figure 12. Evenness index (Pielou's *J*) calculated from phytoplankton cell density and plotted on each sampling date from August to October 2012 in the four study lakes.
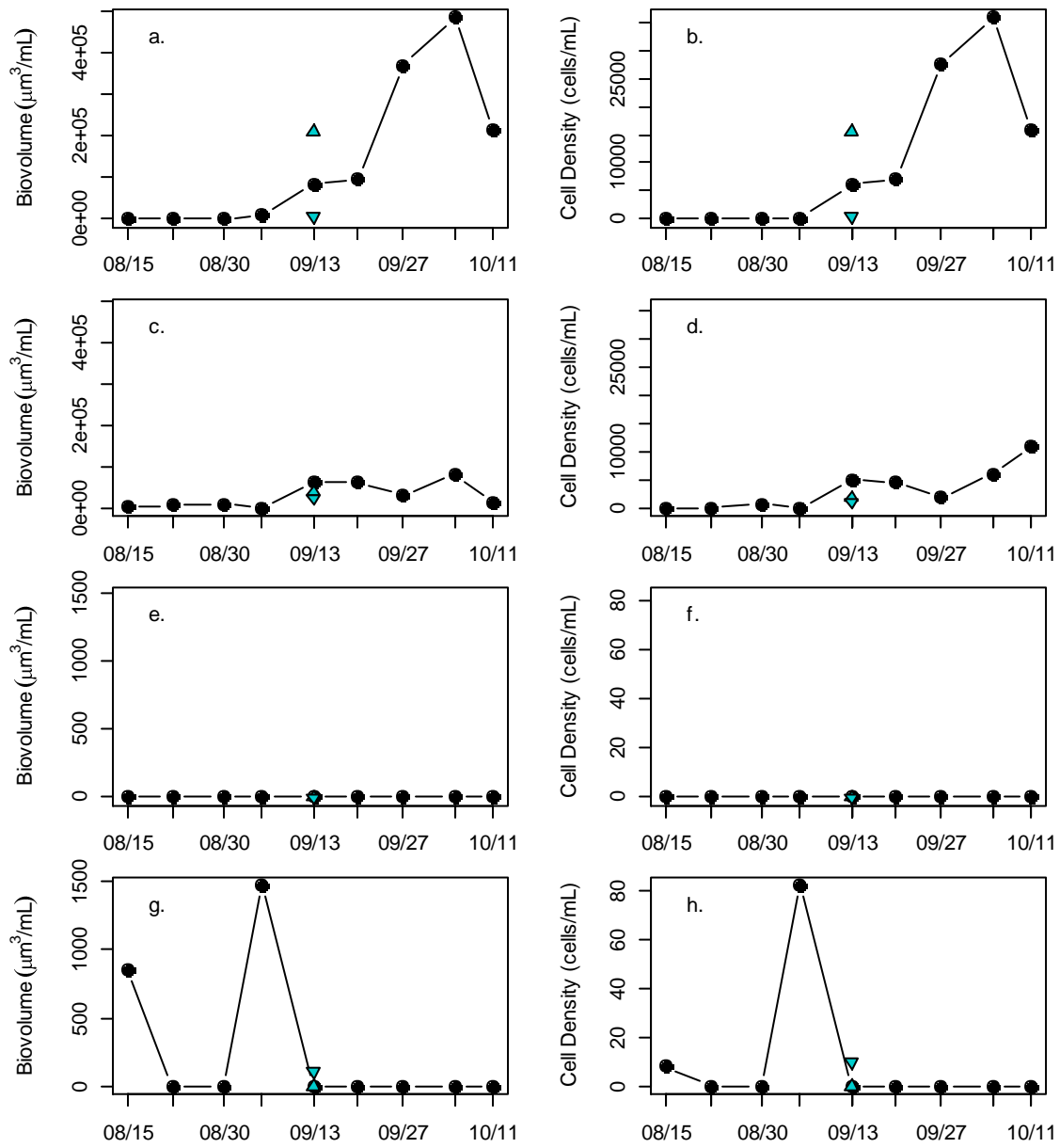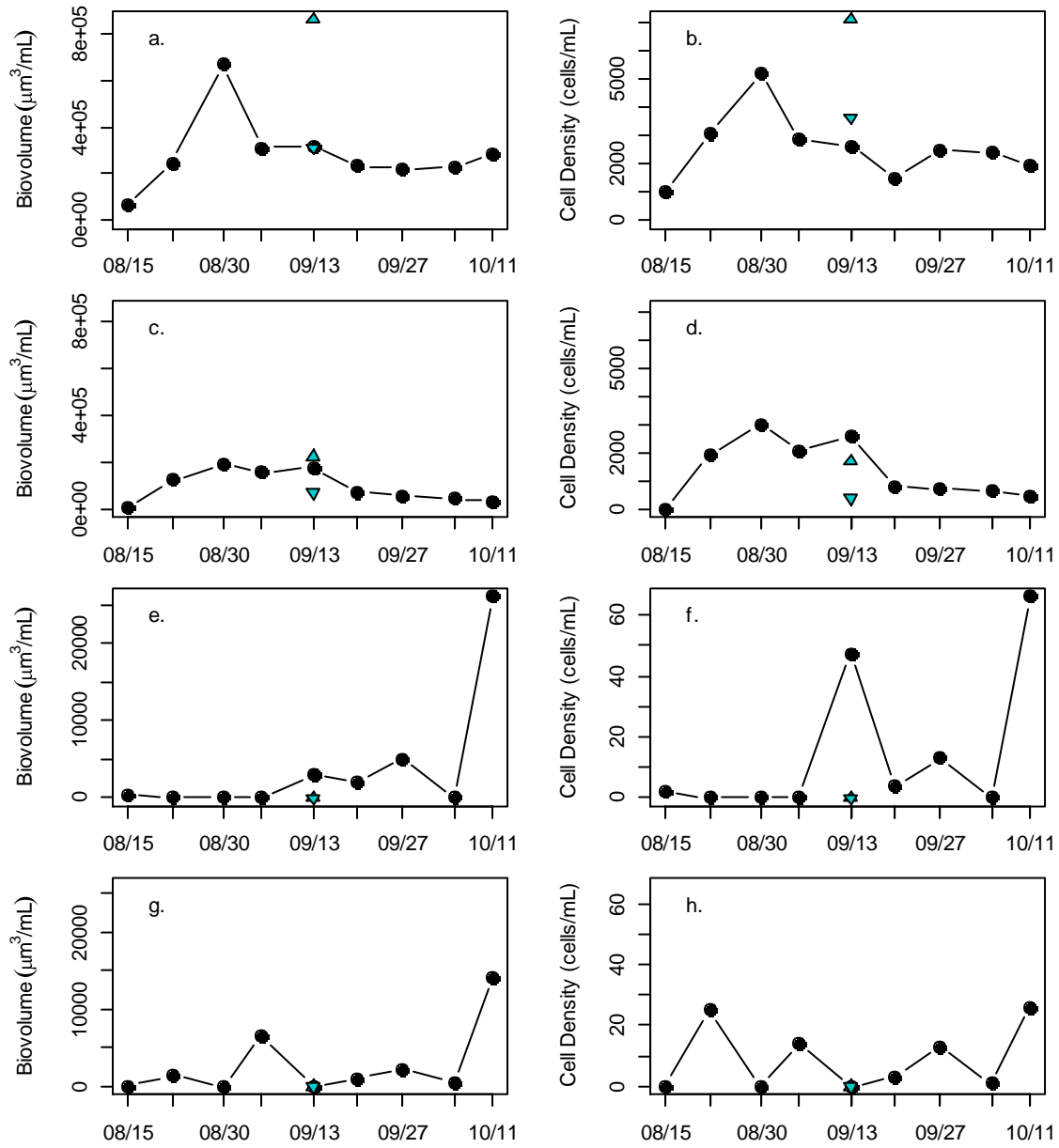
Figure 13. Cyanobacteria biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
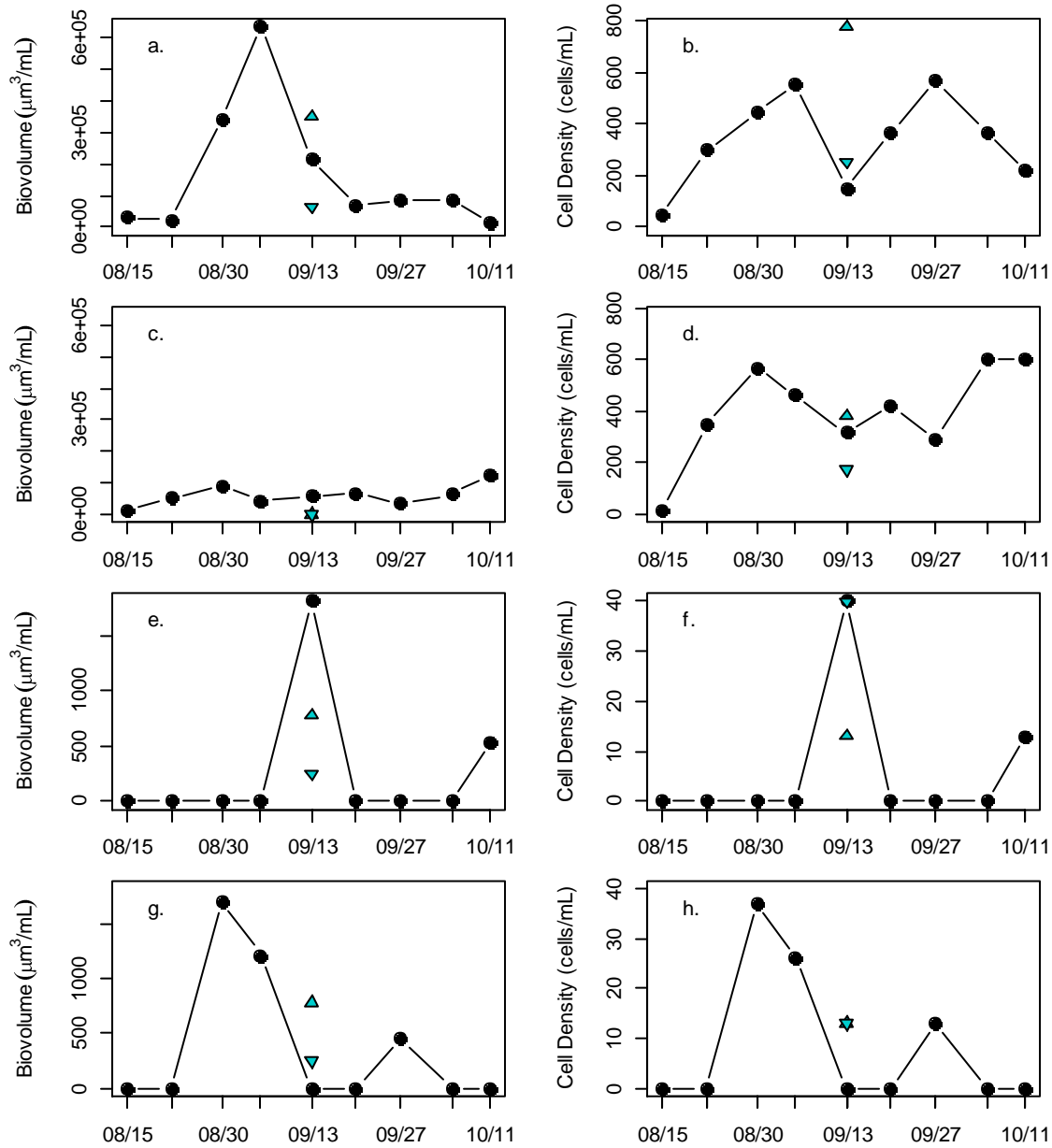
Figure 14. Chlorophyta biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
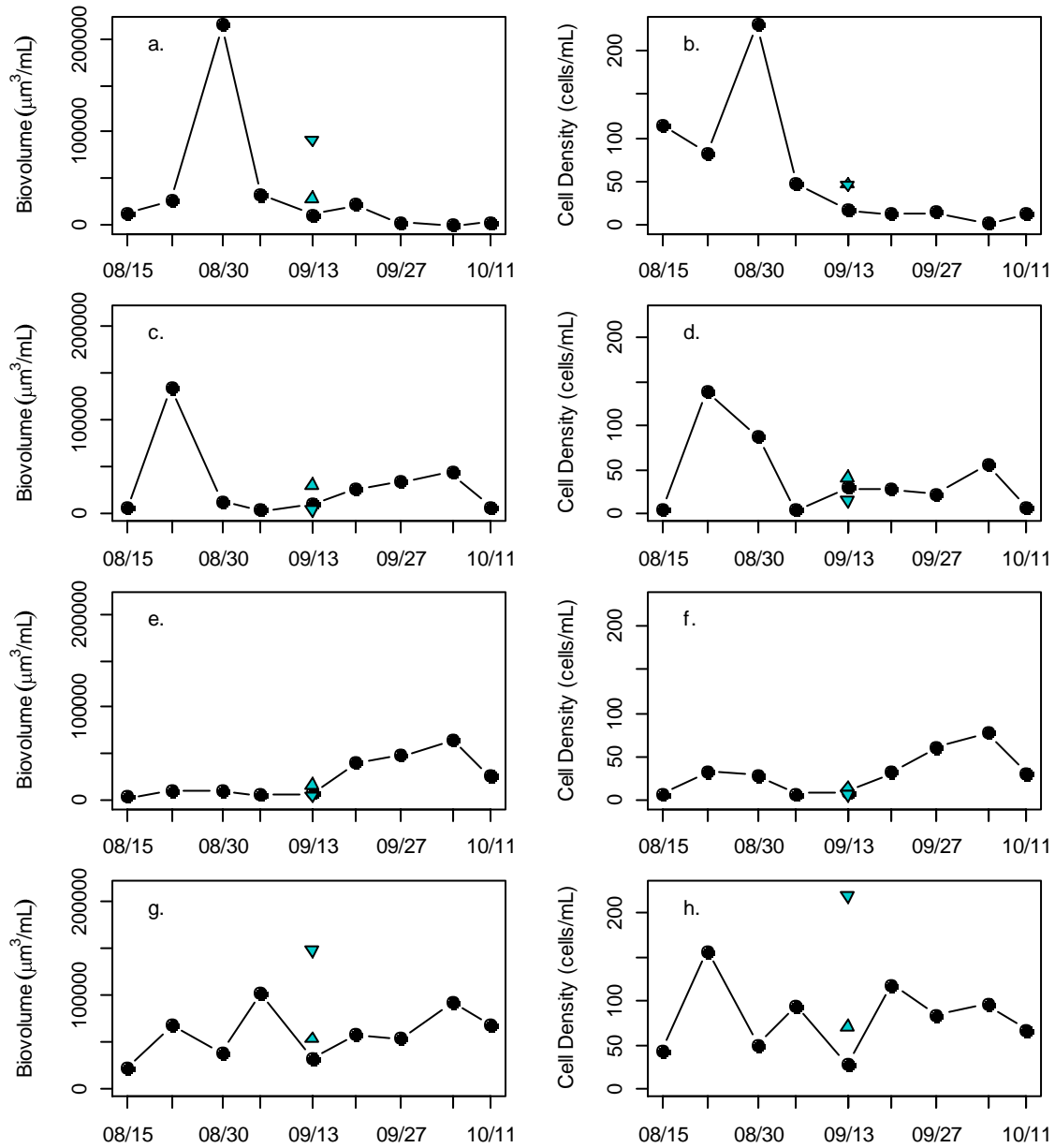
Figure 15. Ochrophyta biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).

51

Figure 16. Bacillariophyceae biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
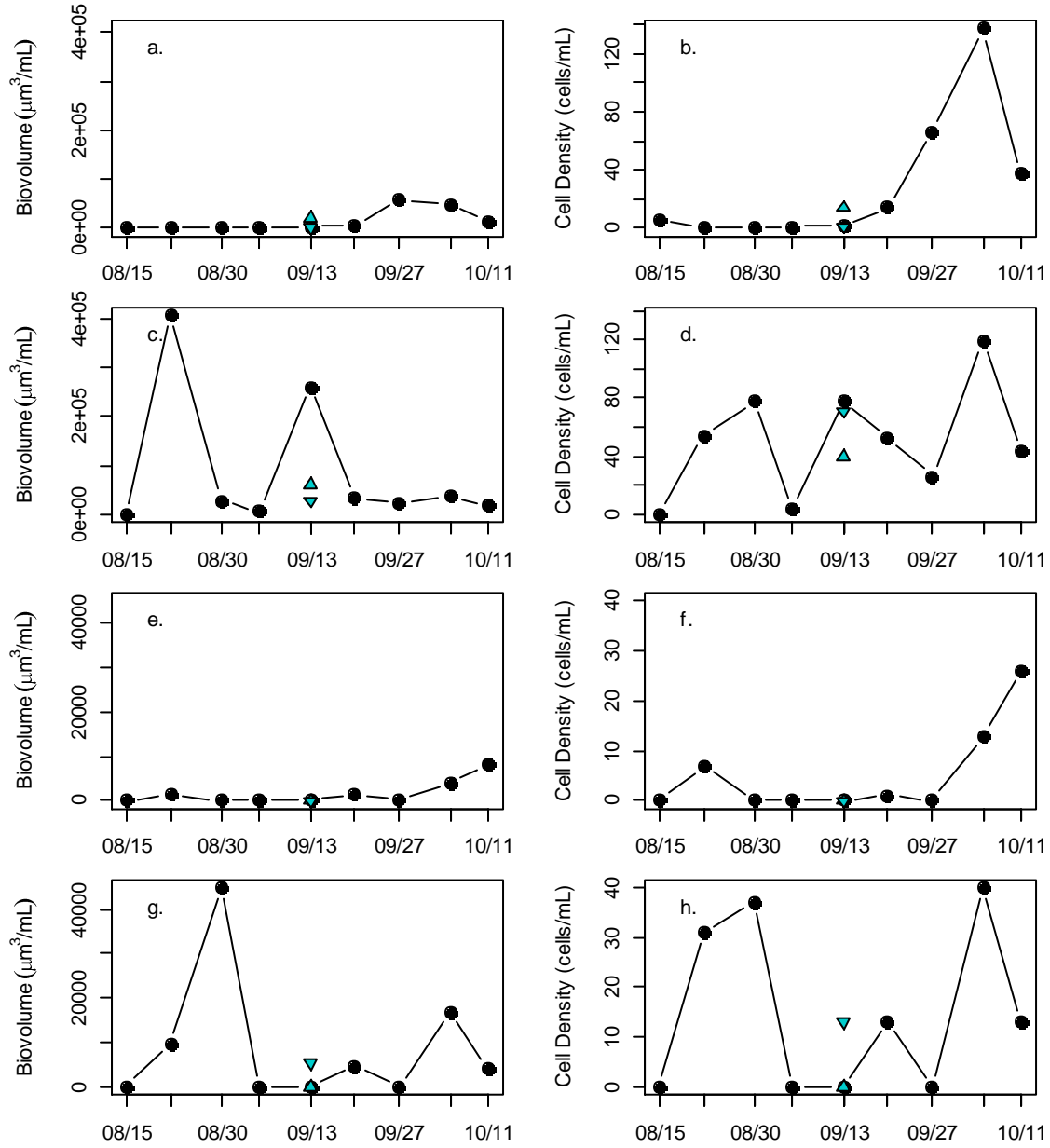
Figure 17. Myzozoa biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
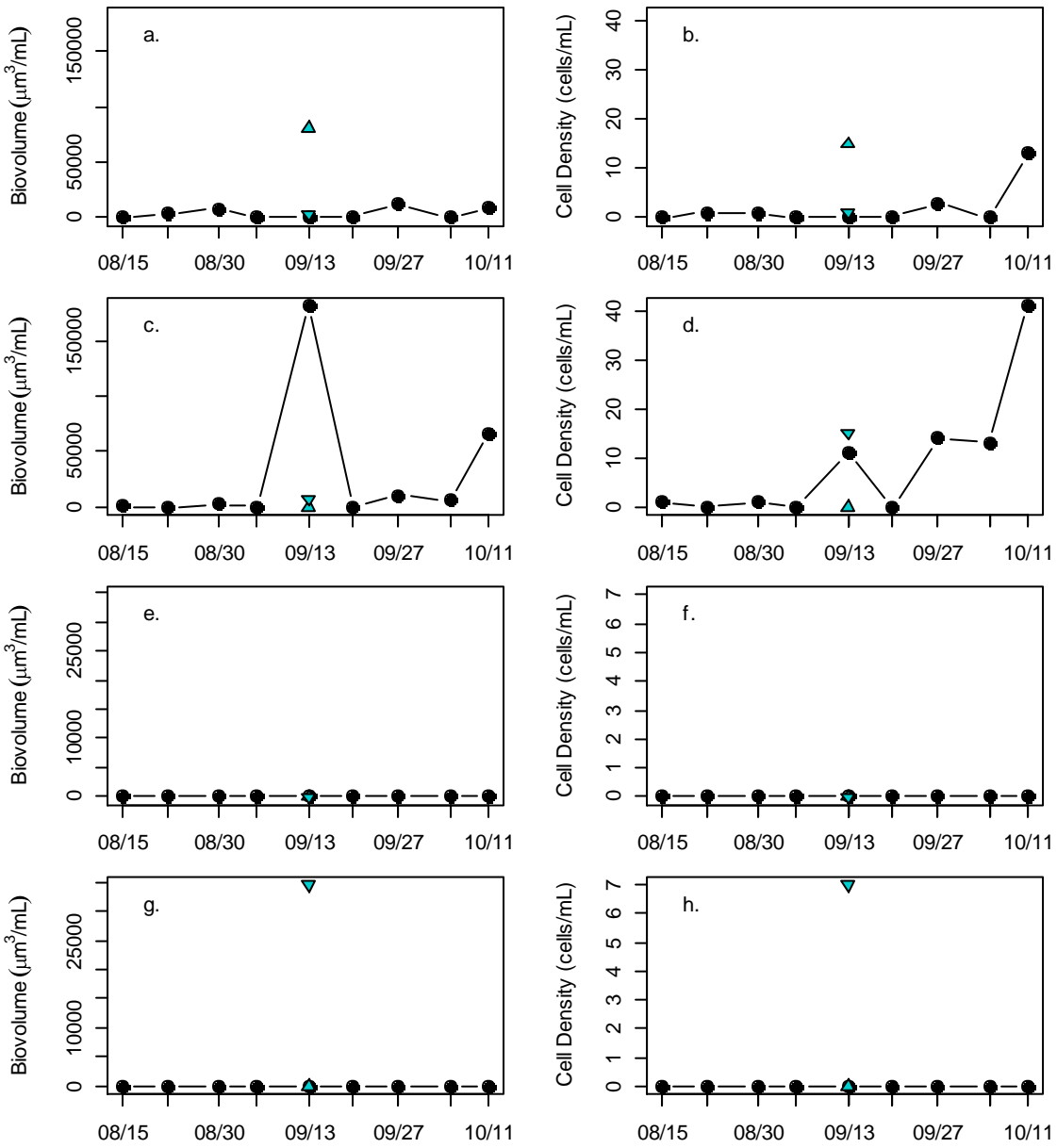
53

