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ORIGINAL PAPER

Temporal changes in diatom valve diameter indicate shifts in lake trophic status

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Abstract When diatoms undergo vegetative cell division the new siliceous wall components are slightly smaller than those of the parent because they are produced within the confines of the parent wall. Thus, with continued growth the mean size of cells in a population declines. Given this unique feature of diatom cell division, if the growth of a species in a lake increases (decreases) under more (less) favorable conditions, then the mean size of the resulting population will decline (increase). Numerous paleolimnological investigations rely on shifts in the relative abundances of diatom species over time to infer lake conditions. Although relative abundance data yield information about the dominance of species in the community, they do not necessarily provide evidence about growth of a given species. For instance, a species could have increased in growth, but simply to a lesser extent than other taxa, resulting in a decline in relative abundance. In a similar fashion, relative abundance values can be misleading when used to infer environmental change, such as trophic status change in lakes. We propose that including data on mean size of diatom valves can yield greater insight into changes in growth and improve observations and conclusions based on relative abundance data. To test this concept, we examined changes in the mean diameter of *Aulacoseira ambigua* (Grunow) Simonsen valves relative to known shifts in lake trophic status in a core from Bantam Lake, Connecticut, representing ~ 130 years of sediment accumulation. The mean valve diameter of *A. ambigua* declined from 9.7 to 7.6 µm, with the largest declines clearly tracking significant increases in trophic status. We conclude that changes in the mean size of diatom frustules over time can provide valuable information for understanding long-term environmental changes.

Keywords *Aulacoseira* · Diatoms · Eutrophication · Inference · Paleolimnology · Size reduction · Trophic status

Introduction

Over the course of an annual cycle in most freshwater lakes and ponds, there is a succession of algal species in the planktic environment that often repeats each year if conditions remain stable (Reynolds 2006). In

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general, the longer a species is exposed to conditions that favor its growth, the larger will be the resultant population. Theoretically, if the same favorable conditions are repeated each year, similar-size populations would form. A change in any single variable, or combination of variables, that further enhance growth of the species could yield an even denser population, whereas shifts in variables that reduce growth would decrease the resulting size of the population (Reynolds 2006). An assumption commonly made in paleolimnological research is that past changes in growth patterns are archived in the sediment record.

Indeed, changes in the relative abundances of algal species archived in lake sediments over time, especially of diatom species, are routinely used as proxies for inferring past environmental change in paleolimnological research (Smol and Stoermer 2010). A classic example is the enhanced growth of specific diatom taxa, which results from increased influx of nutrients associated with cultural eutrophication, coupled with declines of other species that are typical of oligotrophic conditions (Hall and Smol 2010). Changes in the composition of diatoms have also been used successfully to reconstruct other past lake-water variables, including pH (Chen et al. 2008; Finkelstein et al. 2014), nitrogen (Siver 1999) and phosphorus concentrations (Chen et al. 2008; Whitmore et al. 2015), and to examine proliferation of cyanobacteria (Riedinger-Whitmore et al. 2005), changes in land use (Siver et al. 1999), shifts in thermal structure (Brown et al. 2017) and impacts of climate change (Boeff et al. 2016). Although most studies rely on relative diatom abundance data, paleolimnological inferences could be strengthened by including absolute concentration data, which can be estimated by addition of a known quantity of an internal marker, such as spores, pollen or microspheres (Laws 1983; Kaland and Stabell 1981). In a survey of papers published in the *Journal of Paleolimnology* from 2017 to 2019, all 30 articles that included diatoms as paleoenvironmental proxies used only relative abundance data.

In addition to shifts in species composition, identified using relative abundances, we hypothesize that the unique way in which diatoms undergo cell division offers another potentially valuable means to track the impact of environmental variables on diatom growth over time. When a diatom undergoes vegetative cell division, the new siliceous valves are formed within the confines of the parent cell and each becomes a hypovalve on the two new daughter cells (Round et al. 1990; Jewson 1992). This results in a decline in the mean size of cells as a population grows. Even though the reduction in size caused by a cell division is small, it becomes more significant and noticeable with successive divisions (Round et al. 1990). Given size reduction with continued growth, we propose that this metric could provide valuable information on shifts in population growth, and because the siliceous frustules become part of the sediment record, this metric could also be linked to long-term environmental changes.