Figure 18. Charophyta biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
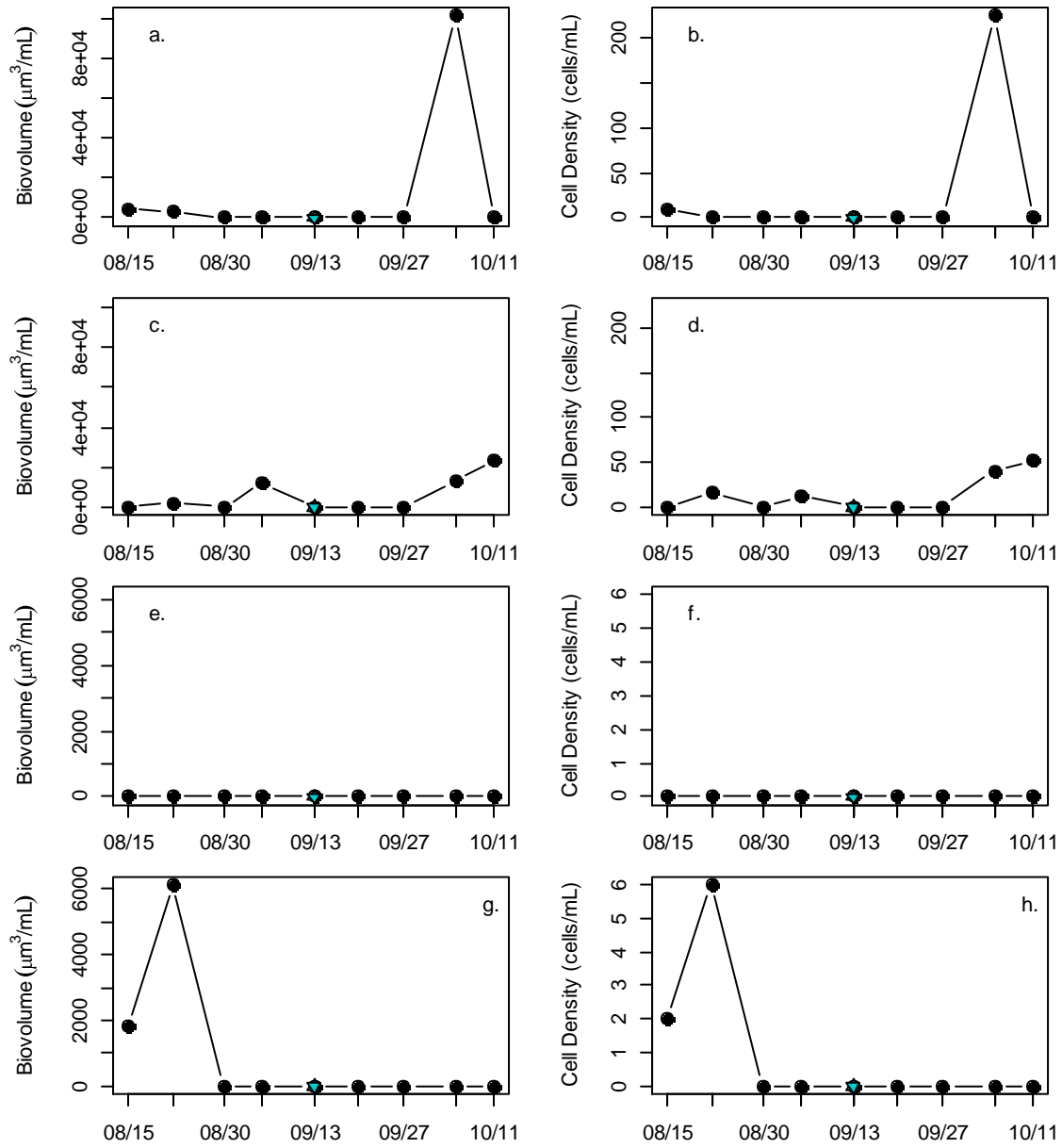
Figure 19. Cryptophyta biovolume (left panels) and cell density (right panels) in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
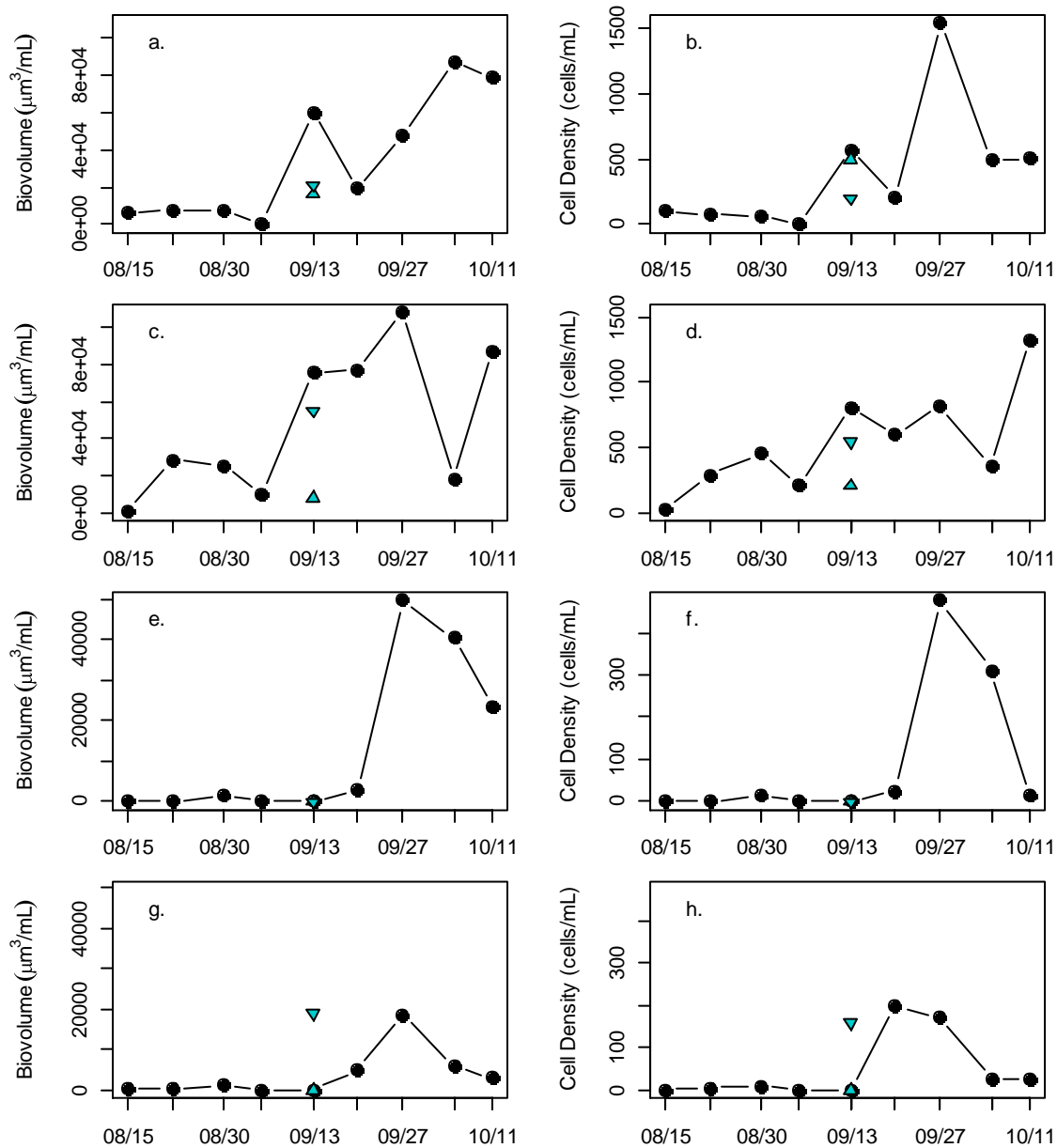
Figure 20. Biovolume (left panels) and cell density (right panels) of unidentified genera categorized as "Undetermined" in (a–b) Picture Lake; (c–d) Highwood Lake; (e–f) Upper Bagley Lake; and (g–h) Lower Bagley Lake from August to October 2012. *Note different scales.* Two additional samples were collected in each lake on September 13 to assess site variability (triangles).
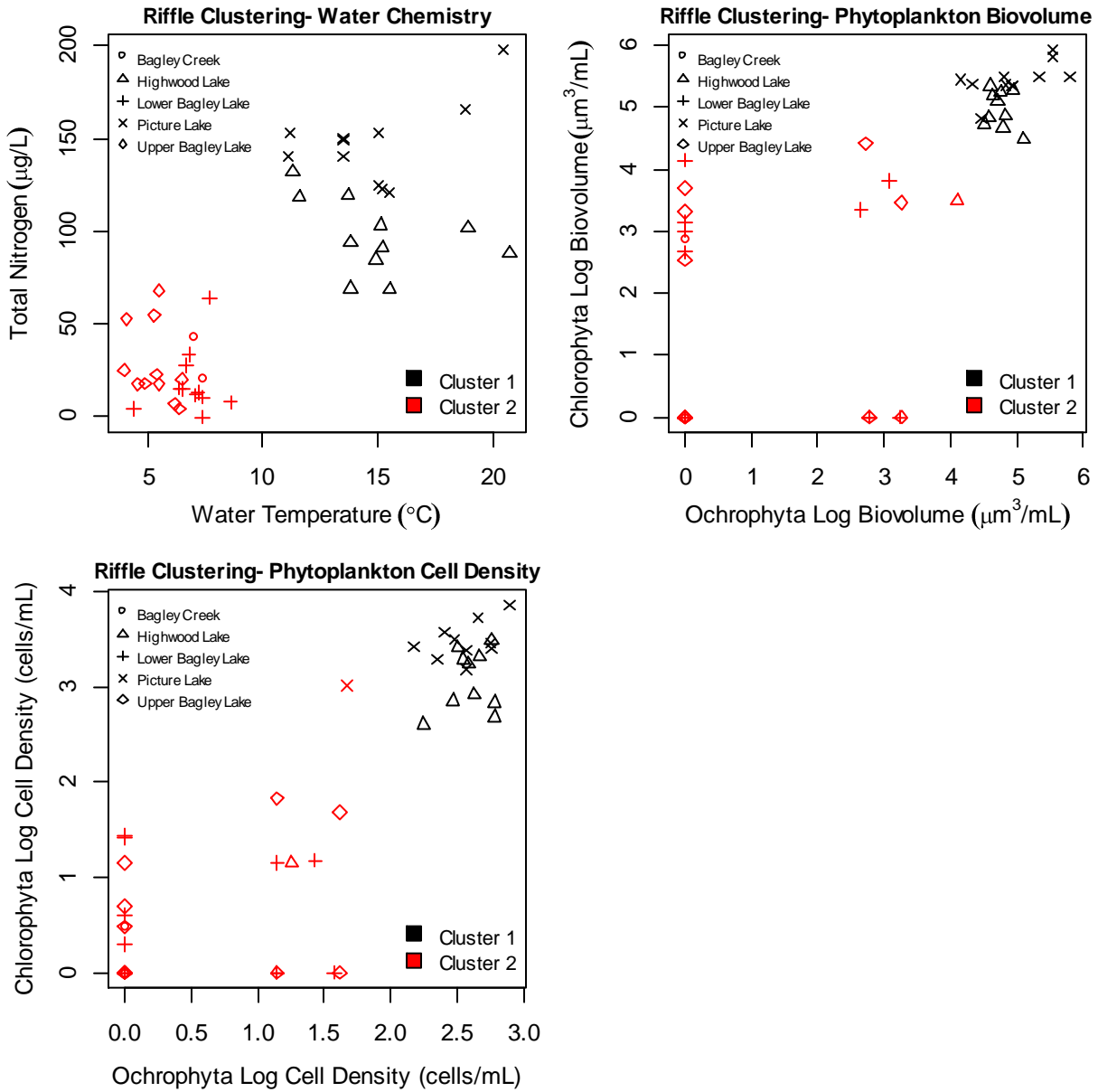
Figure 21. Nonmetric clustering plots of water chemistry variables, phytoplankton biovolume, and phytoplankton cell density. The two variables with the highest PRE scores were plotted against each other.
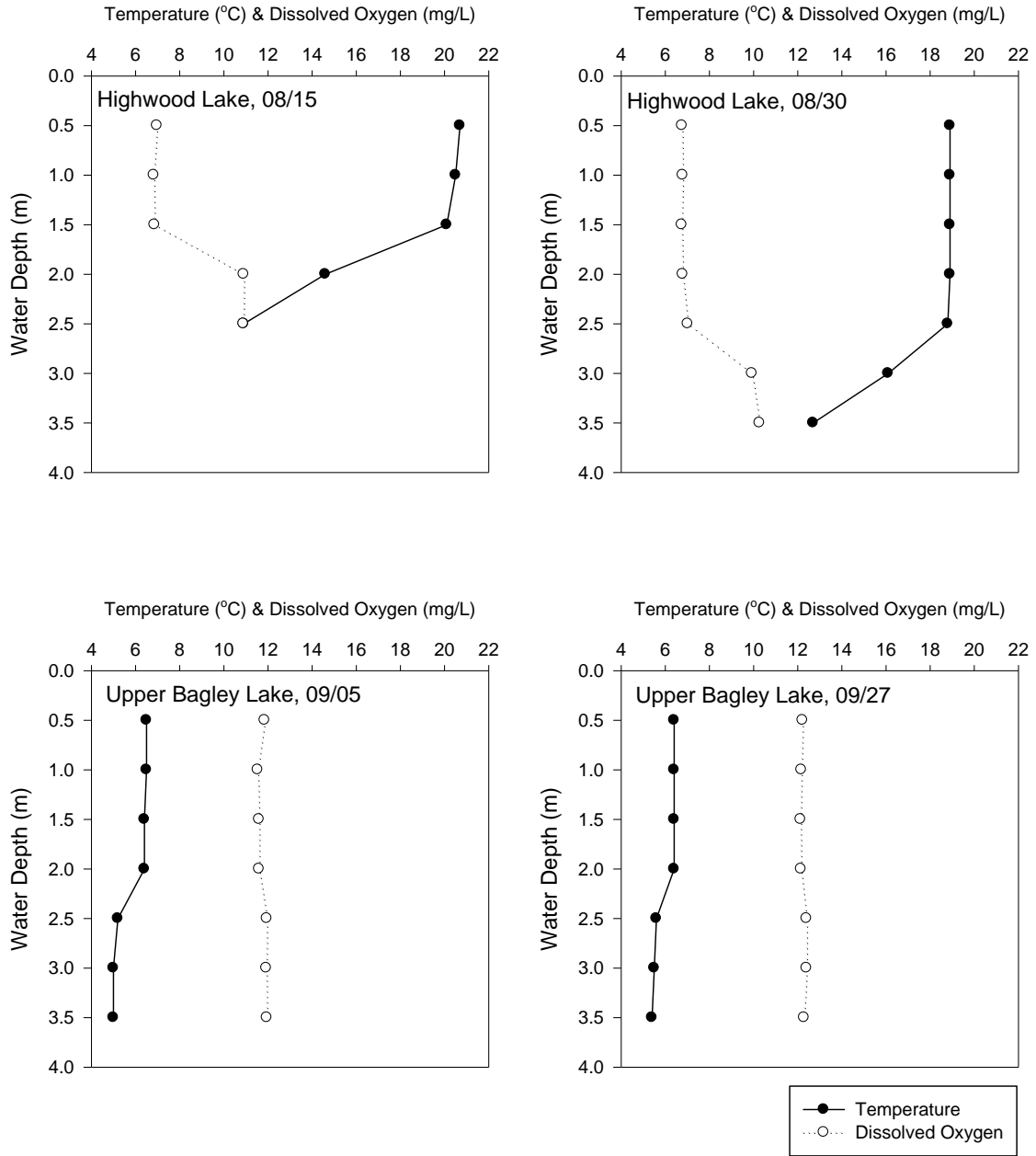
Figure 22. Depth profiles of water temperature and dissolved oxygen showing unstable stratification in Highwood and Upper Bagley Lakes on several dates during the 2012 sampling period.
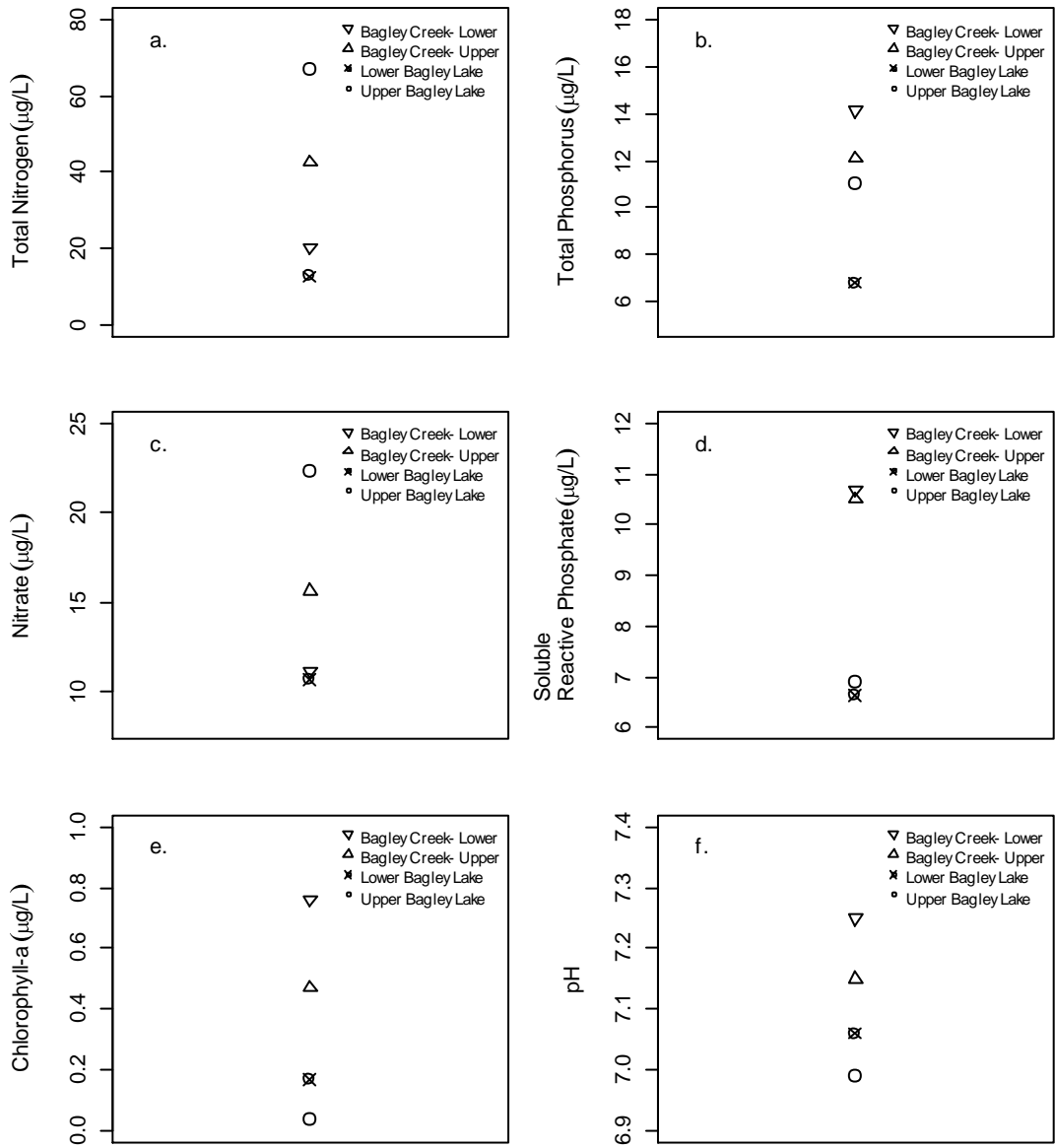
Figure 23. Selected water chemistry results in Bagley Creek and Upper and Lower Bagley Lakes on September 13, 2012, including (a) total nitrogen; (b) total phosphorus; (c) nitrate; (d) soluble reactive phosphate; (e) chlorophyll-*a*; and (f) pH.
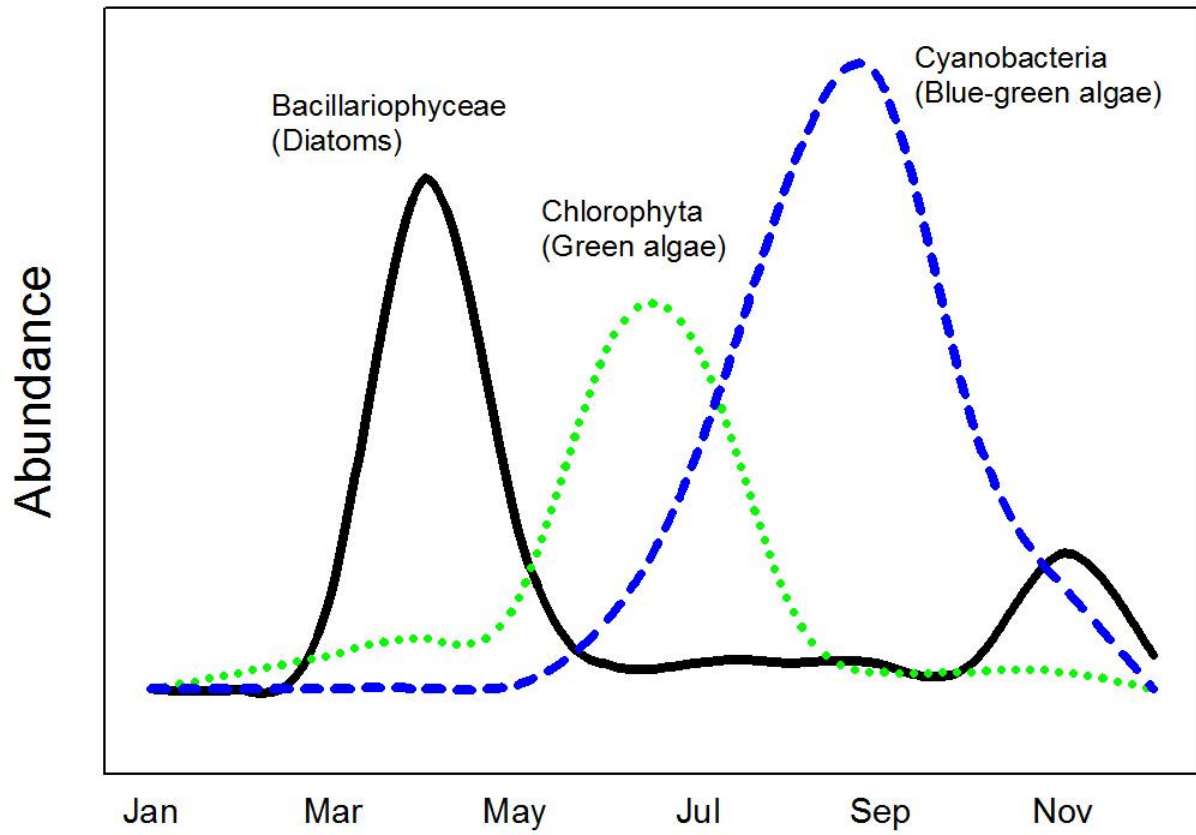
Figure 24. Theoretical model showing seasonal succession of major phytoplankton groups in north temperate lakes (Wetzel 1983).

TABLES

Table 1. Characteristics of the four study lakes.

	Elevation[1] (m)	Surface Area[2] (ha)	Shoreline Perimeter[2] (m)	Maximum Depth[3] (m)	Lake Type	Surface Outflow	Surrounding Vegetation Characteristics	Dominant Substrate Characteristics[4]	Public Access	Naturally reproducing fish?
Picture Lake	1250	1.2	411	3.5	Kettle	NA	Subalpine forest, shrubs, herbs	Silt, Detritus	Road, surrounding trail	Unknown
Highwood Lake	1252	0.75	322	6.1	Kettle	NA	Subalpine forest, shrubs, herbs	Silt, Detritus	Road	Unknown
Lower Bagley Lake	1279	1.5	687	1.5	Reservoir	Bagley Creek	Subalpine forest, shrubs, rock	Sand, Silt	Surrounding trail	Yes
Upper Bagley Lake	1292	2.6	662	5.5	Cirque	Bagley Creek	Rock, shrubs	Silt, Sand, Boulder, Rubble	Surrounding trail	Yes

[1]Values obtained using Garmin GPSMAP 60CSx
[2]Values determined from Mt. Baker-Snoqualmie National Forest GIS Data Library using ArcGIS 10.1
[3]Values measured from my field study
[4]Unpublished data provided by U.S. Forest Service

Table 2. Median, minimum, and maximum values for surface water chemistry variables in the four study lakes from August–October 2012. Values below method detection limit are denoted as "<".

	Picture Lake			Highwood Lake			Upper Bagley Lake			Lower Bagley Lake		
	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max
Water Temperature (°C)	15.0	11.1	20.4	14.9	11.3	20.7	5.4	4.0	6.5	7.1	4.4	8.6
Dissolved Oxygen (mg/L)	8.1	7.0	8.7	8.3	6.8	8.7	11.8	10.8	13.2	11.2	10.5	12.5
pH	6.7	6.6	6.7	6.6	6.1	6.7	7.0	6.9	7.3	7.1	7.0	7.2
Specific Conductance (μS/cm)	10.4	9.8	10.6	10.4	10.1	14.3	12.9	11.5	13.7	13.6	12.3	14.9
Total Nitrogen (μg/L)	149.1	120.9	198.0	93.8	67.9	131.9	<21.8	<21.8	67.4	<21.8	<21.8	63.7
Nitrate (μg/L)	<5.5	<5.5	7.5	<5.5	<5.5	7.2	24.2	6.5	28.1	9.3	6.9	16.8
Ammonium (μg/L)	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9	<9.9
Total Phosphorus (μg/L)	7.6	4.9	11.7	<4.8	<4.8	11.5	10.2	<4.8	15.9	9.2	<4.8	11.8
Soluble Reactive Phosphate (μg/L)	2.8	1.5	4.7	2.3	1.4	5.3	8.5	5.3	11.4	7.5	3.7	9.9
Chlorophyll-*a* (μg/L)	0.8	0.4	1.8	0.7	0.1	1.3	<0.1	<0.1	1.7	0.2	<0.1	2.5

Table 3. List of all phytoplankton taxa found in 44 lake surface samples. For each taxon, the number of samples in which the taxon was observed, relative percent abundance in the entire dataset, and presence (X) in the four study lakes are also included.

	Taxonomic Authority	Occur-rences	Relative Cell Density (%)	Relative Bio-volume (%)	Picture Lake	High-wood Lake	Lower Bagley Lake	Upper Bagley Lake
Phylum Charophyta			**0.06**	**3.06**				
Cosmarium cf. *humile*	Nordstedt ex De Toni	1	<0.01	0.03	X			
Cosmarium regnellii	Wille	1	<0.01	0.04		X		
Cosmarium	Corda ex Ralfs	5	0.01	0.71	X	X		
Euastrum elegans	Ralfs	2	<0.01	0.10	X			
Mougeotia	C.Agardh	2	0.01	1.55		X	X	
Spondylosium	Brébisson ex Kützing	4	0.04	0.21		X		
Staurodesmus triangularis	(Lagerheim) Teiling	4	0.01	0.08	X	X		
Xanthidium	Ehrenberg ex Ralfs	1	<0.01	0.34		X		
Phylum Chlorophyta			**22.78**	**35.61**				
Botryosphaerella sudetica	(Lemmermann) P.C.Silva	21	16.66	15.77	X	X		X
Chlamydomonas cf. *globosa*	J.W.Snow	5	0.02	0.16	X		X	
Chlamydomonas globosa	J.W.Snow	3	0.01	0.06			X	X
Chlamydomonas sp. 1	Ehrenberg	7	0.23	1.34	X	X		X
Chlamydomonas sp. 2	Ehrenberg	1	0.01	0.03		X		
Crucigenia tetrapedia	(Kirchner) Kuntze	18	0.54	1.37	X	X		
Crucigeniella rectangularis	(Nägeli) Komárek	11	1.42	1.61	X	X		
Elakatothrix	Wille	15	0.44	0.43	X	X		X
Oocystis cf. *marssonii*	Lemmermann	14	1.55	8.98	X	X	X	
Oocystis marssonii	Lemmermann	4	0.58	2.76	X	X		
Oocystis parva	West & G.S.West	4	0.30	0.20	X	X	X	
Oocystis sp. 1	Nägeli ex A.Braun	5	0.25	0.20	X			
Oocystis sp. 2	Nägeli ex A.Braun	1	0.17	0.65	X			
Oocystis sp. 3	Nägeli ex A.Braun	9	0.23	0.18	X	X		

63

Table 3 continued.

	Taxonomic Authority	Occur-rences	Relative Cell Density (%)	Relative Bio-volume (%)	Picture Lake	High-wood Lake	Lower Bagley Lake	Upper Bagley Lake
Pediastrum tetras	(Ehrenberg) Ralfs	12	0.10	1.77	X	X		
Scenedesmus cf. *ellipticus*	Corda	1	0.02	<0.01	X			
Scenedesmus ellipticus	Corda	14	0.21	0.10	X	X		
Scenedesmus	Meyen	3	0.03	0.01	X	X		
Phylum Cryptophyta			**0.17**	**1.20**				
Cryptomonas erosa	Ehrenberg	3	0.01	0.14		X	X	
Cryptomonas marssonii	Skuja	3	0.15	1.04	X	X		
Rhodomonas	Karsten	1	0.01	0.01		X		
Phylum Cyanobacteria			**66.01**	**13.08**				
Anabaena	Bory de Saint-Vincent ex Bornet & Flahault	14	0.60	0.22	X	X	X	
Aphanocapsa	Nägeli	11	62.70	12.25	X	X		
Aphanothece	Nägeli	2	0.12	0.08	X	X		
Merismopedia punctata	Meyen	13	2.57	0.46	X	X		
Oscillatoria		1	<0.01	0.01		X		
Oscillatoria tenuis	C.Agardh	1	<0.01	0.05		X		
Pseudanabaena catenata	Lauterborn	4	0.02	0.01	X	X	X	
Phylum Euglenozoa			**0.01**	**0.05**				
Euglena	Ehrenberg	2	0.01	0.05	X	X		
Phylum Myzozoa			**0.49**	**8.31**				
Dinoflagellate Cyst		21	0.38	1.80	X	X	X	X
Gymnodinium uberrimum	(G.J.Allman) Kofoid & Swezy	1	0.02	2.90		X		
Peridinium cf. *inconspicuum*	Lemmermann	1	<0.01	0.04		X		
Peridinium umbonatum	F.Stein	15	0.09	3.58	X	X	X	X

Table 3 continued.

	Taxonomic Authority	Occur-rences	Relative Cell Density (%)	Relative Bio-volume (%)	Picture Lake	High-wood Lake	Lower Bagley Lake	Upper Bagley Lake
Phylum Ochrophyta			**3.96**	**18.31**				
Bitrichia chodatii	(Reverdin) Chodat	15	0.11	0.38	X	X		
Characiopsis curvata		25	2.61	1.66	X	X	X	X
Chrysosphaerella	Lauterborn	1	0.17	0.21		X		
Dinobryon cylindricum	O.E.Imhof	5	0.05	1.45	X			
Dinobryon sertularia	Ehrenberg	12	0.46	10.80	X	X		
Dinobryon		16	0.36	3.26	X	X		
Synura uvella	Ehrenberg	2	0.21	0.56		X		
Class Bacillariophyceae			**1.16**	**12.99**				
Achnanthes lanceolata	(Brébisson ex Kützing) Grunow	4	0.01	0.03		X	X	X
Achnanthes sp. 1	Bory de Saint-Vincent	37	0.35	0.81	X	X	X	X
Achnanthes sp. 2	Bory de Saint-Vincent	1	0.01	0.02	X			
Achnanthidium minutissimum	(Kützing) Czarnecki	5	0.01	0.06			X	X
Asterionella formosa	Hassall	3	0.02	0.21	X		X	X
Aulacoseira cf. *distans*	(Ehrenberg) Simonsen	2	<0.01	0.02	X			X
Aulacoseira distans	(Ehrenberg) Simonsen	5	0.02	0.15	X		X	X
Aulacoseira	Thwaites	5	0.06	0.52	X	X	X	X
Cyclotella	(Kützing) Brébisson	4	0.01	0.17	X	X	X	
Diatoma mesodon	(Ehrenberg) Kützing	22	0.24	3.86			X	X
Encyonema cf. *silesiacum*	(Bleisch) D.G.Mann	15	0.04	0.42	X		X	X
Encyonema silesiacum	(Bleisch) D.G.Mann	4	0.01	0.02			X	X
Encyonema	Kützing	4	<0.01	0.01		X	X	
Eunotia bilunaris	(Ehrenberg) Schaarschmidt	1	<0.01	0.07			X	
Eunotia	Ehrenberg	3	<0.01	<0.01		X	X	
Fragilaria crotonensis	Kitton	5	0.04	0.43	X		X	
Frustulia rhomboides	(Ehrenberg)	7	0.02	0.42	X	X		X

Table 3 continued.

	Taxonomic Authority	Occur-rences	Relative Cell Density (%)	Relative Bio-volume (%)	Picture Lake	High-wood Lake	Lower Bagley Lake	Upper Bagley Lake
Gomphonema	Ehrenberg	3	<0.01	0.04			X	X
Hannaea arcus	(Ehrenberg) R.M.Patrick	6	0.01	0.33			X	X
Hannaea	(Ehrenberg) R.M.Patrick	1	<0.01	0.01				X
Meridion circulare	(Greville) C.Agardh	6	<0.01	0.02		X	X	X
Navicula capitata	Ehrenberg	1	<0.01	0.01				X
Navicula capitatoradiata	Germain	5	0.02	0.18			X	X
Navicula cf. *cryptocephala*	Kützing	9	0.03	0.36	X	X	X	X
Navicula cf. *cryptotenella*	Lange-Bertalot	3	0.01	0.17			X	X
Navicula	Bory de Saint-Vincent	22	0.09	0.74	X	X	X	X
Neidium	(Ehrenberg) Pfizer	1	0.02	1.32	X			
Nitzschia palea	(Kützing) W.Smith	2	0.01	0.06	X	X		
Nitzschia	Hassall	15	0.05	0.19	X	X	X	X
Pinnularia	Ehrenberg	1	<0.01	0.01			X	
Stauroneis	Ehrenberg	1	<0.01	0.08		X		
Synedra	Ehrenberg	5	<0.01	0.04		X	X	X
Synedra ulna	(Nitzsch) Ehrenberg	5	<0.01	0.23	X		X	X
Tabellaria fenestrata	(Lyngbye) Kützing	3	0.03	1.25	X	X		
Tabellaria flocculosa	(Roth) Kützing	9	0.02	0.72	X	X	X	
Undetermined			**5.35**	**7.38**				
Unknown Cyst		2	0.12	0.19		X		X
Unknown Flagellate		10	0.48	0.73	X	X	X	X
Unknown Flagellate Uroglena Cells		1	0.69	0.28	X			
Unknown Flagellate with One Flagella		5	0.61	0.67	X	X		X
Unknown Flagellate with Two Flagella		1	0.07	0.09		X		
Unknown Small Spherical		4	0.27	0.52	X	X	X	X
Unknown Spherical		20	1.81	3.70	X	X	X	X

66

Table 3 continued.

	Taxonomic Authority	Occur-rences	Relative Cell Density (%)	Relative Bio-volume (%)	Picture Lake	High-wood Lake	Lower Bagley Lake	Upper Bagley Lake
Unknown Spherical with One Flagella		8	0.58	0.87	X	X	X	X
Unknown Spherical with One Flagella (Chlorophyta)		1	0.01	0.01	X			
Unknown Tetrad		12	0.69	0.27	X	X		X
Unknown Tetrad (Chlorophyta)		1	<0.01	<0.01	X			

Table 4. Correlation matrix showing Kendall's tau statistics for water chemistry variables. Significant correlations were determined at $p < 0.05$ (shaded boxes).

	Dissolved Oxygen	Water Temperature	pH	Specific Conductance	Total Phosphorus	Soluble Reactive Phosphate	Total Nitrogen	Nitrate
Water Temperature	$\tau = -0.62$ $p = 1.50\text{x}10^{-9}$							
pH	$\tau = 0.53$ $p = 3.39\text{x}10^{-7}$	$\tau = -0.47$ $p = 5.05\text{x}10^{-6}$						
Specific Conductance	$\tau = 0.48$ $p = 4.00\text{x}10^{-6}$	$\tau = -0.49$ $p = 2.16\text{x}10^{-6}$	$\tau = 0.51$ $p = 8.86\text{x}10^{-9}$					
Total Phosphorus	$\tau = 0.22$ $p = 0.03$	$\tau = -0.25$ $p = 0.01$	$\tau = 0.34$ $p = 1.26\text{x}10^{-4}$	$\tau = 0.26$ $p = 2.82\text{x}10^{-3}$				
Soluble Reactive Phosphate	$\tau = 0.44$ $p = 2.04\text{x}10^{-5}$	$\tau = -0.42$ $p = 4.62\text{x}10^{-5}$	$\tau = 0.48$ $p = 6.12\text{x}10^{-8}$	$\tau = 0.37$ $p = 2.24\text{x}10^{-5}$	$\tau = 0.39$ $p = 8.03\text{x}10^{-6}$			
Total Nitrogen	$\tau = -0.47$ $p = 3.49\text{x}10^{-6}$	$\tau = 0.44$ $p = 2.01\text{x}10^{-5}$	$\tau = -0.52$ $p = 3.11\text{x}10^{-9}$	$\tau = -0.54$ $p = 8.41\text{x}10^{-10}$	$\tau = -0.12$ $p = 0.16$	$\tau = -0.44$ $p = 4.20\text{x}10^{-7}$		
Nitrate	$\tau = 0.51$ $p = 5.17\text{x}10^{-7}$	$\tau = -0.61$ $p = 2.68\text{x}10^{-9}$	$\tau = 0.37$ $p = 2.53\text{x}10^{-5}$	$\tau = 0.38$ $p = 2.01\text{x}10^{-5}$	$\tau = 0.26$ $p = 2.75\text{x}10^{-3}$	$\tau = 0.49$ $p = 1.67\text{x}10^{-8}$	$\tau = -0.35$ $p = 6.26\text{x}10^{-5}$	
Chlorophyll-*a*	$\tau = -0.20$ $p = 0.05$	$\tau = 0.16$ $p = 0.13$	$\tau = -0.02$ $p = 0.85$	$\tau = -0.02$ $p = 0.82$	$\tau = 0.10$ $p = 0.25$	$\tau = -0.26$ $p = 3.58\text{x}10^{-3}$	$\tau = 0.25$ $p = 4.72\text{x}10^{-3}$	$\tau = -0.33$ $p = 1.62\text{x}10^{-4}$

Table 5. Proportional reduction in error (PRE) generated from nonmetric clustering (Riffle), and association analysis results showing cluster group memberships of 46 samples based on water chemistry variables, phytoplankton biovolume, and phytoplankton cell density. Chi-squared was considered significant at $p < 0.05$.

Water Chemistry

Riffle Results		Association Analysis Results		
	PRE Score		Cluster Group	
			1	2
Dissolved Oxygen	0.96			
Water Temperature	0.96	Bagley Creek	0	2
pH	0.96	Highwood Lake	11	0
Total Nitrogen	0.96	Lower Bagley Lake	0	11
Soluble Reactive Phosphate	0.96	Picture Lake	11	0
Nitrate	0.87	Upper Bagley Lake	0	11
Specific Conductance	0.87			
Chlorophyll-*a*	0.36			
Total Phosphorus	0.33	$\chi^2 = 46.0$, $p = 2.46 \times 10^{-9}$		

Phytoplankton Biovolume

Riffle Results		Association Analysis Results		
	PRE Score		Cluster Group	
			1	2
Chlorophyta	0.95			
Ochrophyta	0.95	Bagley Creek	0	2
Cyanobacteria	0.79	Highwood Lake	10	1
Myzozoa	0.46	Lower Bagley Lake	0	11
Charophyta	0.35	Picture Lake	11	0
Bacillariophyceae	0.18	Upper Bagley Lake	0	11
Cryptophyta	0.12			
Euglenozoa	0.05	$\chi^2 = 42.3$, $p = 1.42 \times 10^{-8}$		

Phytoplankton Cell Density

Riffle Results		Association Analysis Results		
	PRE Score		Cluster Group	
			1	2
Ochrophyta	1.00			
Chlorophyta	0.90	Bagley Creek	0	2
Cyanobacteria	0.84	Highwood Lake	10	1
Myzozoa	0.47	Lower Bagley Lake	0	11
Charophyta	0.39	Picture Lake	10	1
Cryptophyta	0.08	Upper Bagley Lake	0	11
Euglenozoa	0.05			
Bacillariophyceae	0.04	$\chi^2 = 38.6$, $p = 8.42 \times 10^{-8}$		

Table 6. Results of association analysis performed on cluster memberships for water chemistry variables and phytoplankton biovolume, and water chemistry variables and phytoplankton cell density. Chi-squared was considered significant at $p < 0.05$.

		Cluster Group (Biovolume)		Cluster Group (Cell Density)	
		1	2	1	2
Cluster Group (Water Chemistry)	1	21	1	20	2
	2	0	24	0	24
		$\chi^2 = 38.4$ $p = 5.78 \times 10^{-10}$		$\chi^2 = 35.0$ $p = 3.31 \times 10^{-9}$	

APPENDICES

Appendix A. Standard method and method detection limit[1] (MDL) for nitrogen and phosphorus constituents and chlorophyll-*a*. Nitrogen and phosphorus concentrations were expressed in units of μg-N/L or μg-P/L, respectively. Also shown are acceptable ranges and control limits for laboratory duplicates[2].

	Standard Method	MDL (μg/L)	Lab Duplicate Differences (μg/L) Acceptable Range	Control Limit
Total Nitrogen	4500-P J and 4500-NO$_3$ I	21.8	55.0	82.0
Total Phosphorus	4500-P J and 4500-P G	4.8	13.7	20.6
Nitrate + Nitrite	SM 4500-NO$_3$ I	5.5	13.1	19.6
Ammonium	SM 4500-NH$_3$ H	9.9	6.6	9.9
Soluble Reactive Phosphate	SM 4500-P G	1.1	2.6	3.9
Chlorophyll-*a*	SM 10200 H	0.1	NA	NA

[1] Values were analytically determined by the Institute for Watershed Studies, Western Washington University using Method 1030 C in APHA (2012)
[2] Values were determined by the Institute for Watershed Studies, Western Washington University

Appendix B. Quality assurance results for laboratory and field duplicate water chemistry samples. All laboratory duplicate samples for nitrogen and phosphorus constituents were within acceptable range as defined in Appendix A. One field duplicate collected in Lower Bagley Lake for total phosphorus was high (absolute difference 36.1 µg/L); I considered all other field duplicates acceptable.

	Laboratory Duplicates (Absolute Difference, µg/L)				Field Duplicates (Absolute Difference, µg/L)			
	n	Med	Min	Max	n	Med	Min	Max
Total Nitrogen	9	14.5	0.9	40.6	9	9.4	0.7	46.3
Total Phosphorus	9	1.7	0.3	7.7	9	1.7	0.8	36.1
Nitrate	10	0.2	0.1	1.2	9	1.5	0.4	3.0
Soluble Reactive Phosphate	10	0.3	0.1	1.6	9	1.8	0.1	4.3
pH	8	0.0	<0.1	0.1	9	<0.1	<0.1	0.1
Specific Conductance	8	0.1	<0.1	0.2	9	0.1	<0.1	0.7
Chlorophyll-*a*	61	0.1	<0.1	0.6	9	<0.1	<0.1	0.6

Appendix C. Photos of the four lakes during the 2012 sampling period.

Picture Lake, August 12

Highwood Lake, September 13

Upper Bagley Lake, August 15

Lower Bagley Lake, August 15

Appendix D. Images of selected phytoplankton taxa encountered in water samples from Picture, Highwood, Upper Bagley, and Lower Bagley Lakes. Images are my own unless otherwise noted.
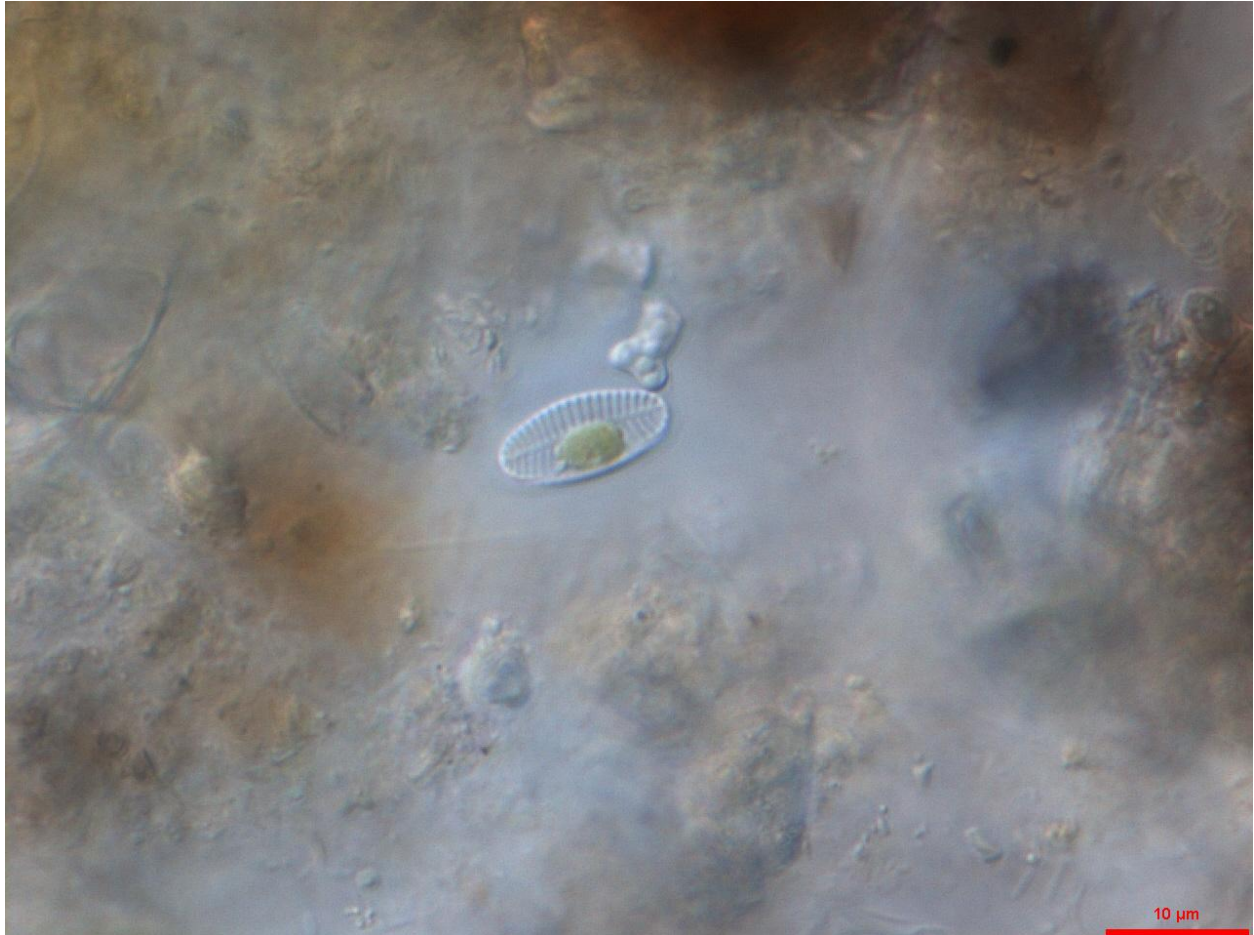
Achnanthes lanceolata. Highwood Lake, October 11, 2012. Image by Karl Bruun.