In a recent study of diatom remains in a long core from the early Eocene Giraffe Pipe fossil locality, *Aulacoseira giraffensis* Siver, Wolfe & Edlund was found to be the dominant taxon over a 10-m section that represented tens of hundreds of years of accumulation (Siver et al. 2019). The sudden appearance of *Aulacoseira giraffensis* within the core, along with shifts in other microfossil taxa, are believed to correlate with deepening of the ancient lake contained within the Giraffe crater (Barber et al. 2013; Siver et al. 2019). Although the relative abundances of *A. giraffensis* were high over this section of the core, absolute concentrations of microfossils within the mudstones were highly variable. A preliminary investigation confirmed a cyclic pattern in the mean diameter of *A. giraffensis* valves along this section of the core (Siver et al. 2019). Presence and shifts in abundances of *A. giraffensis* are key elements in reconstructing the ancient history of the Giraffe waterbody. The impetus behind that study was to determine whether the cyclic pattern in valve diameter of *A. giraffensis* could shed additional light on paleoenvironmental inferences at the Giraffe Pipe locality.

The objective of this study was to test whether valve diameter of a related diatom species, *Aulacoseira ambigua*, which has grown in a modern lake for at least the last 160 years, changed significantly over that period, given documented shifts in trophic status. Our hypothesis was that eutrophication of the lake resulted in enhanced growth of *A. ambigua* that was coupled with a decline in the mean valve diameter, and that evidence for shifts in valve diameter would be preserved in the sediment record. A second objective was to compare how well changes in mean valve diameter and shifts in relative abundance of *A. ambigua* tracked trophic state condition over time.

Study site

Bantam Lake, situated in the Towns of Morris and Litchfield, Connecticut (Fig. 1), ranks as the largest natural freshwater lake in the state, covering 3.91 km^2 (966 acres). The lake lies in the Western Uplands region of Connecticut, which is largely characterized by highly resistant crystalline rocks (Canavan and Siver 1995). The watershed spans over 80.9 km^2, yielding a ratio of watershed to lake surface area of 21. The lake is relatively shallow, with a mean depth of 4.4 m and a maximum depth of 7.6 m, and has a short water retention time of approximately 106 days (Canavan and Siver 1995). Since at least the 1980s, the waterbody has suffered from widespread phytoplankton blooms during summer and autumn periods, and extensive summer growth of invasive macrophytes, including *Myriophyllum spicatum* (Eurasian watermilfoil), *Cabomba caroliniana* (Fanwort) and *Potamogeton crispus* (curly-leaf pondweed) (Aquatic Ecosystem Research (AER) 2020). A detailed paleolimnological study of Bantam Lake was previously completed by our laboratory in 1992 for the Town of Morris (Siver 1992). Although never officially published, data from that study on scaled chrysophytes and planktic diatoms, and archived materials from a dated gravity core, were used in the current investigation. Physical and chemical variables used to characterize lake trophic status are based on Frink and Norvell (1984) and appear in Table 1.

Materials and methods

Field and laboratory procedures

A 42-cm-long core was taken from the center of Bantam Lake in 1991 (Fig. 1) with a Glew gravity corer (Glew 1989). The core was cut on site using an extruder (Glew 1988) into 0.5-cm, 1-cm, and 2-cm sections within the intervals 0–10 cm, 10–30 cm and below 30 cm depth, respectively. The core was dated using ^{210}Pb by Jack Cornett and Bert Risto at AECL, Chalk River, Ontario, Canada, using methods outlined in Cornett et al. (1984), and reported by Siver et al. (1999). The constant rate of supply (CRS) model