Anabaena. Highwood Lake, August 22, 2012.
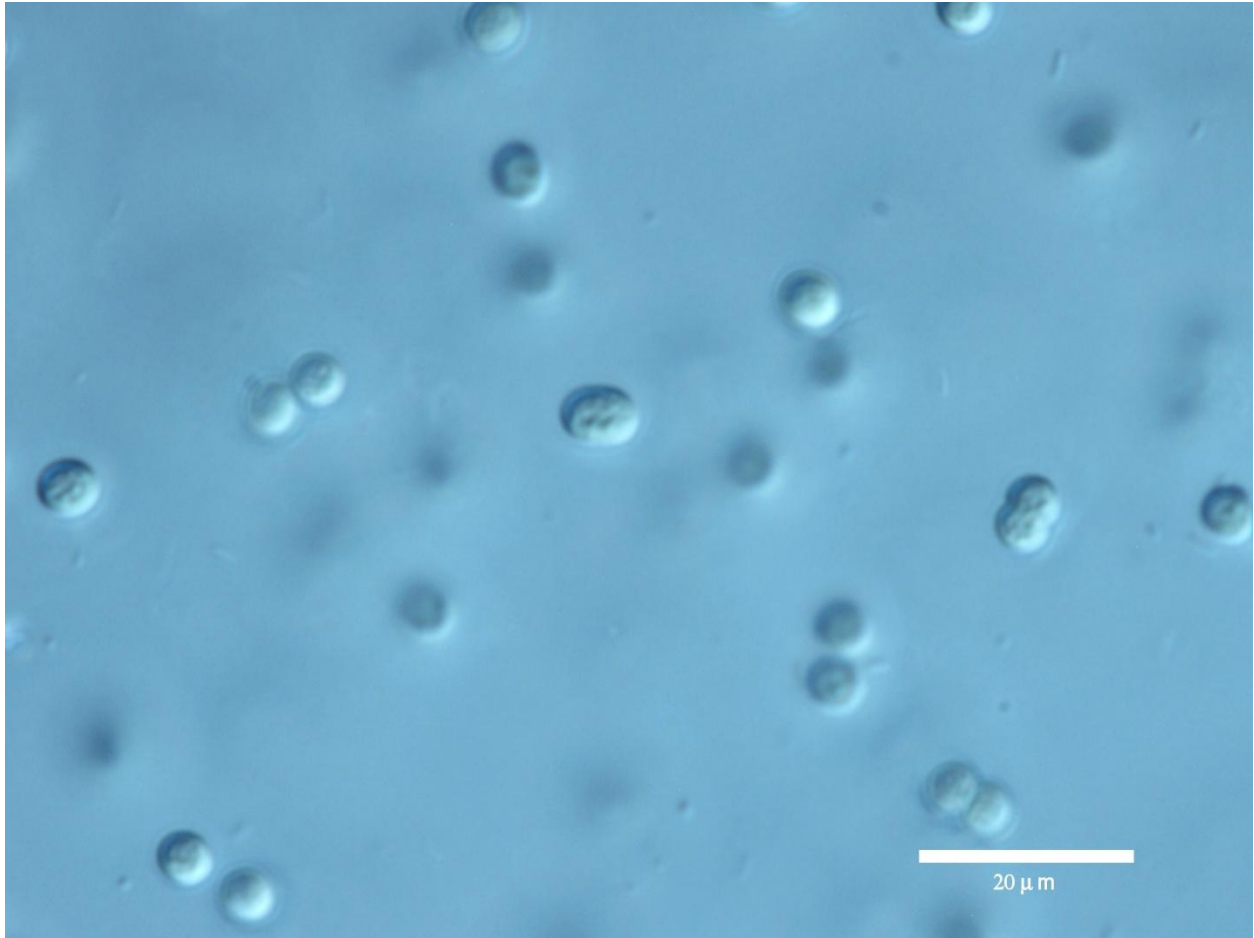
Aphanocapsa cf. *delicatissima*. Highwood Lake, September 27, 2012.
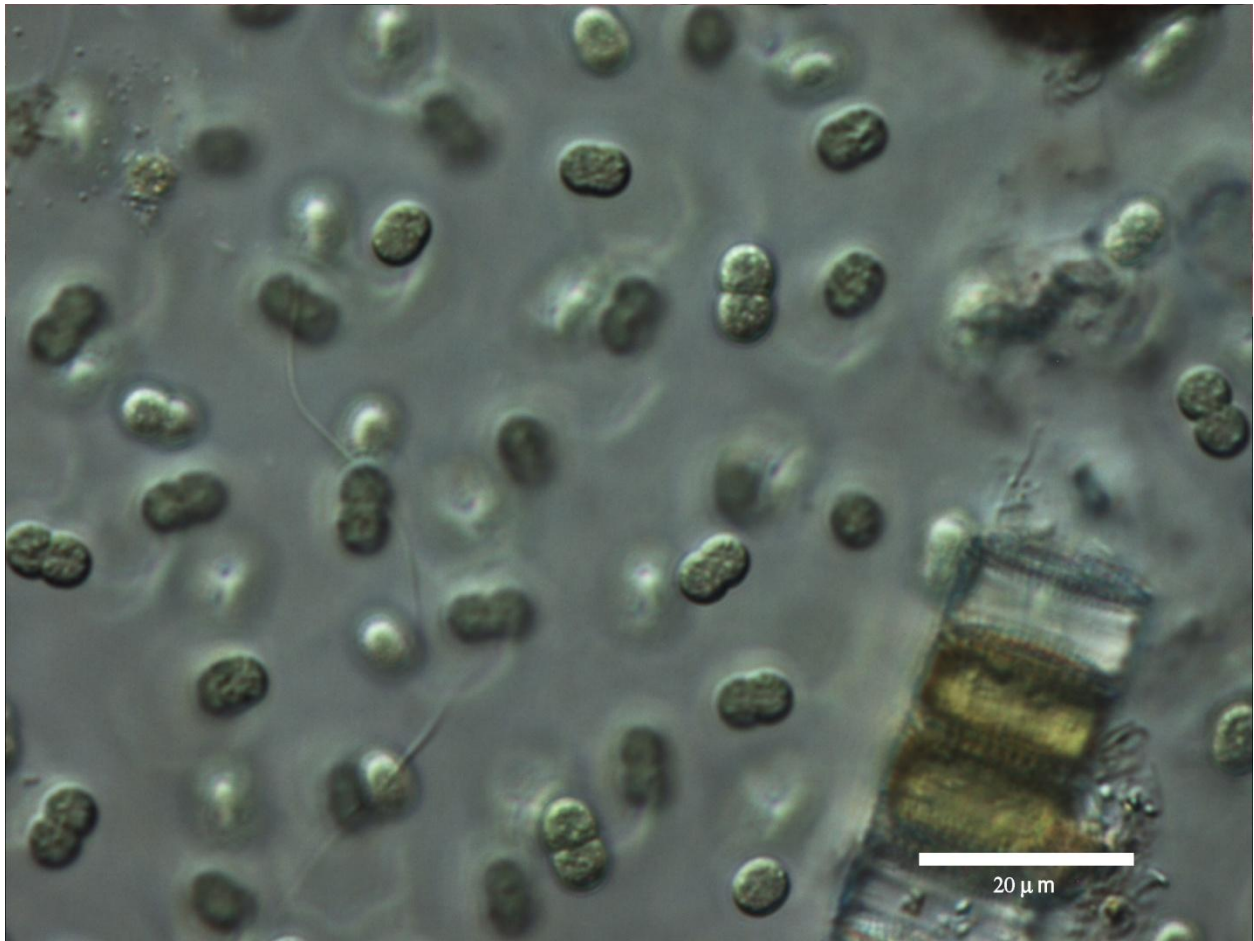
Aphanothece. Picture Lake, August 31, 2011. Image by Robin Matthews.
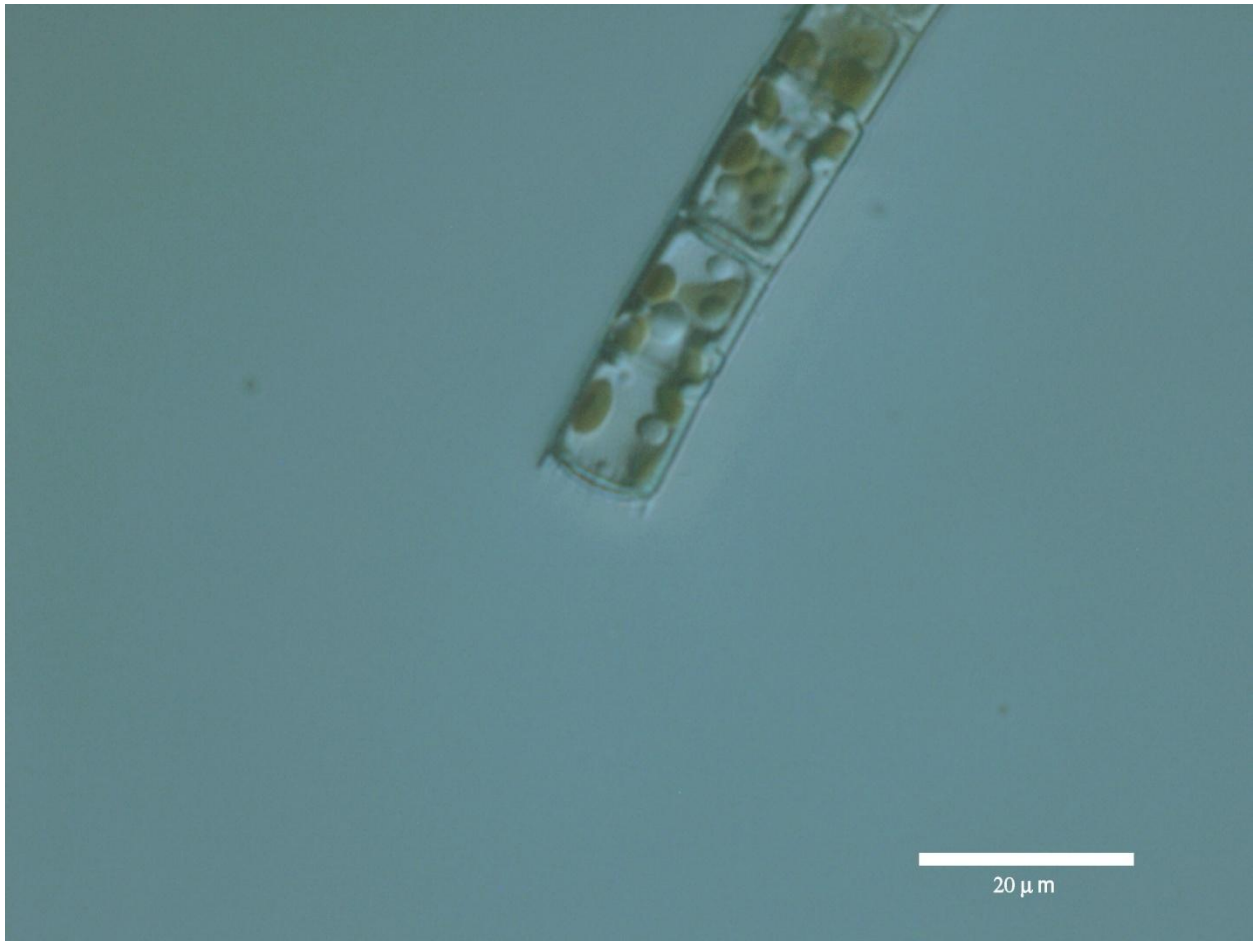
Aulacoseira cf. *distans*. Upper Bagley Lake, August 22, 2012.
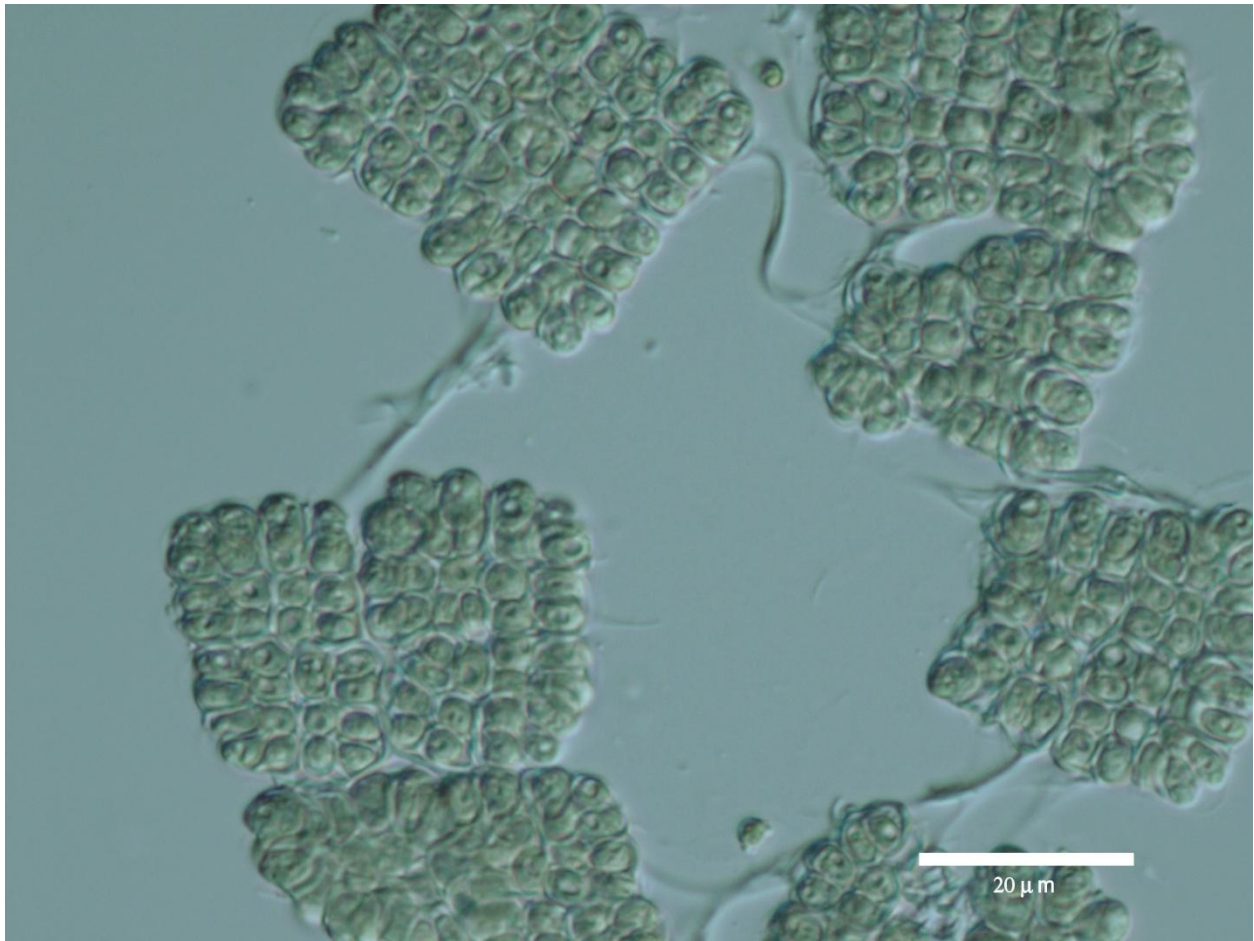
Botryosphaerella sudetica. Highwood Lake, September 27, 2012.
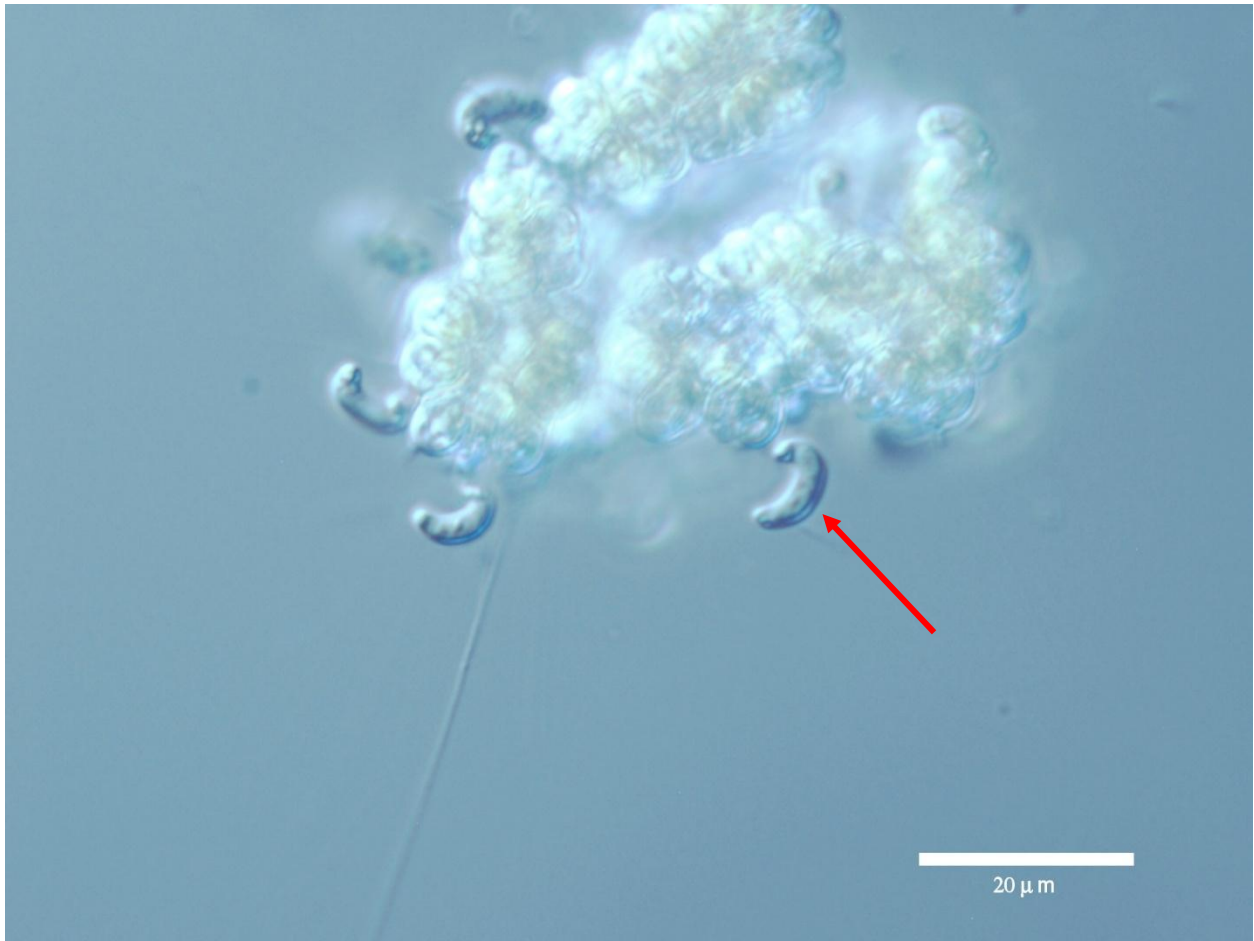
Characiopsis curvata. Highwood Lake, September 27, 2012.
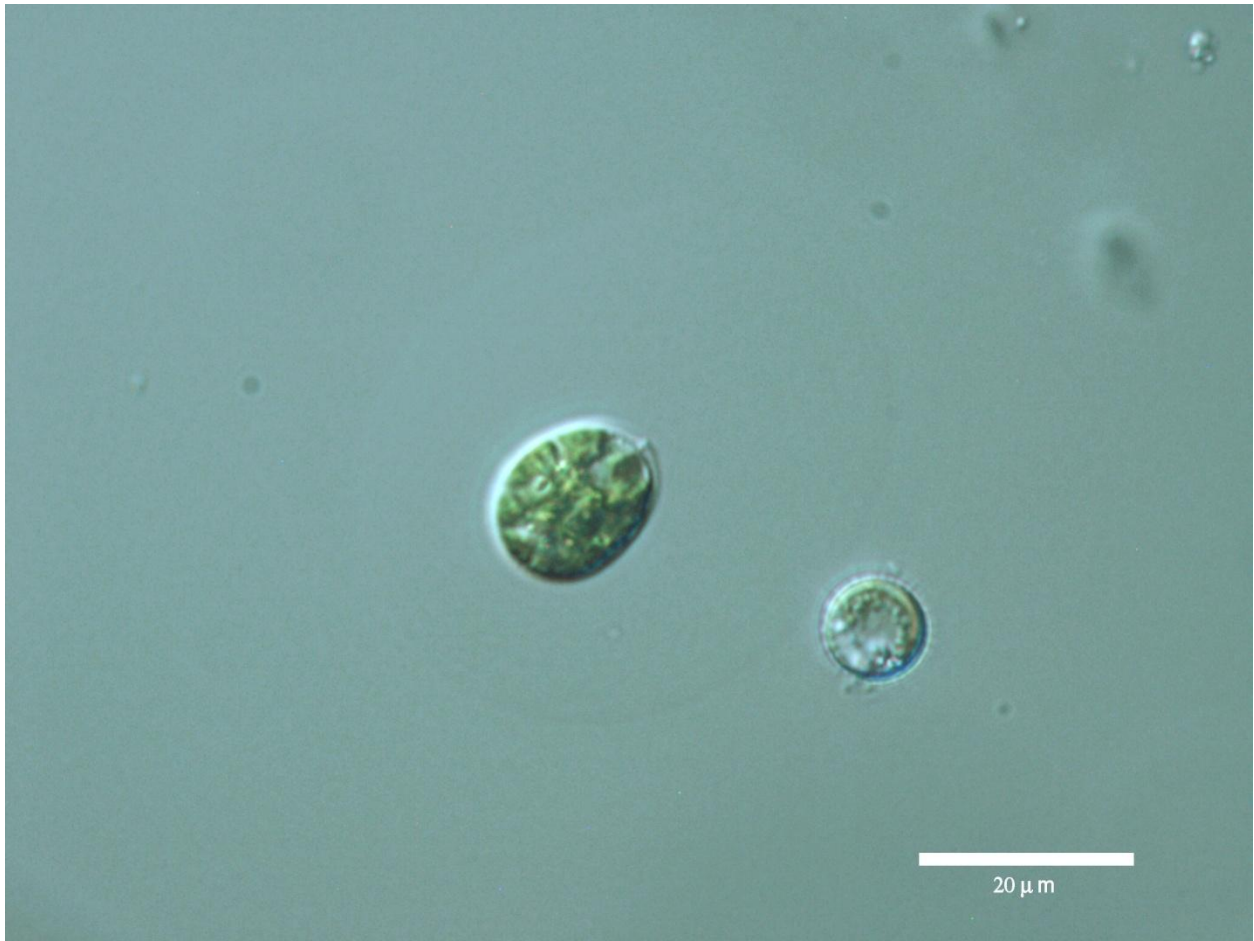
Chlamydomonas sp. A. Picture Lake, September 20, 2012.
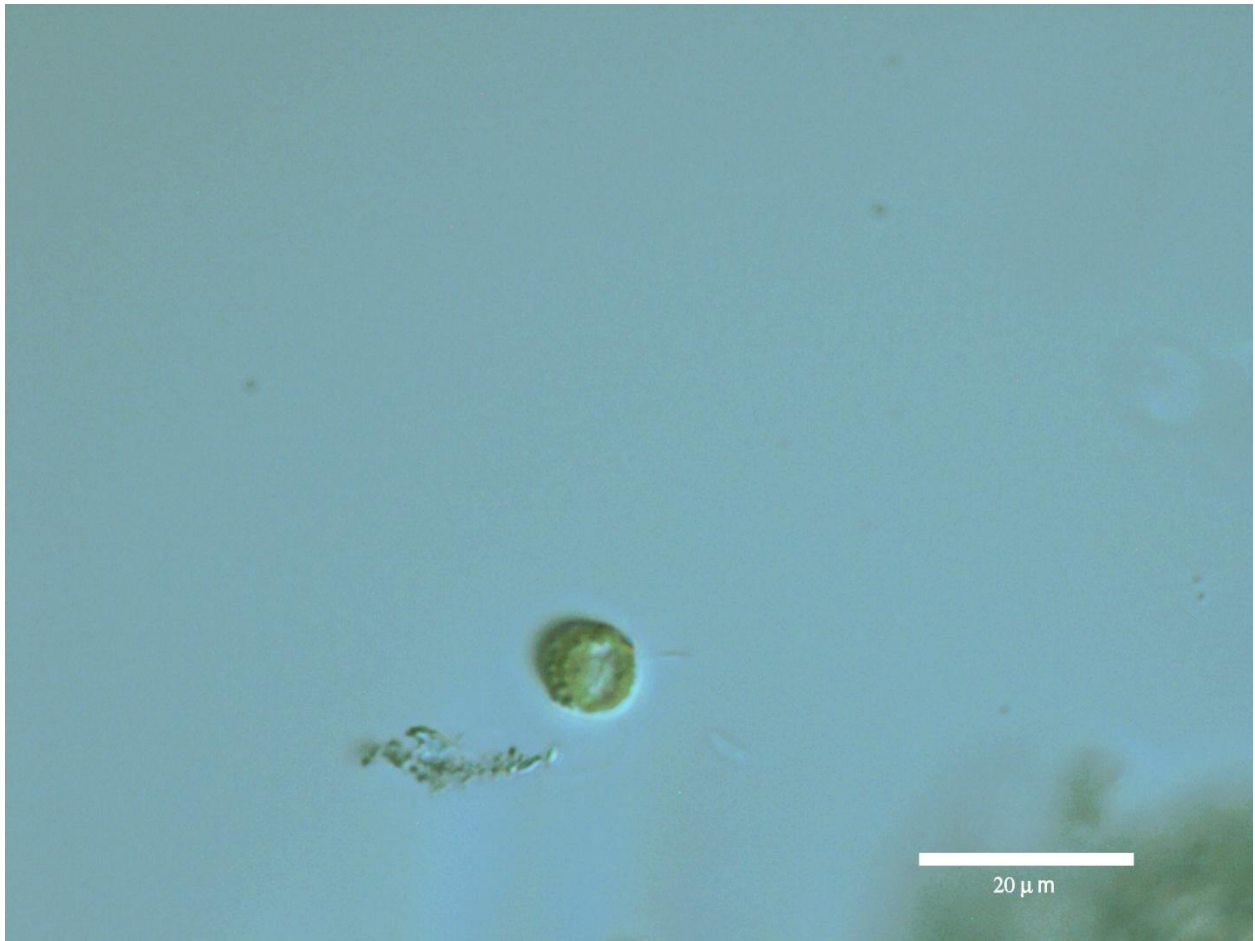
Chlamydomonas sp. B. Upper Bagley Lake, August 22, 2012.
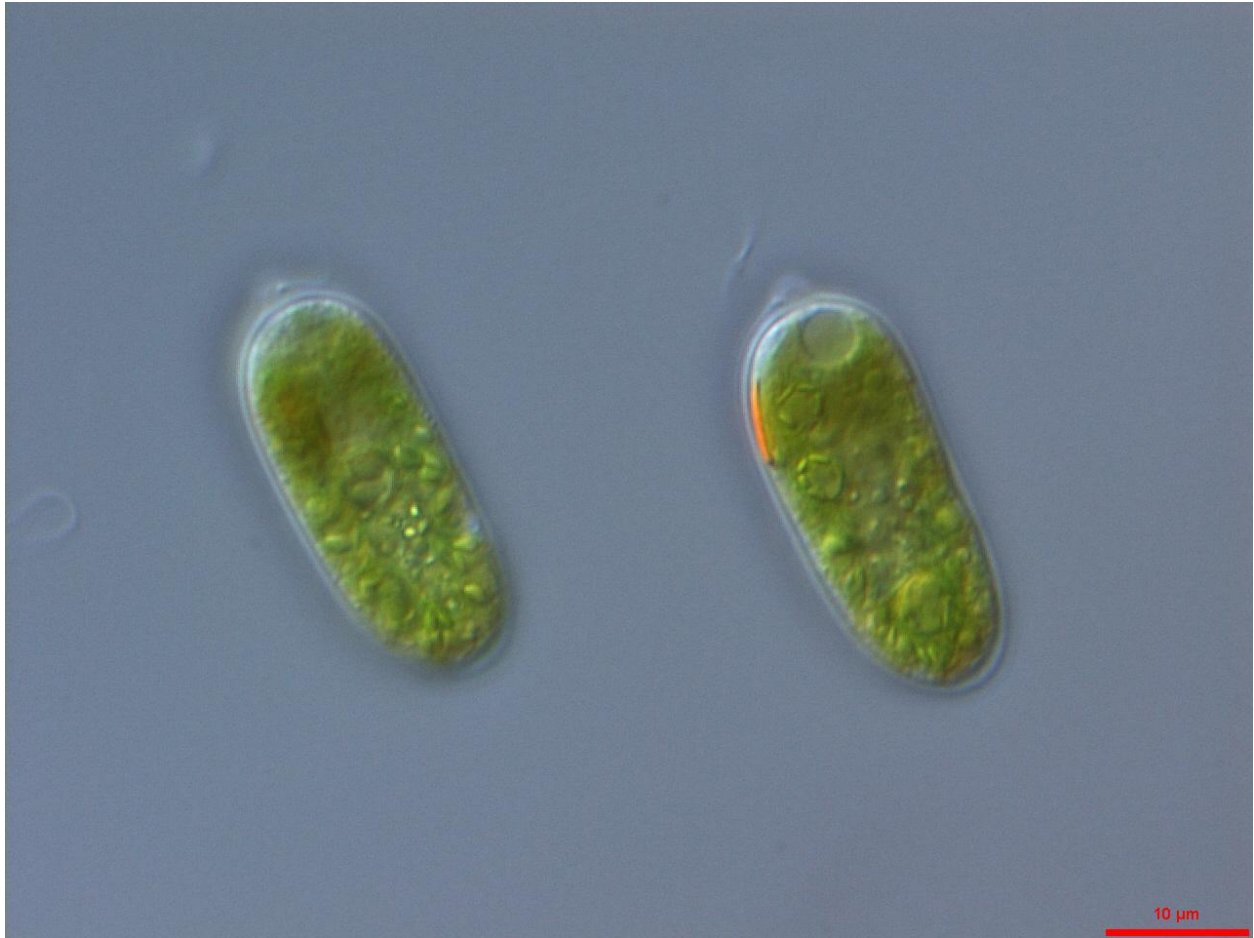
Chlamydomonas sp. C. Picture Lake, October 11, 2012. Image by Karl Bruun.