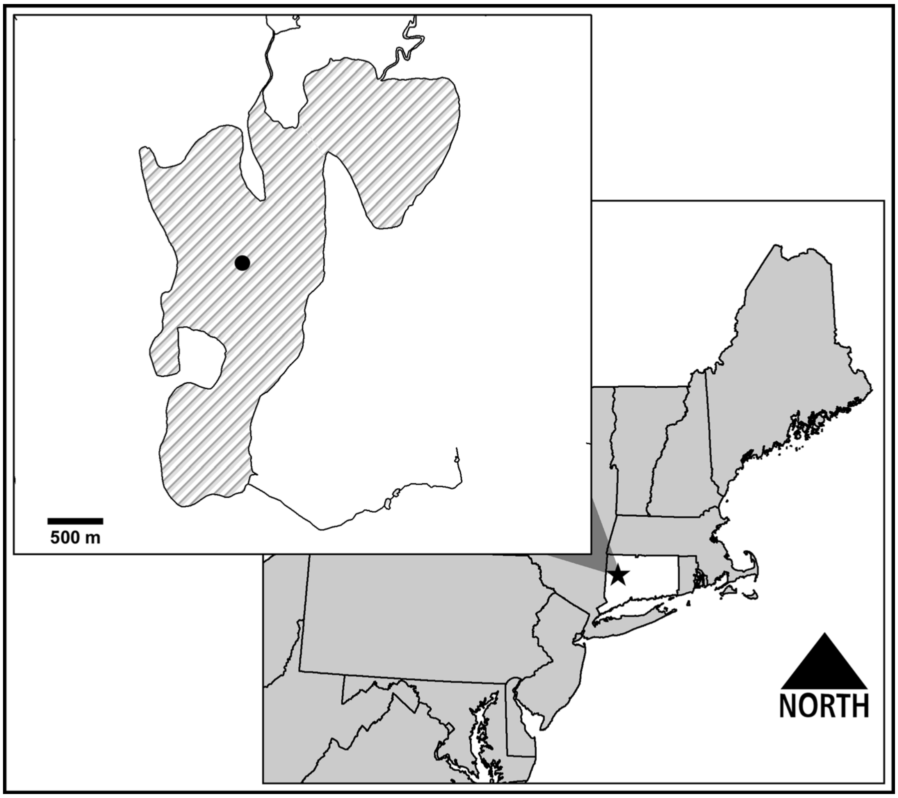

Fig. 1 Map of Bantam Lake showing the coring site (circle), and the location of the lake in the State of Connecticut (star) within the northeastern United States (41°42′17″N; 73°13′16″W)

Table 1 Water quality guidelines used by Frink and Norvell (1984) to characterize the trophic status of a freshwater lake with respect to total phosphorus (TP), total nitrogen (TN), chlorophyll-*a* (Chl), and Secchi Disk depth (SD)

Trophic state category	TP (μg L^{-1})	TN (μg L^{-1})	Chl (μg L^{-1})	SD (m)	Trophic state Score
Oligotrophic	0–10	0–200	0–2	> 6	(− 204) to (− 112)
Early Mesotrophic	10–15	200–300	2–5	4–6	(− 111) to (− 43)
Mesotrophic	15–25	300–500	5–10	3–4	(− 42) to 45
Late Mesotrophic	25–30	500–600	10–15	2–3	44–108
Eutrophic	30–50	600–1000	15–30	1–2	107–293
Hyper eutrophic	> 50	> 1000	> 30	0–1	> 293

Ranges of trophic state scores for each lake type derived from principal component analysis based on the four variables are given

(Appleby and Oldfield 1978) was used to generate dates for each stratum. The CRS model was selected over the constant initial concentration (CIC) model because of changes in sedimentation rate that resulted from watershed development. The raw counts from the ^{210}Pb analysis are no longer accessible for computing error terms. The dates previously generated from the analysis provide a general chronological framework for the study, and align nicely with known changes that took place in the watershed. The correlation between mean valve size and other trophic state indicators is not compromised by inaccuracies in the dating. The age of the core bottom (40–42 cm) dated to 1857. Fourteen sections from the core were used in the current study (Table 2), including 11 for analysis of community structure and trophic status, and 13 for estimating morphometrics of *A. ambigua* valves.

Preparation of core samples for identification and quantification of scaled chrysophytes and planktic diatoms were as outlined in Marsicano and Siver (1993) and Siver (1999). Briefly, we mixed approximately 1 g wet weight of sediment from each section with a mixture of sulfuric acid–potassium dichromate and heated it on a hot plate to oxidize organic matter. At the completion of the oxidation step, the material was transferred to a centrifuge tube and washed with deionized water a minimum of 5 times. We transferred the resulting slurry to a glass vial and brought the volume to 10 mL by adding deionized water.

For scanning electron microscopy (SEM) observation, an aliquot from each slurry was mixed with deionized water and air-dried onto a piece of heavy-duty aluminum foil. The aluminum foil was trimmed, mounted onto an aluminum stub with Apiezon® wax, and coated with gold for two minutes with a Polaron Model E sputter coater. For quantification and observation with light microscopy (LM), another aliquot was mixed with 30 ml of deionized water and added to Battarbee trays (Battarbee 1986) containing wells fitted with 22-mm-diameter circular cover slips. The mixture was allowed to dry on a vibration-free table. The cover slips were mounted onto glass slides using Hyrax® mounting medium. The permanent slides were used to enumerate a minimum of 300 chrysophyte scales and 500 diatom valves from each core interval (Siver 1992), which in turn were used to analyze community structure and infer trophic status. Although counts of chrysophyte scales and diatom valves were done with LM, each sample was first examined with a Coates and Welter field emission SEM to identify organisms that would be difficult to separate from similar species using LM. Ratios of such species were made with SEM and used to separate counts subsequently made with LM (Siver and Marsicano 1996).