Chrysosphaerella. Highwood Lake, October 5, 2012. Image by Karl Bruun.
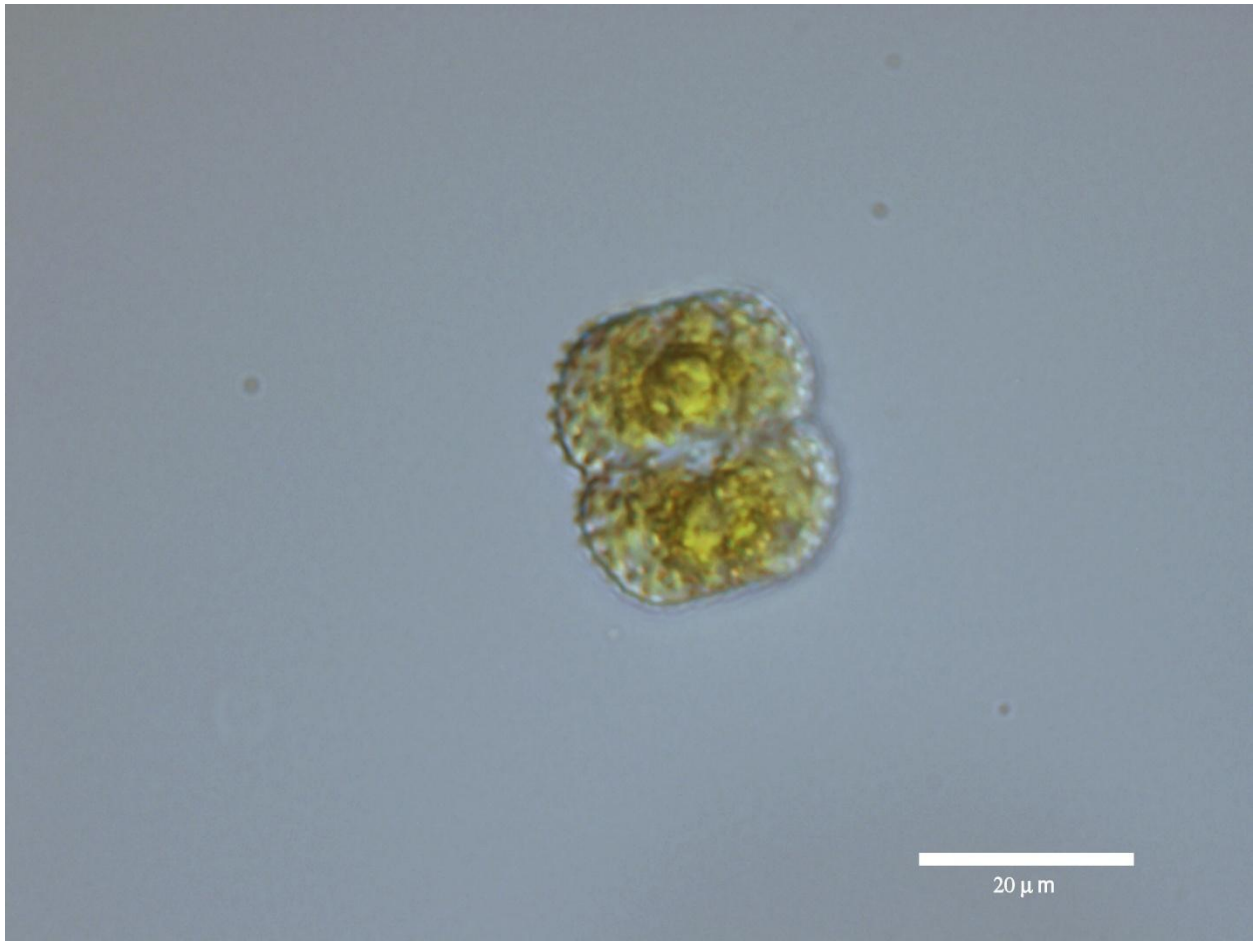
Cosmarium. Upper Bagley Lake, September 20, 2012.
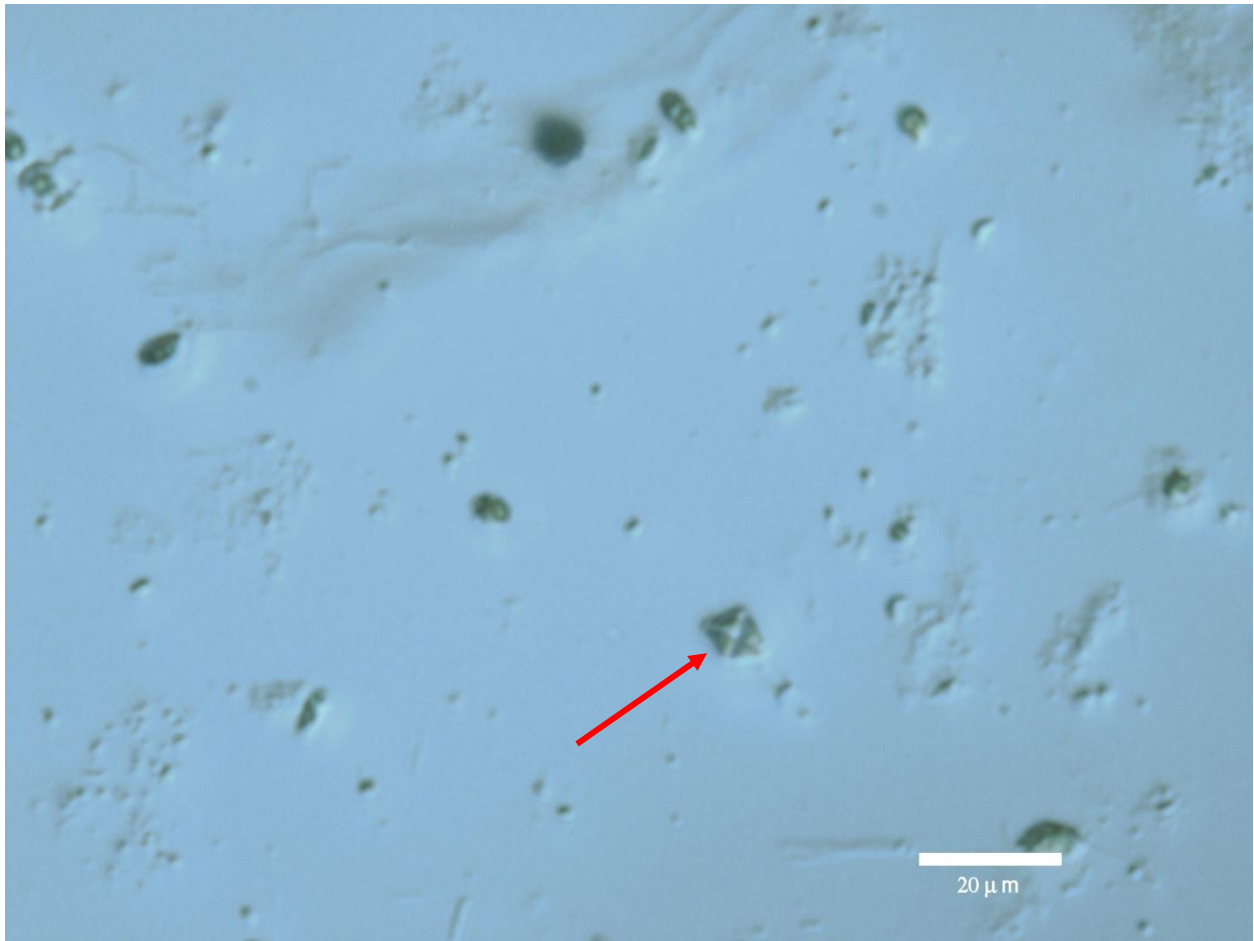
Crucigenia tetrapedia. Highwood Lake, September 13, 2012.

Crucigeniella rectangularis. Picture Lake, September 5, 2012. 400X.

Cyclotella. Picture Lake, September 13, 2012.
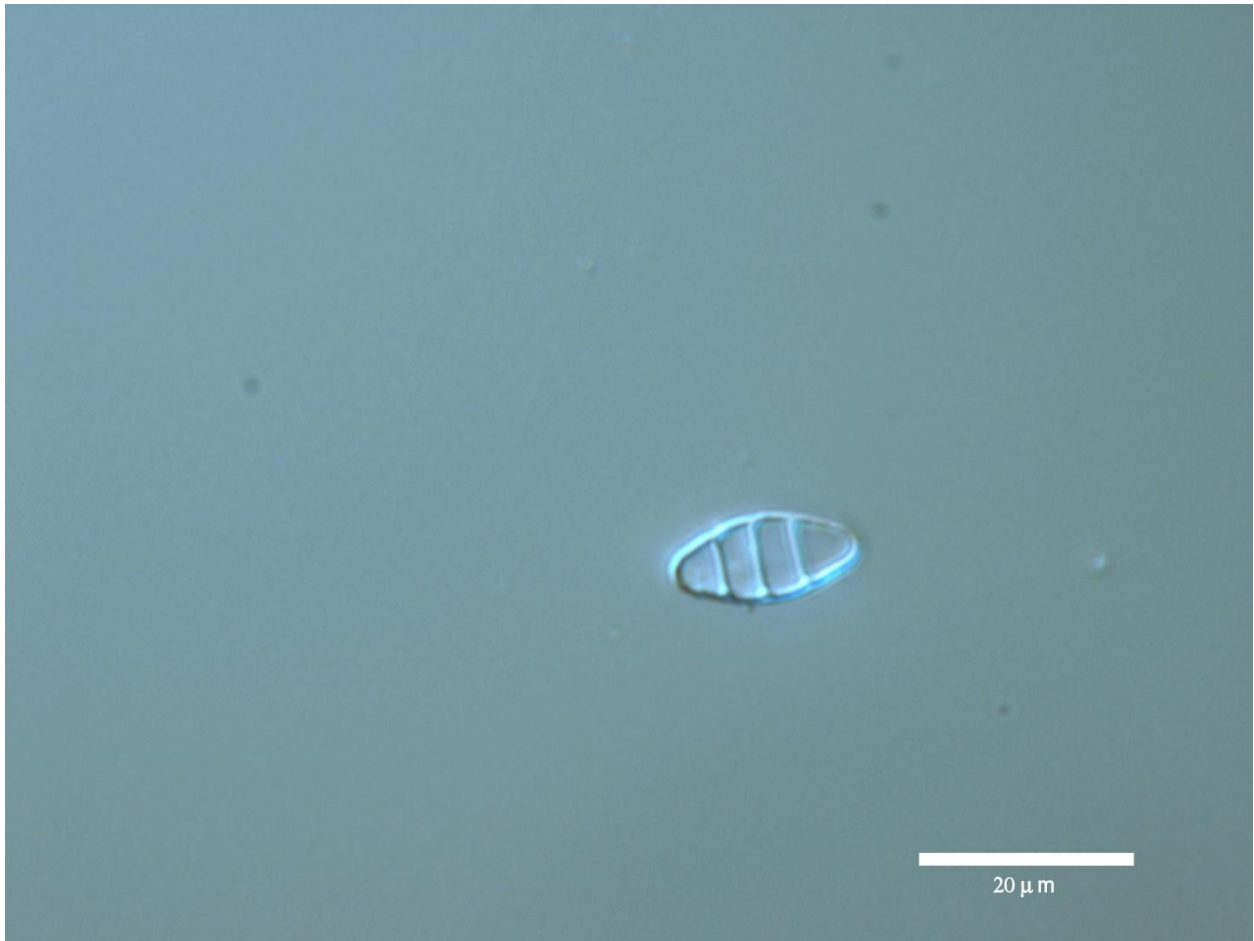
Diatoma mesodon. Bagley Creek, September 13, 2012. Valve view.

Diatoma mesodon. Lower Bagley Lake, September 27, 2012. Girdle view.
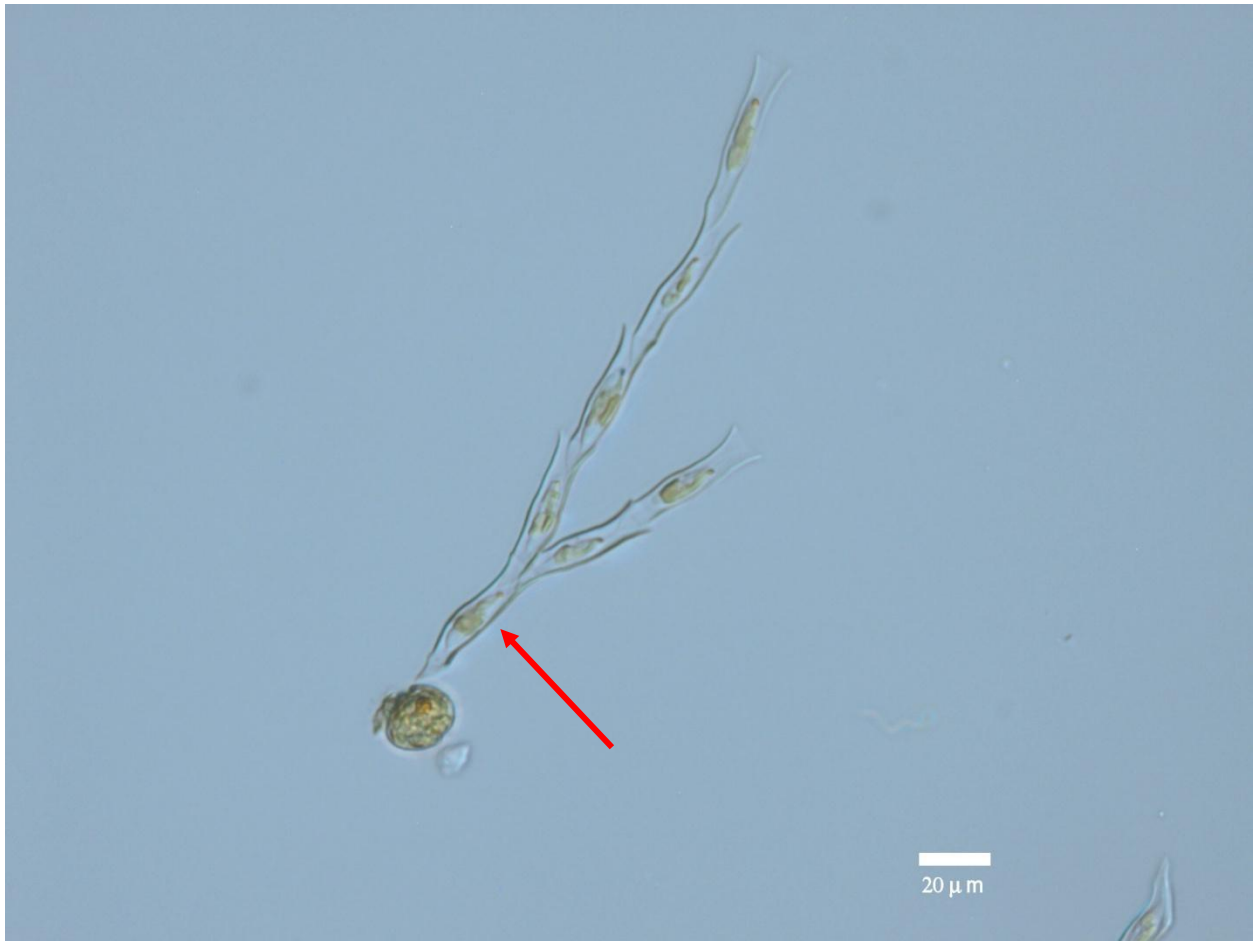
Dinobryon cylindricum var. *alpinum*. Picture Lake, August 30, 2012.

Dinoflagellate cyst. Upper Bagley Lake, October 11, 2012. Image by Karl Bruun.
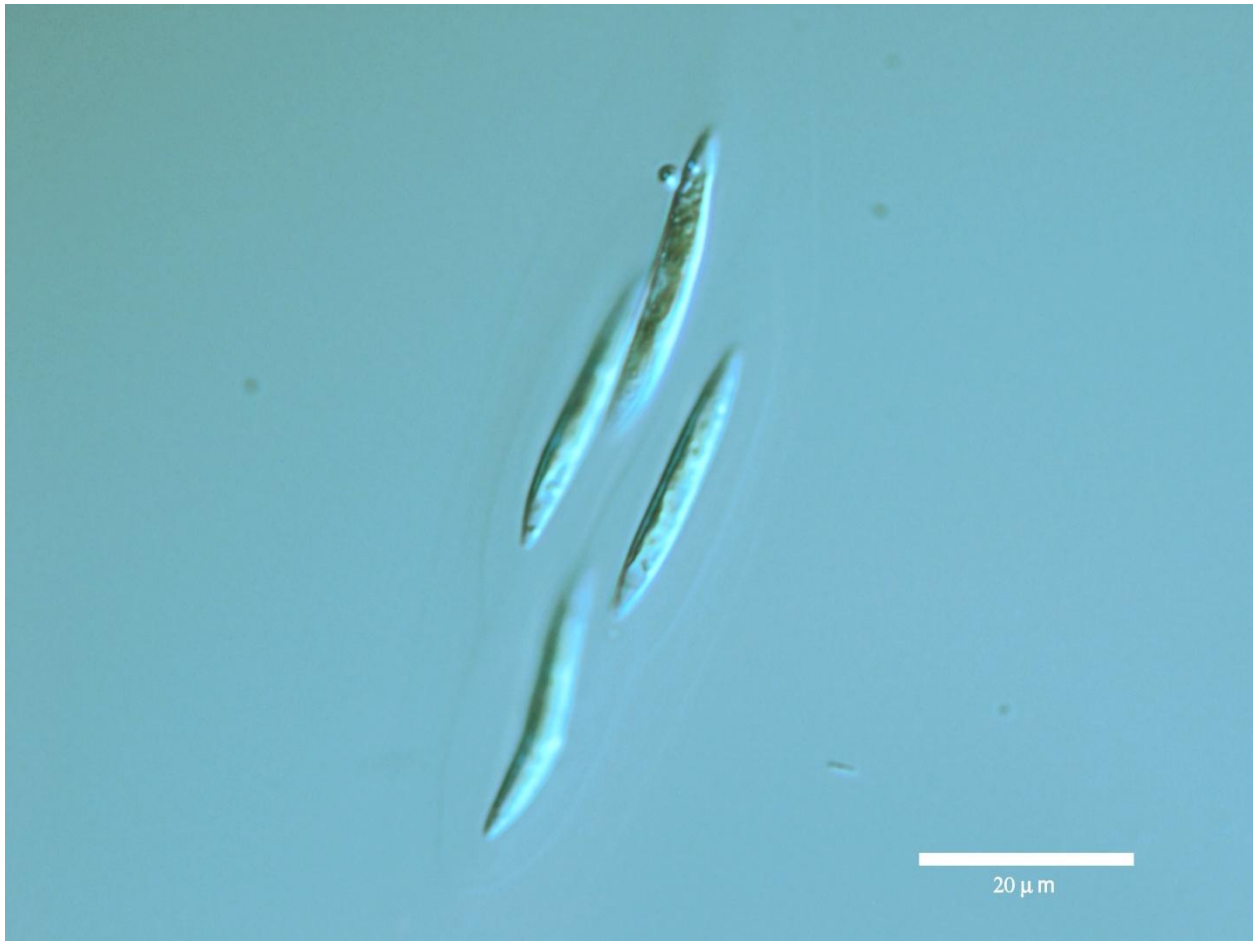
Elakatothrix. Picture Lake, September 27, 2012.
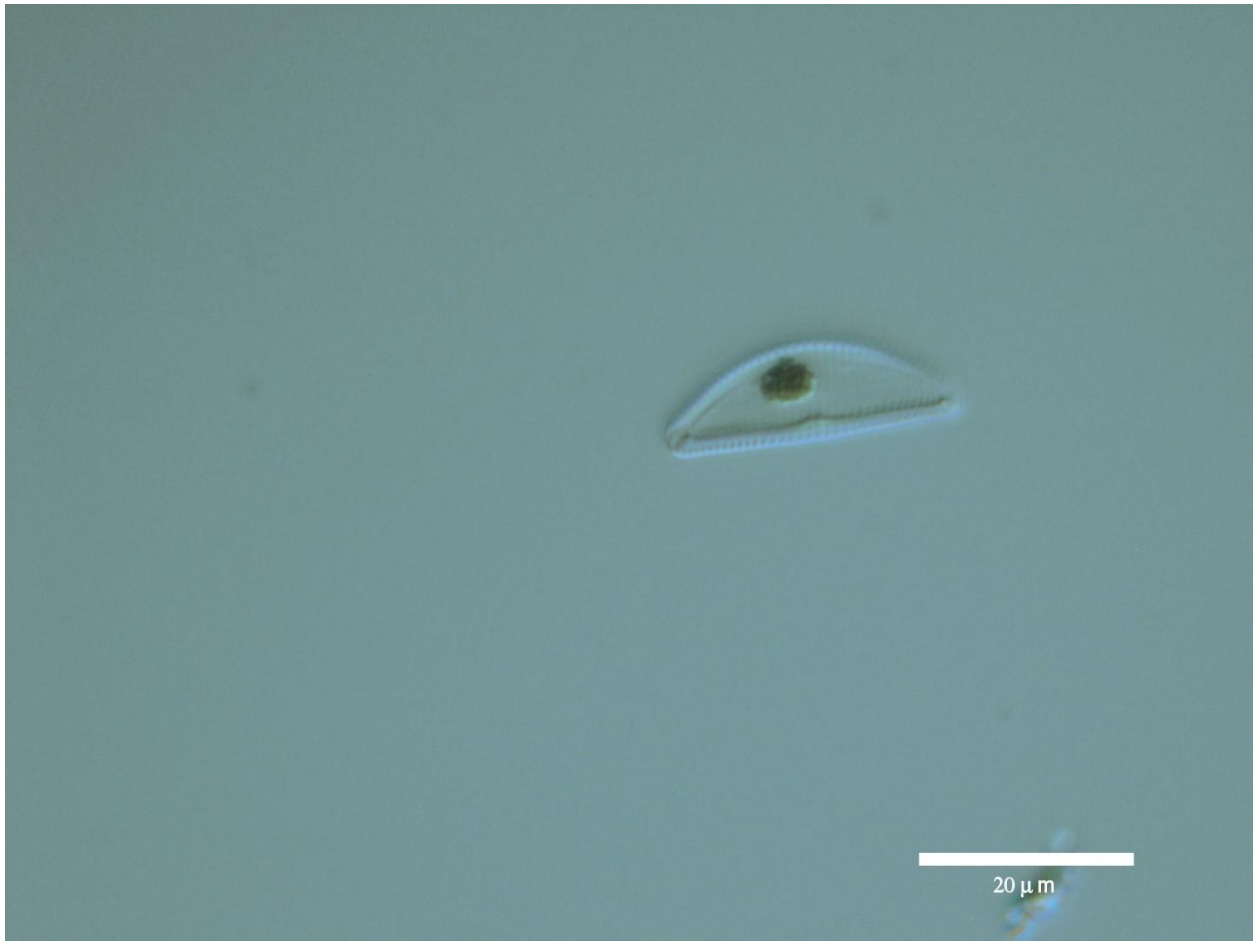
Encyonema cf. *silesiacum*. Lower Bagley Lake, September 27, 2012.

Euastrum elegans. Highwood Lake, August 22, 2012.

Euglena. Highwood Lake, October 11, 2012. Image by Karl Bruun.
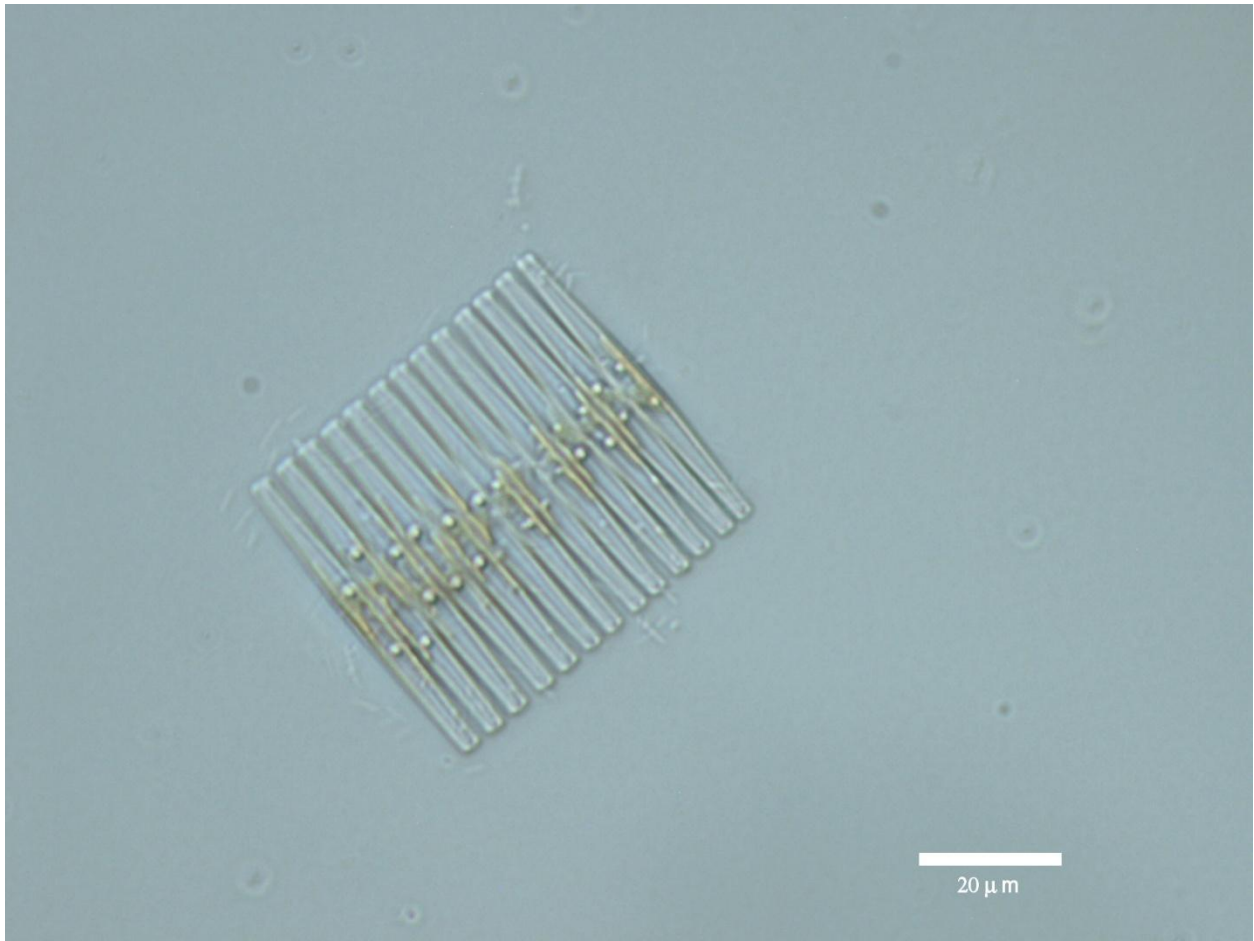
Fragilaria crotonensis. Lower Bagley Lake, September 20, 2012.

Frustulia rhomboides var. *amphipleuroides*. Lower Bagley Lake, August 15, 2012.
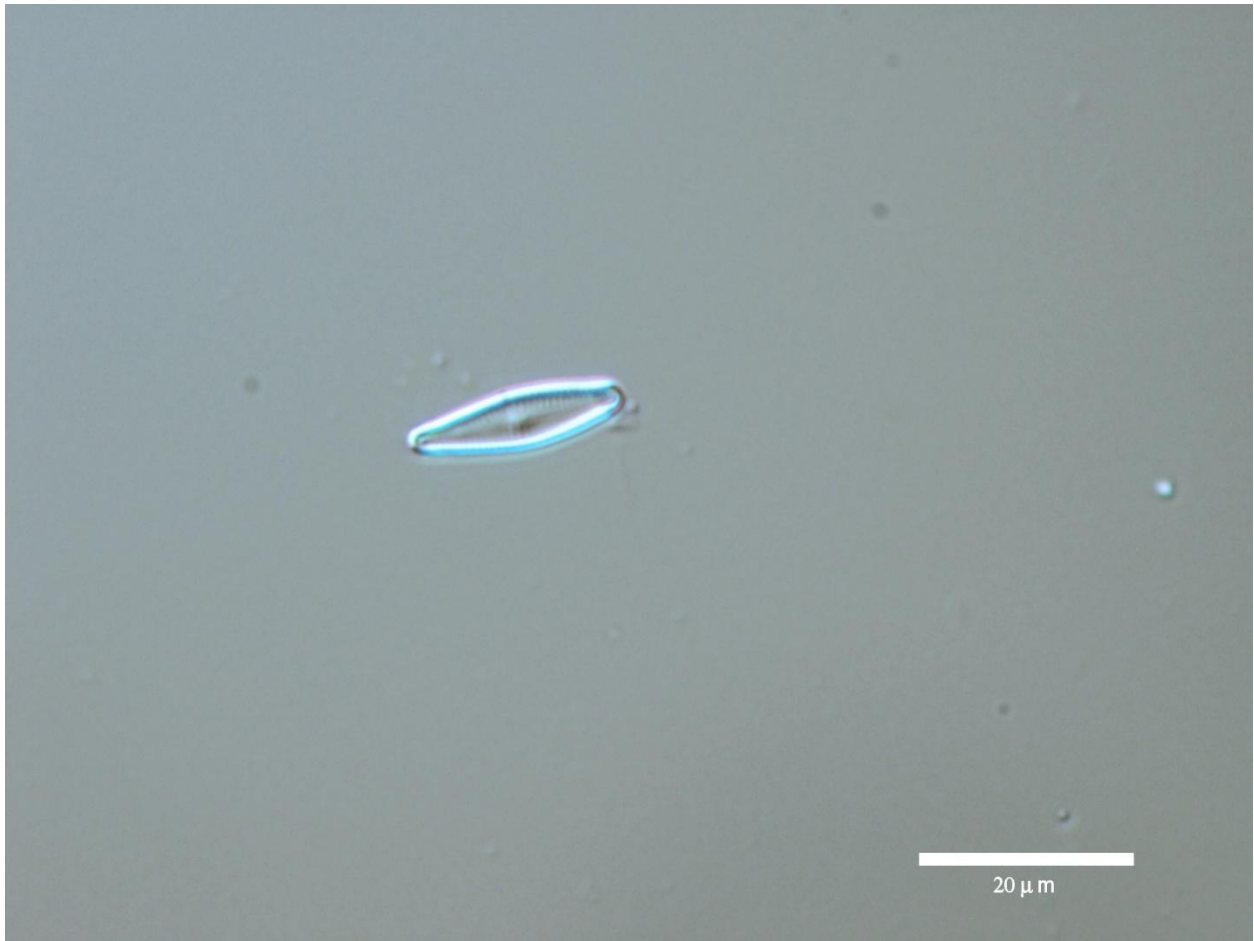
Gomphonema. Upper Bagley Lake, October 11, 2012.
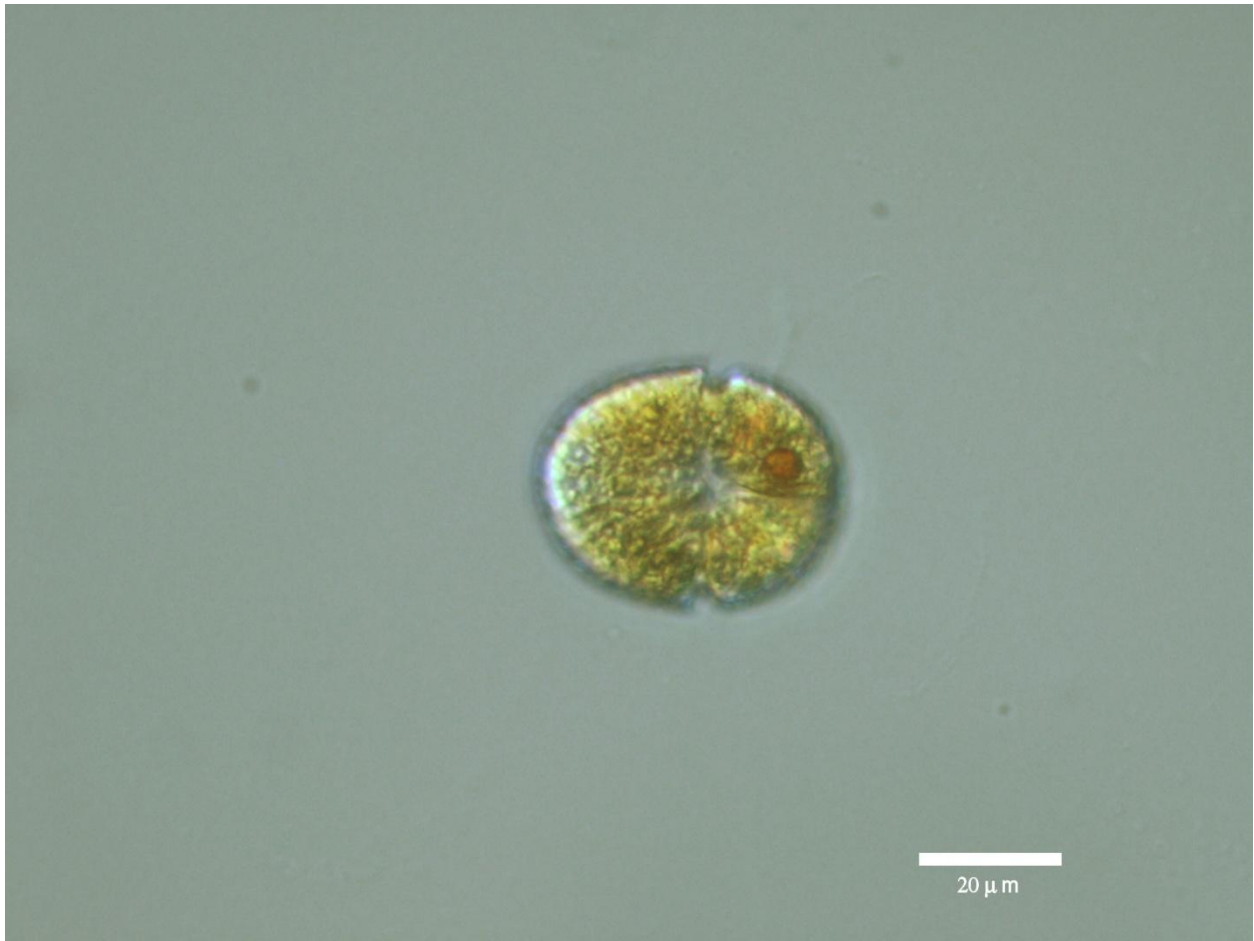
Gymnodinium uberrimum. Picture Lake, September 20, 2012.
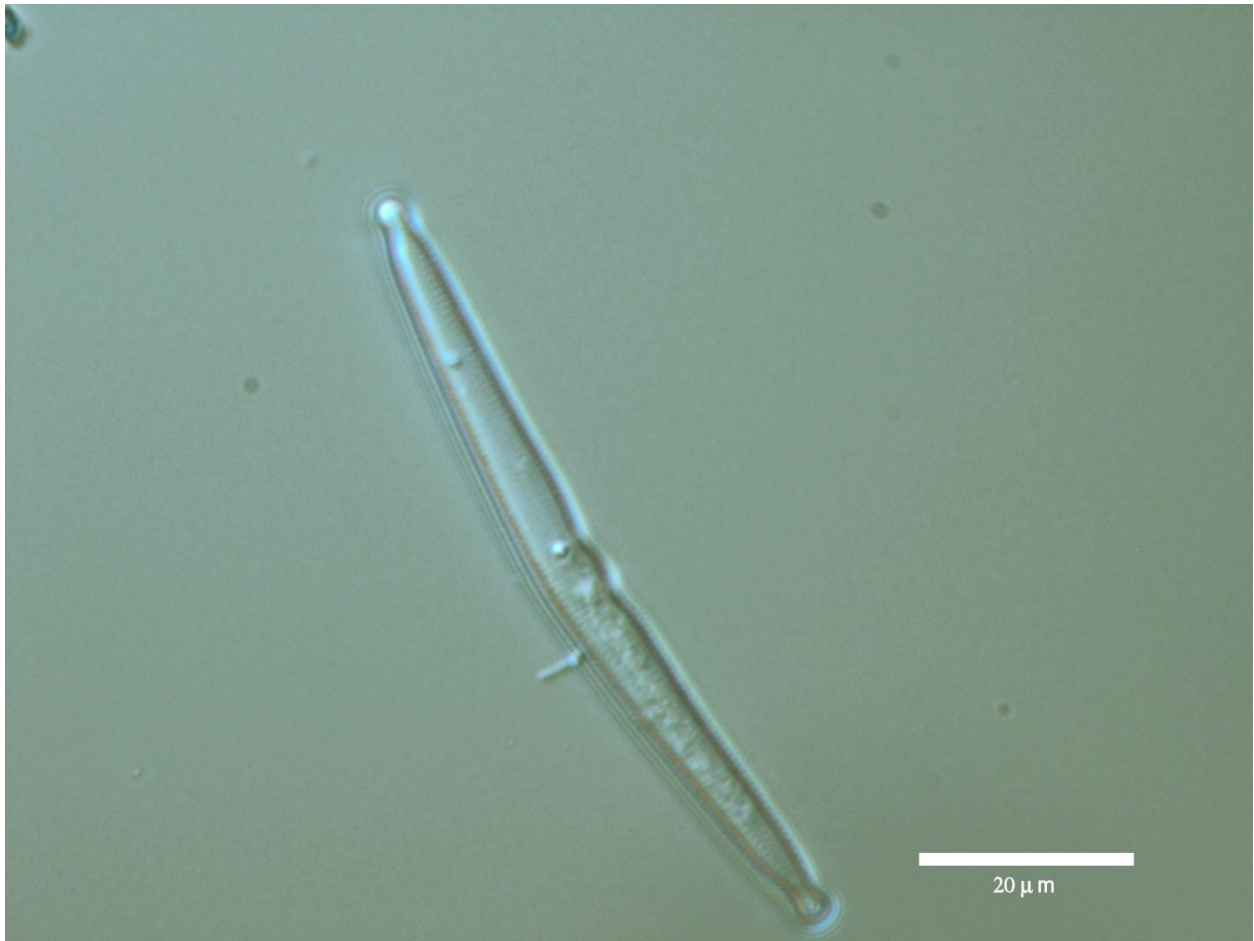
Hannaea arcus. Lower Bagley Lake, September 27, 2012.
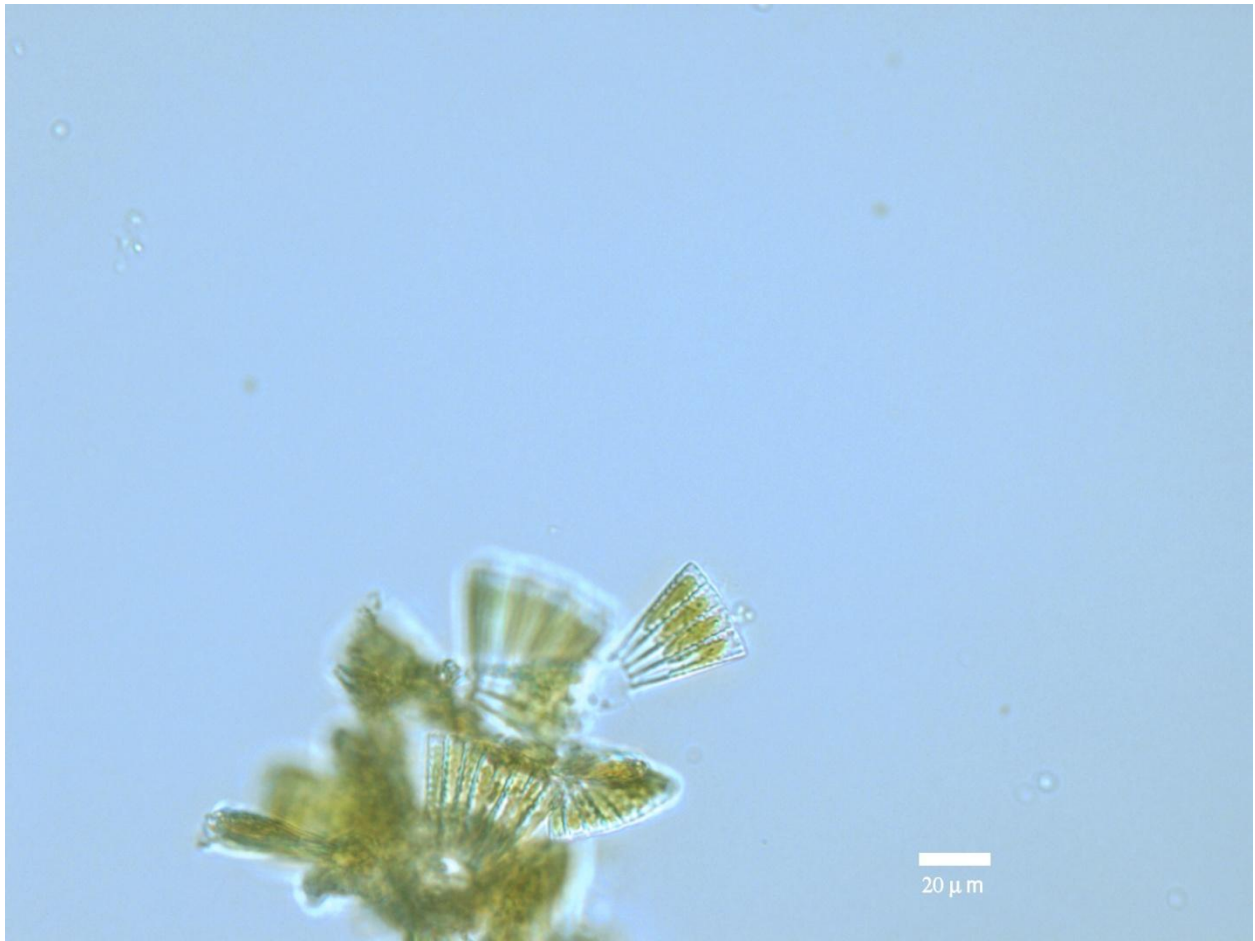
Meridion circulare. Upper Bagley Lake, September 13, 2012.