Aulacoseira ambigua morphometrics

The archived set of prepared slides was used to estimate *Aulacoseira ambigua* valve diameter for 13 sections of the core (Table 3). The surface sample (0–0.5 cm) was not analyzed for valve diameter because ^{210}Pb analysis indicated it probably represented less than one year of accumulation. Valve diameters and mantle heights were measured for 200 *A. ambigua* valves per section (n = 2600 valves total) using a Zeiss Axiocam 503 color camera and accompanying software. Specimens were imaged with an Olympus BX51 light microscope using a 100× lens with a numerical aperture of 1.25. Mantle height was

Table 2 The inferred trophic scores and status of Bantam Lake for the time period 1857–1991 derived from the remains of scaled chrysophytes in a 42-cm-long sediment core using the model of Siver and Marsicano (1996)

Core depth (cm)	Age	Trophic score	Trophic status	nMDS score axis I	RelAb (%)
0–0.5	1991	213	Eutrophic	− 23	12
1–1.5	1989	202	Eutrophic	− 10	29
2.5–3	1987	222	Eutrophic	− 21	19
4.5–5	1984	188	Eutrophic	− 27	30
7–8	1980	196	Eutrophic	− 9	28
9–10	1975	na	na	na	na
13–14	1964	200	Eutrophic	− 18	18
18–19	1946	35	Mesotrophic	− 9	17
20–21	1938	4	Mesotrophic	0	13
24–25	1926	− 43	Mesotrophic	31	30
30–32	1910	na	na	na	na
34–36	1898	− 108	Oligo/Early Meso	36	17
36–38	1889	na	na	na	na
40–42	1857	− 136	Oligo/Early Meso	50	10

Corresponding scores along Axis I of an nMDS analysis based on the relative abundances of scaled chrysophytes and planktic diatoms, and the relative abundances of *Aulacoseira ambigua* valves, are given for each depth in the core

measured from the valve face to the end of the collum, and diameter was estimated at mid-mantle height. Three measurements were taken for each metric and the mean for each specimen was recorded. Morphometric data were analyzed for descriptive statistics, ANOVA analysis, and graphic representation, using SigmaPlot (version 12.5; Systat Software, Inc., San Jose, California).

Data and statistical analysis

The trophic status of Bantam Lake between 1857 and 1991 was inferred using the model developed by Siver and Marsicano (1996), based on scaled chrysophyte remains. Briefly, trophic scores for 45 Connecticut lakes were estimated with principal component analysis (PCA), using four variables: total phosphorus, total nitrogen, chlorophyll-*a*, and Secchi disk depth. Values of these same four variables used by Frink and Norvell (1984) to determine lake trophic status were also included in the PCA analysis to yield trophic state scores for lake types ranging from oligotrophic to hypereutrophic (Table 1). Trophic state scores are equivalent to the distances along PCA axis 1. The PCA analysis was done using CANOCO (ter Braak 1990). Weighted averaging, with tolerance downweighting using WACALIB (version 2.1; Line and Birks 1990), was then used to model lake trophic state scores based on scaled chrysophytes. Scaled chrysophyte abundances for different strata in the Bantam Lake core were added as passive variables in the weighted averaging analysis.

We used PRIMER-E (version 7; Clarke and Warwick 2001; Anderson et al. 2008) to conduct non-metric multidimensional scaling (nMDS) to examine shifts in diatom and scaled chrysophyte community structure over the length of the core. The resemblance matrix used for the nMDS analysis consisted of relative abundance data for both scaled chrysophytes and planktic diatoms, reported in the previous study by Siver (1992). Relative abundances of organisms were square-root transformed, and the resemblance matrix used in the nMDS was developed using a Bray–Curtis similarity measure. Morphometric data were analyzed for descriptive statistics, ANOVA analysis ($p < 0.05$) was used to test for differences in valve diameter between populations from different strata, and graphic representation was done using SigmaPlot version 12.5. Histograms of valve diameter are shown for three representative strata, and data for all strata analyzed are given in Electronic Supplementary Material (ESM) Table S1.