Merismopedia punctata. Highwood Lake, August 22, 2012. 600X.

Navicula cf. *cryptocephala*. Highwood Lake, October 11, 2012. Image by Karl Bruun.

Neidium. Picture Lake, August 30, 2012.
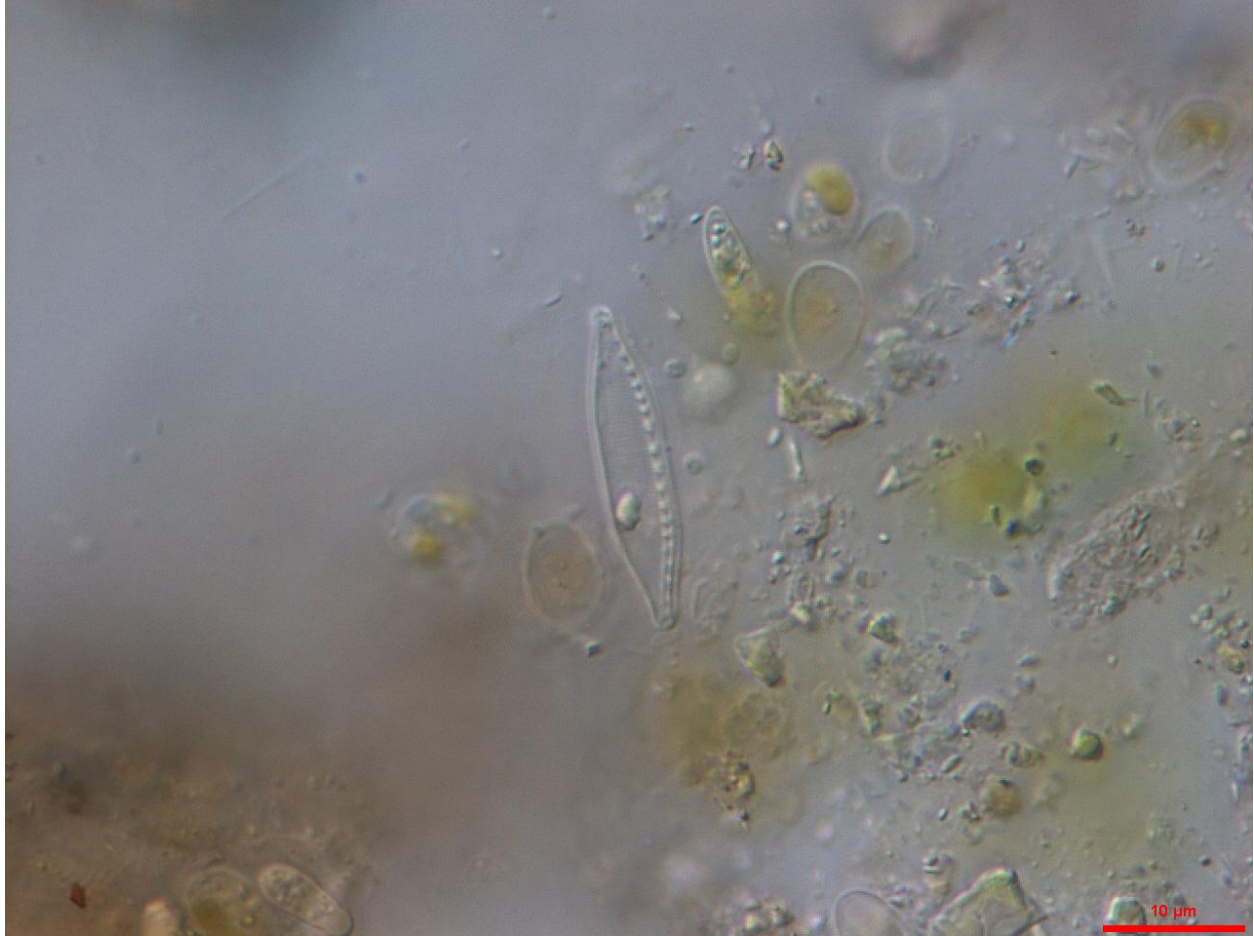
Nitzschia. Highwood Lake, October 11, 2012.
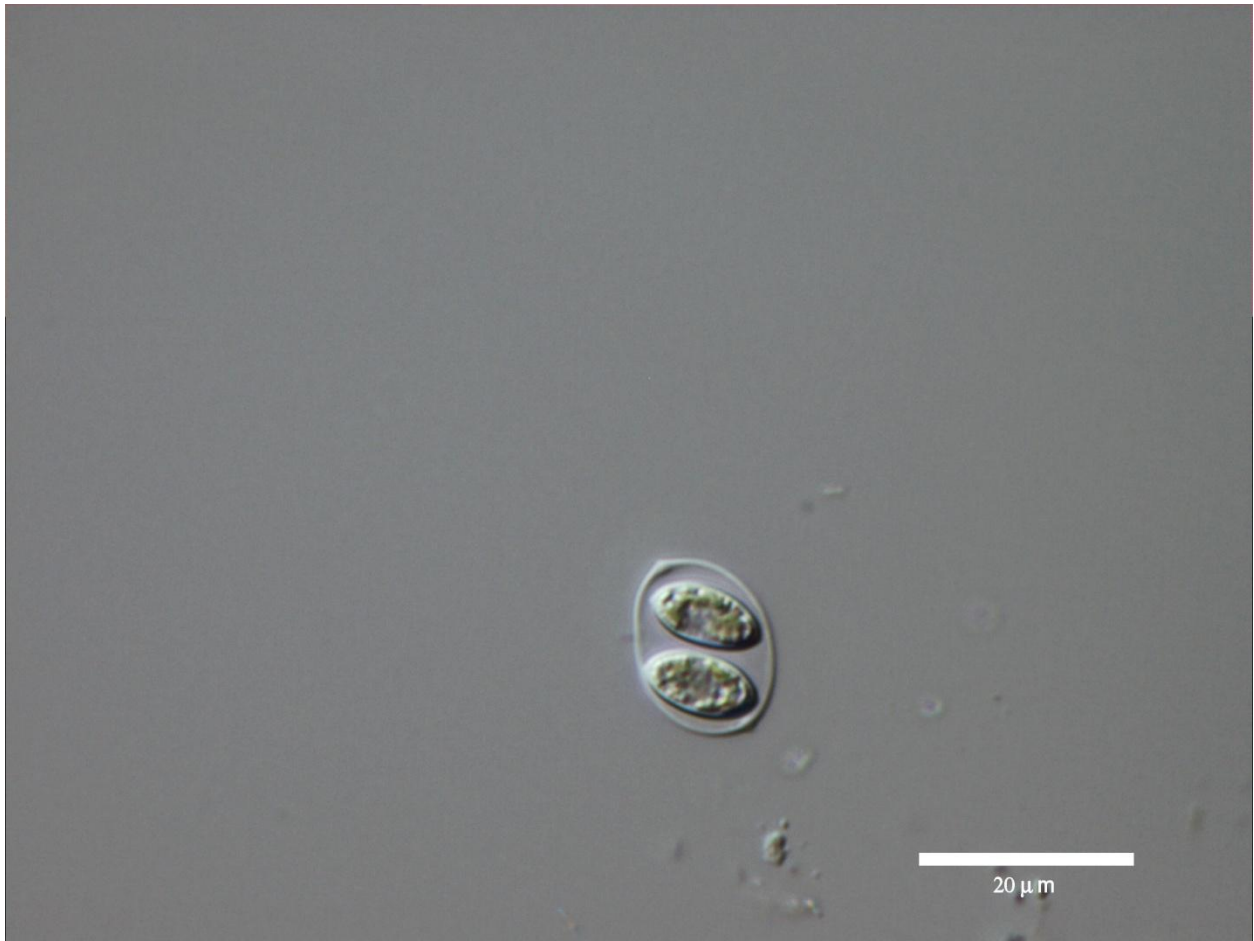
Oocystis cf. *marssonii*. Picture Lake, August 26, 2008. Image by Robin Matthews.
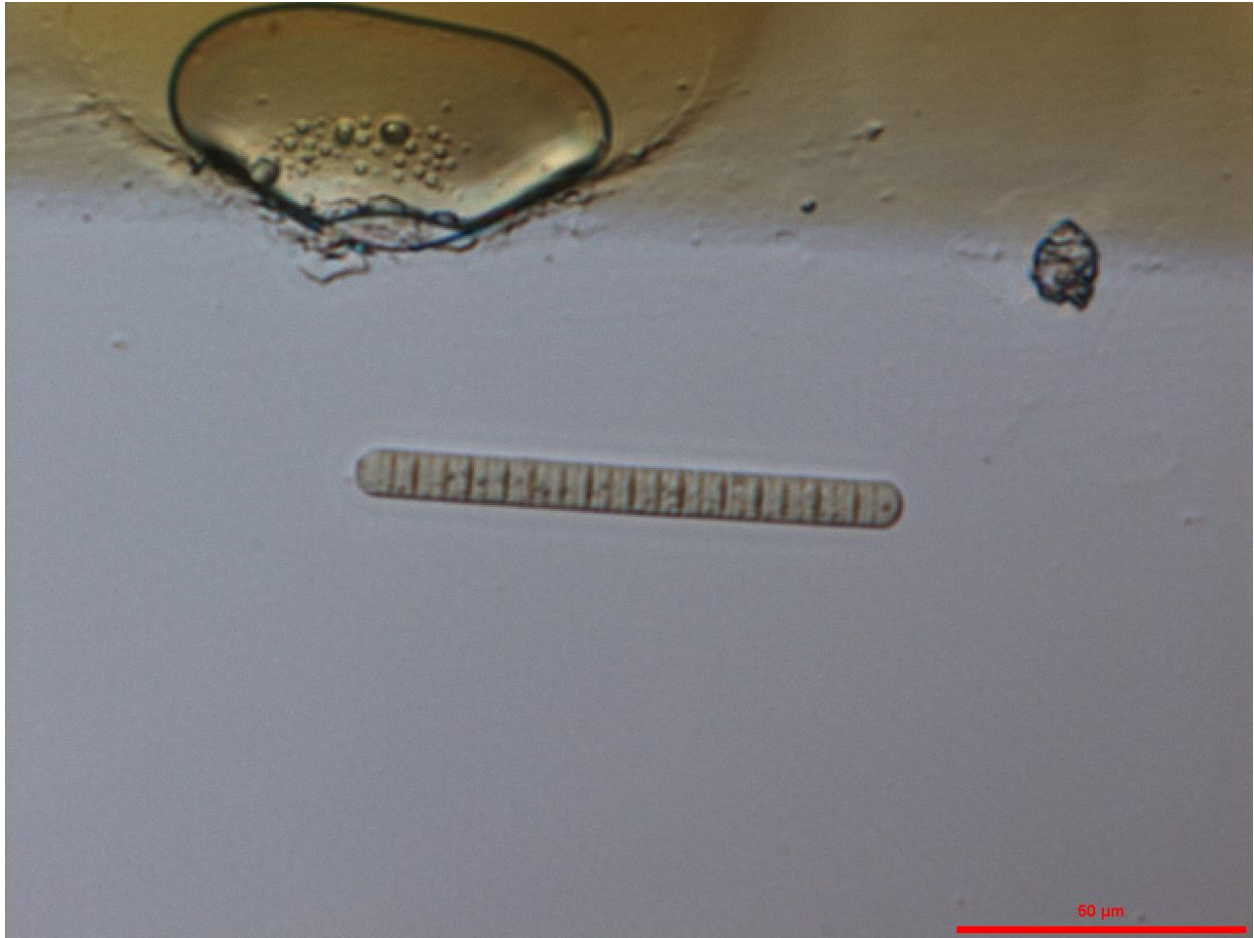
Oscillatoria tenuis. Lower Bagley Lake, September 27, 2012. Image by Karl Bruun.
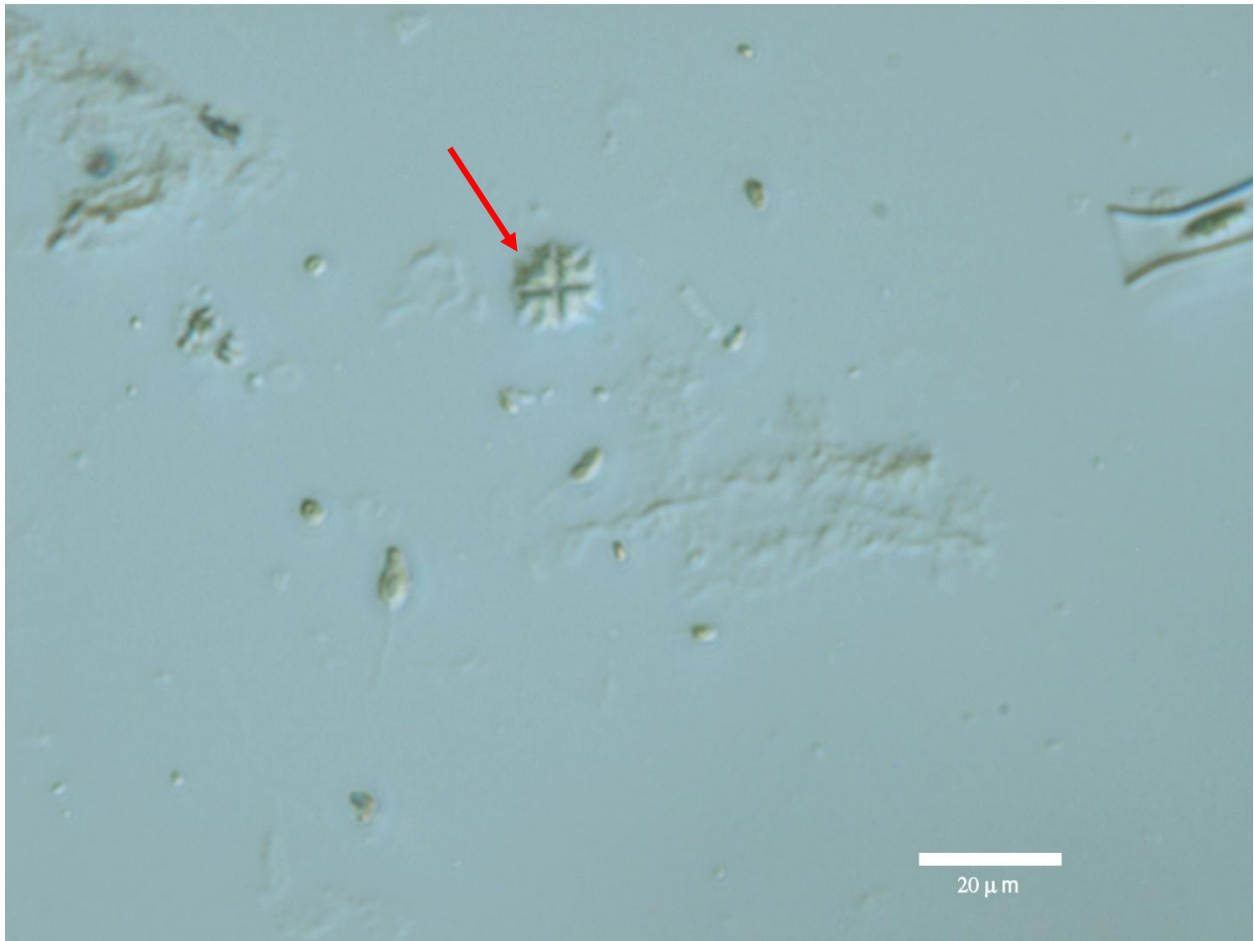
Pediastrum tetras. Picture Lake, September 14, 2012.
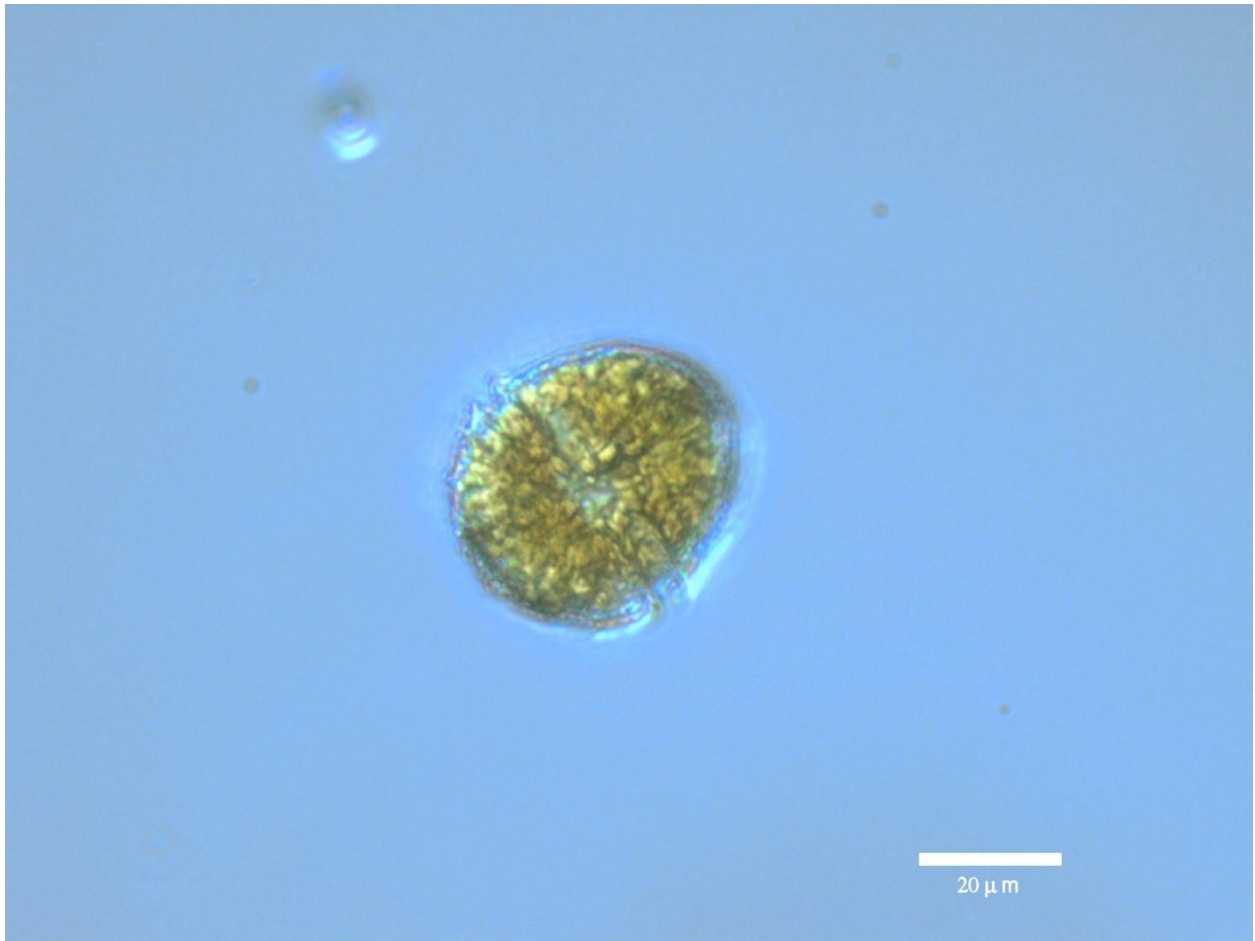
Peridinium. Highwood Lake, September 27, 2012.

Pinnularia. Upper Bagley Lake, August 22, 2012. 200X.

Scenedesmus. Picture Lake, August 22, 2012. 400X.
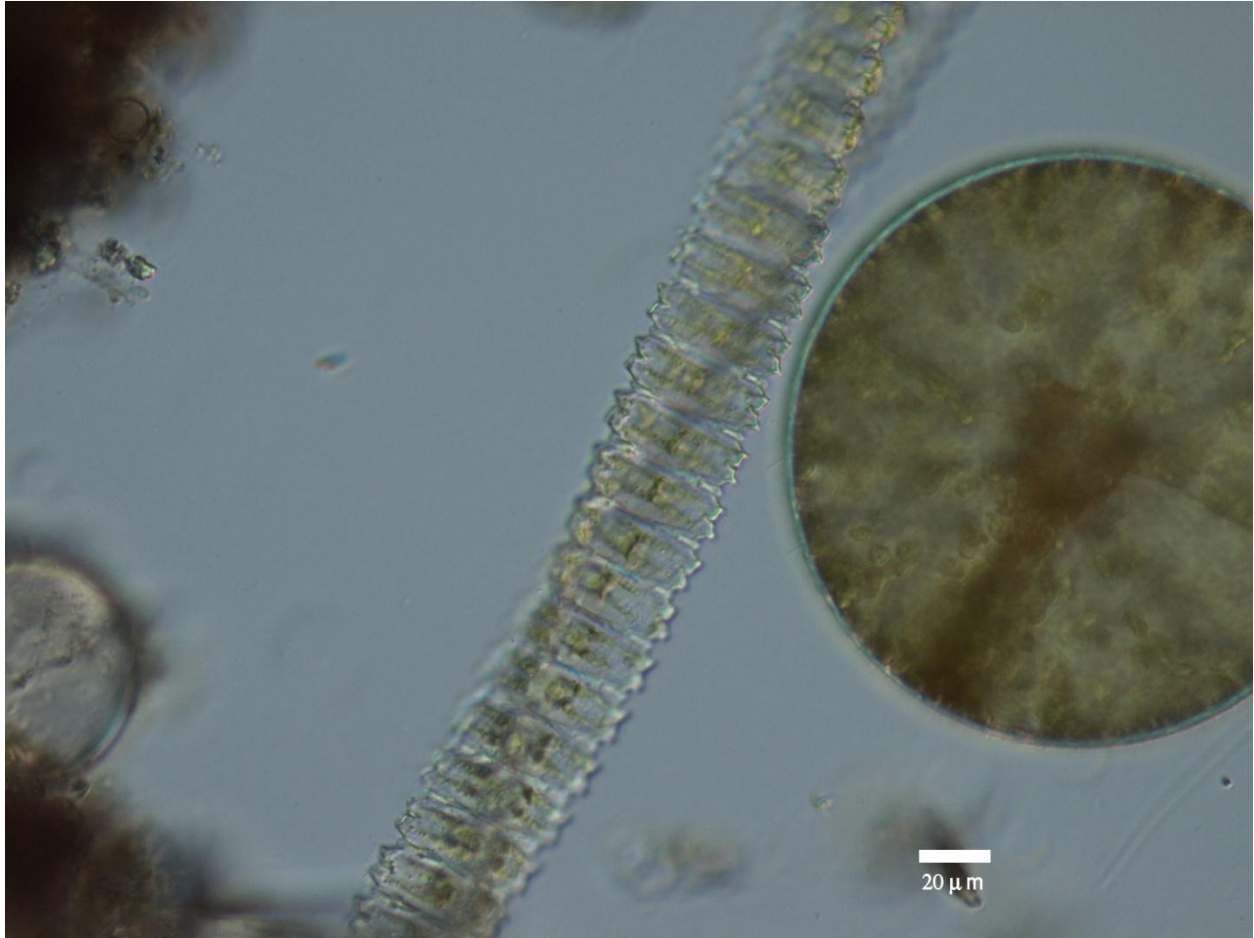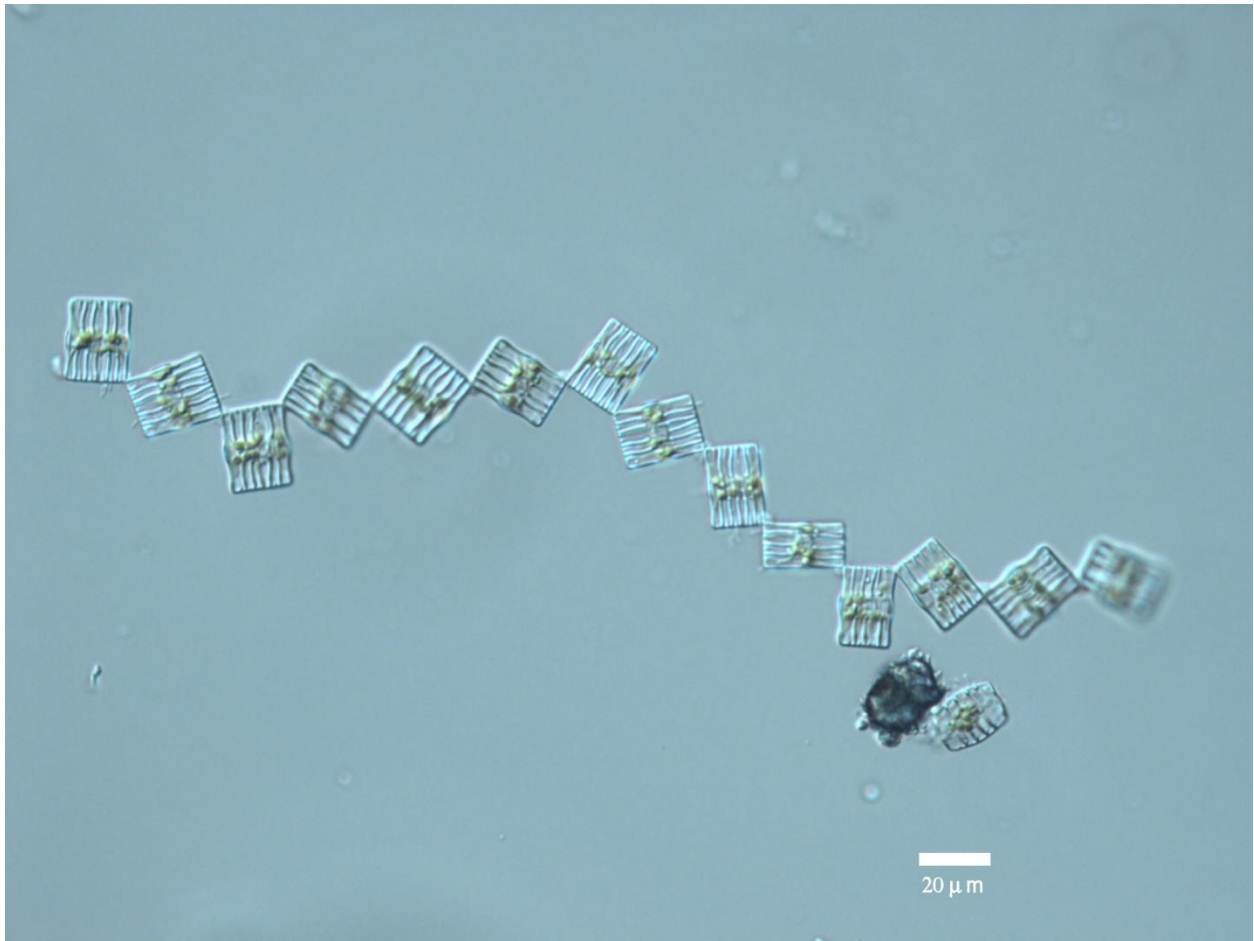
Spondylosium. Highwood Lake, August 17, 2012. Image by Robin Matthews.
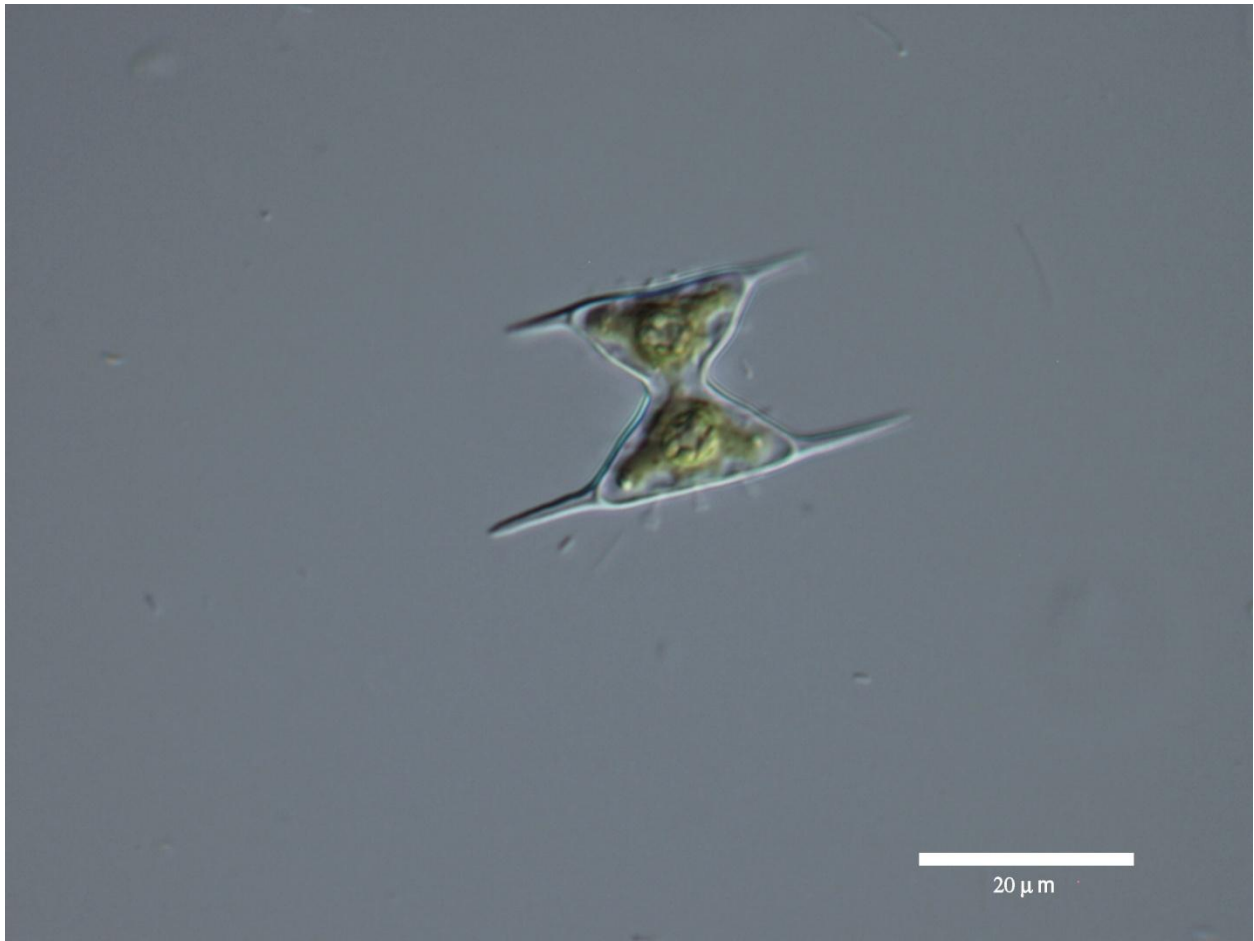
Staurodesmus triangularis. Picture Lake, August 17, 2012. Image by Robin Matthews.
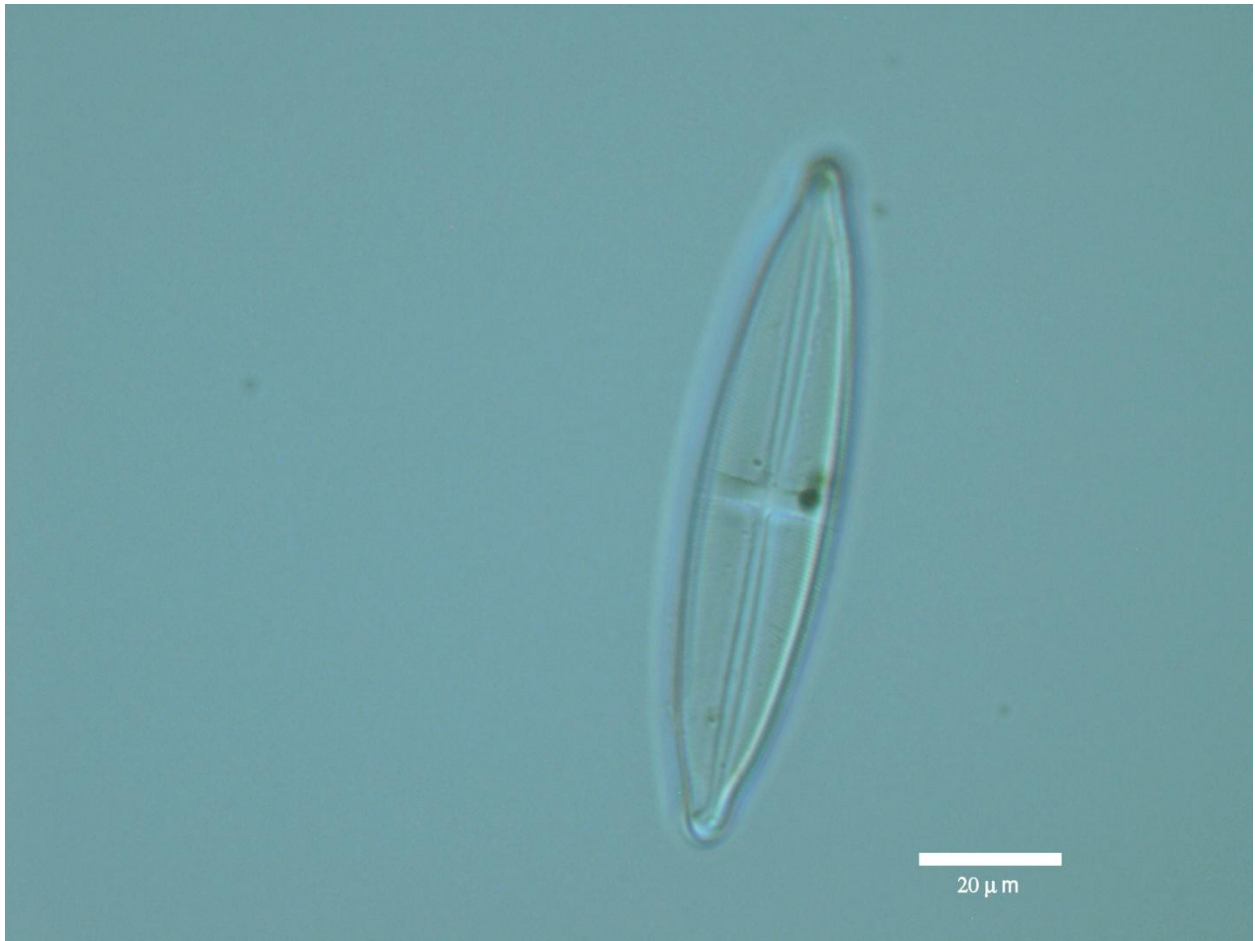
Stauroneis. Highwood Lake, August 22, 2012.

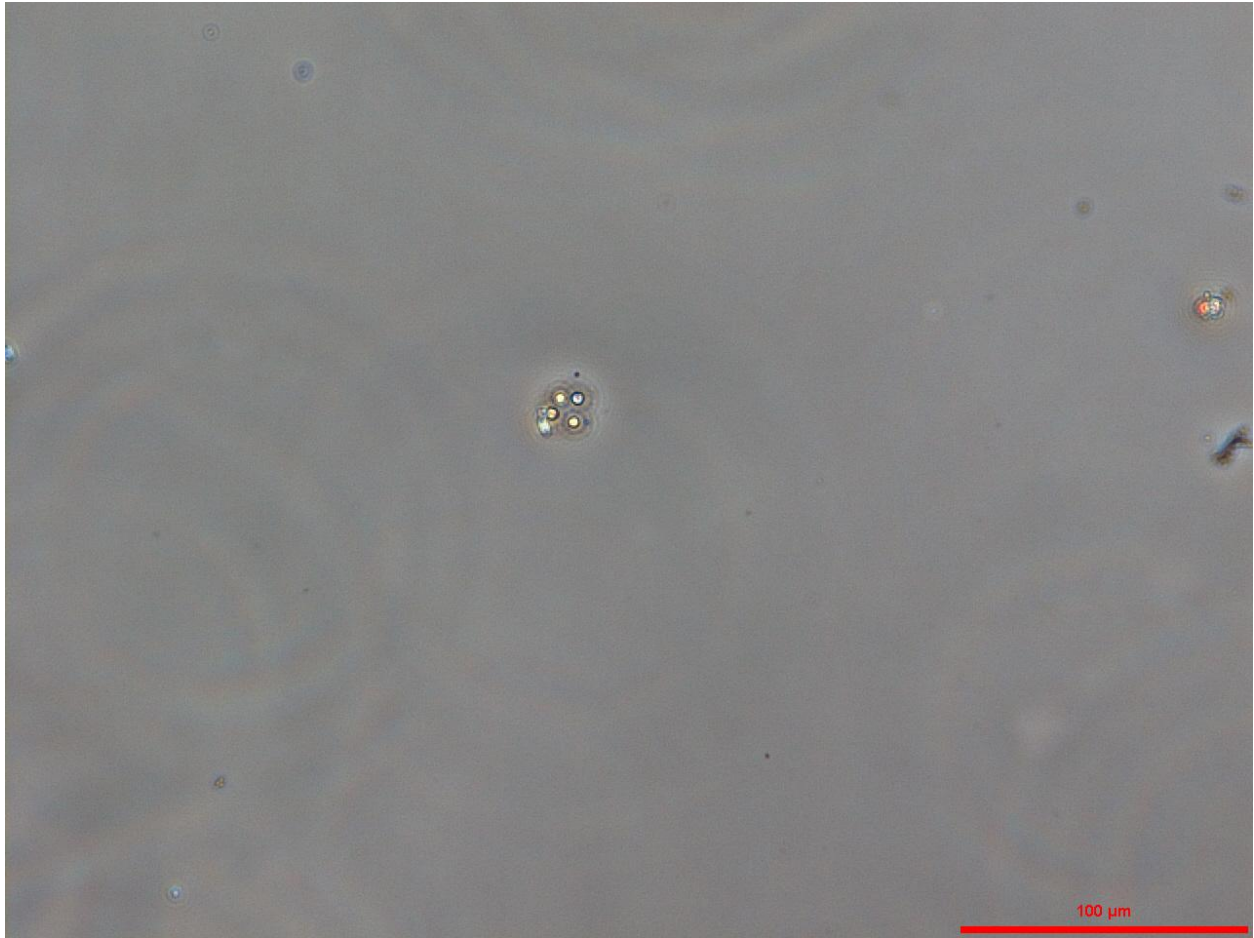*Synura uvella*. Highwood Lake, October 5, 2012.

Tabellaria flocculosa. Lower Bagley Lake, September 20, 2012.
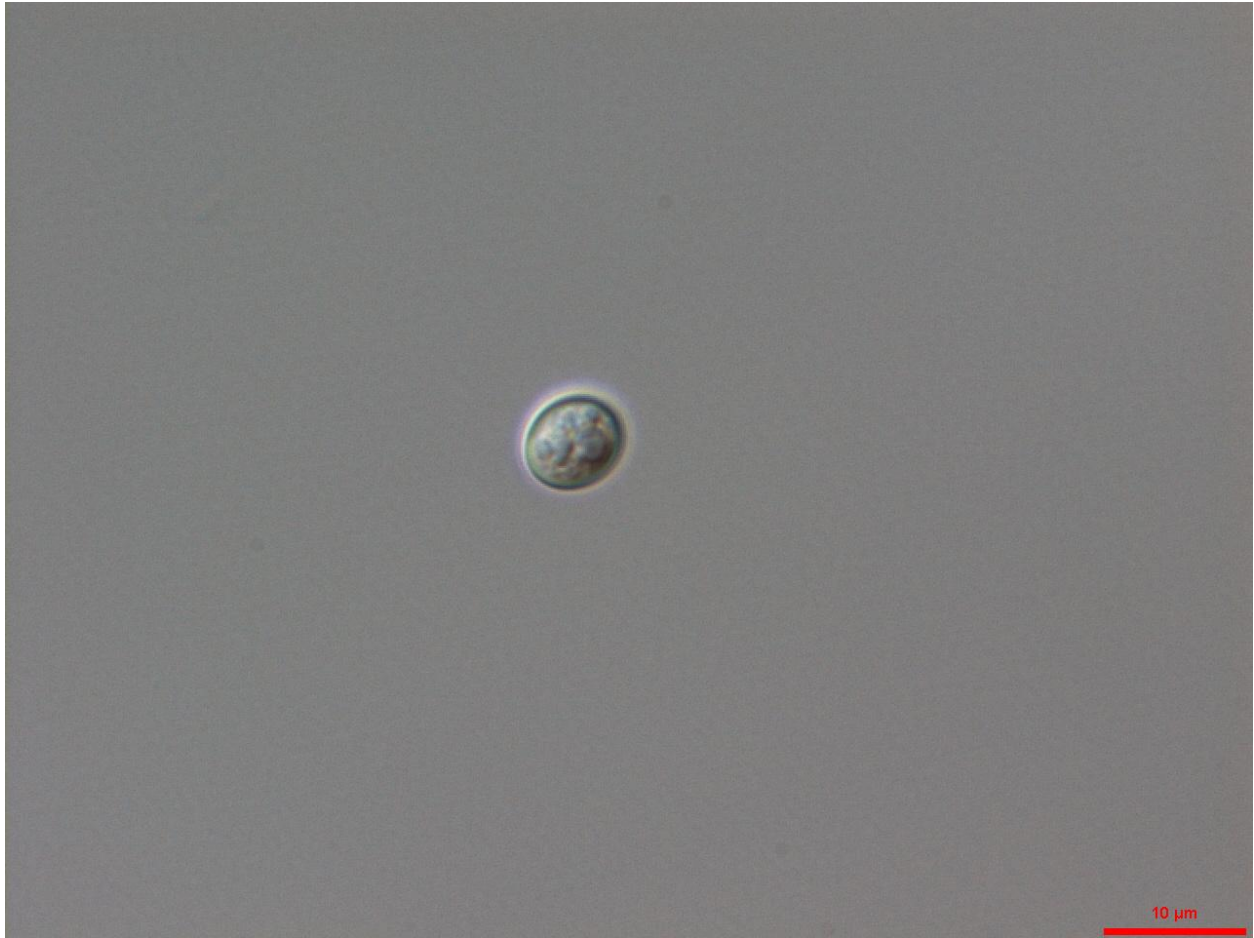
Unknown flagellate, 1 flagella. Highwood Lake, October 5, 2012. Image by Karl Bruun.

Unknown flagellate, 2 flagella. Highwood Lake, October 5, 2012. Image by Karl Bruun.
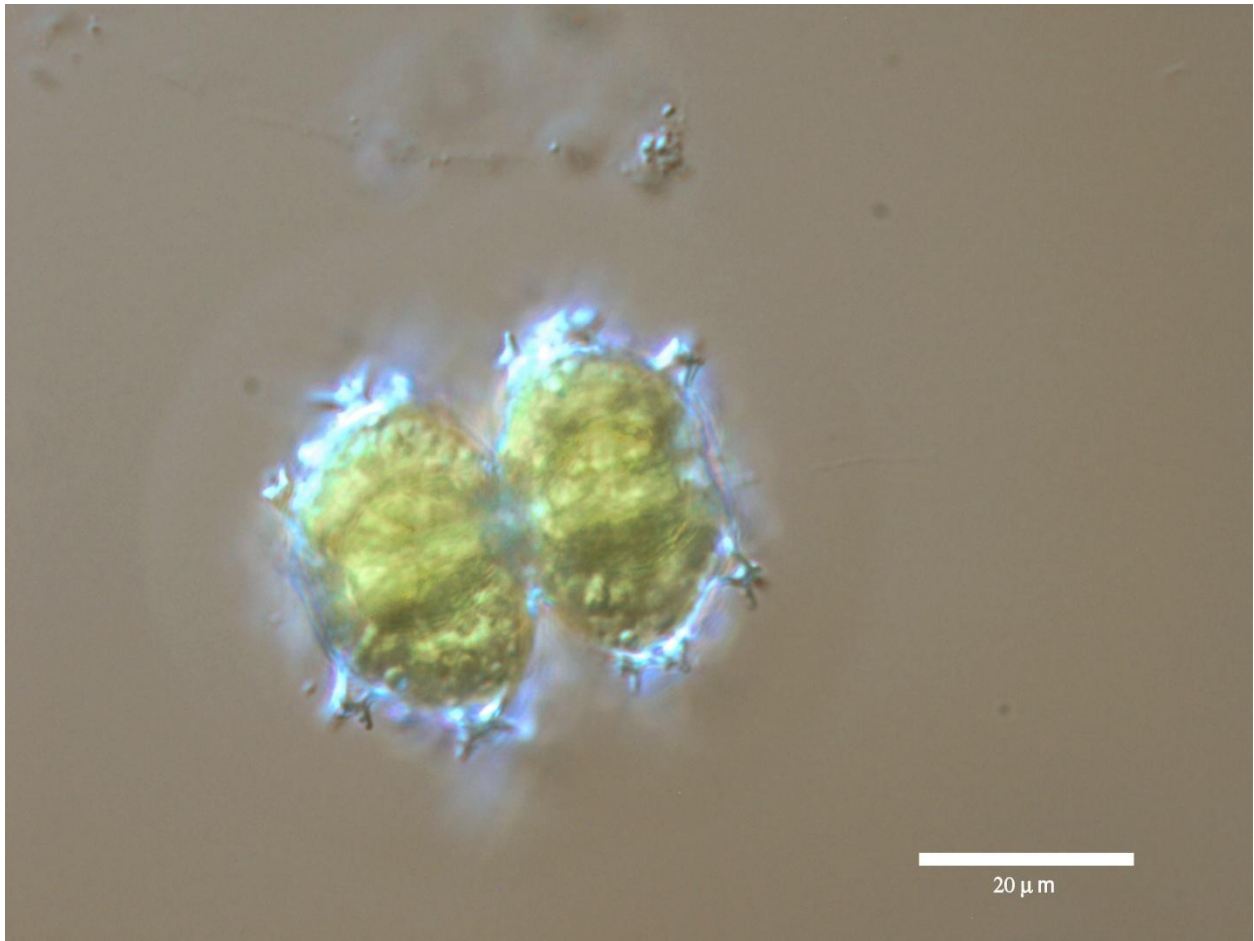
Unknown small spherical. Highwood Lake, August 15, 2012. Image by Karl Bruun.

Unknown spherical. Highwood Lake, August 22, 2012. Image by Karl Bruun.

Unknown spherical, 1 flagella. Highwood Lake, August 30, 2012. 400X. Image by Karl Bruun.

Xanthidium sp. A. Highwood Lake, September 20, 2012.

Xanthidium sp. B. Highwood Lake, August 22, 2012.