Table 3 The mean, minimum, maximum, and 95% confidence interval of the mean values for both the diameter and length of *Aulacoseira ambigua* valves from 13 sections of a gravity corer from Bantam Lake

Core Depth (cm)	Age	Mean diameter ± S.E. (μm)	C.I. of the mean diameter (μm)	Minimum diameter (μm)	Maximum diameter (μm)	Mean length ± S.E. (μm)	C.I. of the mean length (μm)	Minimum length (μm)	Maximum length (μm)	Mean L:D ratio
1–1.5	1989	7.8 ± 0.1	0.28	4.4	16.1	11.9 ± 0.1	0.24	5.6	15.8	1.62
2.5–3	1987	8.2 ± 0.1	0.28	4.6	14.6	11.9 ± 0.1	0.26	5.8	16.4	1.55
4.5–5	1984	7.9 ± 0.1	0.25	4.1	12.7	11.2 ± 0.1	0.28	4.9	16.1	1.48
7–8	1980	7.9 ± 0.1	0.31	4.5	17.8	11.1 ± 0.1	0.27	6.7	16.8	1.48
9–10	1975	7.6 ± 0.1	0.29	4.4	14.3	11.9 ± 0.1	0.29	4.1	17.5	1.67
13–14	1964	7.6 ± 0.1	0.26	4.3	13.2	11.4 ± 0.1	0.27	5.9	17.1	1.59
18–19	1946	8.9 ± 0.2	0.33	3.3	15.9	11.3 ± 0.2	0.31	4.3	17.9	1.38
20–21	1938	8.9 ± 0.1	0.31	4.9	15.0	11.5 ± 0.1	0.27	7.8	17.6	1.38
24–25	1926	9.0 ± 0.2	0.36	4.6	17.7	11.2 ± 0.1	0.25	6.8	16.3	1.32
30–32	1910	8.9 ± 0.2	0.33	5.1	16.0	10.2 ± 0.1	0.20	6.4	13.8	1.22
34–36	1898	9.4 ± 0.1	0.31	5.5	14.6	10.1 ± 0.1	0.21	6.6	14.6	1.13
36–38	1889	9.5 ± 0.1	0.28	4.6	14.9	10.0 ± 0.1	0.23	6.0	15.0	1.14
40–42	1857	9.7 ± 0.2	0.34	4.4	16.4	10.1 ± 0.1	0.26	4.6	19.6	1.13
All specimens		8.5 ± 0.1	0.31	3.3	17.8	11.2 ± 0.1	0.26	4.1	19.6	1.38

The mean length to width ratio (L:W) for each section is also given, as are values across all specimens and samples

We used an Excel spreadsheet to estimate the number of cell divisions that would be needed to decrease the mean valve diameter of a population between two values. The analysis started with a cell of a given diameter that sequentially underwent cell divisions, resulting in an increase in the size of the population. The total number of valves, their estimated diameters, and the mean diameter for the population were calculated at the end of each division. The analysis can be run for many cell division cycles, and results can be used to estimate the number of divisions needed to result in a decrease in the mean valve diameter of a population between any two values of interest. The analysis assumes that every cell in a population divides simultaneously, and that the decrease in diameter per division is the same for all cells. For the purpose of this project, we estimated the additional number of divisions needed to account for the differences we found in the mean valve diameter for populations of *A. ambigua* cells associated with each trophic state shift. The analysis was run using decreases in diameter per cell division of between 0.1 and 0.3 μm, values estimated in previous studies of *Aulacoseria* species (Jewson 1992; Siver et al. 2019).

Results

Historical shifts in trophic state and community structure in Bantam Lake

At the time the core was retrieved from Bantam Lake in 1991, the waterbody was classified as eutrophic to hypereutrophic, with high total phosphorus and total nitrogen concentrations, low Secchi disk depths, and routine summer blooms of cyanobacteria, including species of *Microcystis*, *Aphanizomenon* and *Dolichospermum* (Siver 1992; Canavan and Siver 1995; Siver and Marsicano 1996; Siver et al. 1996). Extensive summer blooms were common in the lake prior to the studies initiated in the 1990s, and despite more public awareness, Bantam Lake remains eutrophic, with elevated nutrient concentrations and continued blooms of cyanobacteria (AER 2020).

Canavan and Siver (1995) summarized data on Bantam Lake from earlier investigations in the 1930s (Deevey 1940), the 1970s (Frink and Norvell 1984), as well as their own findings. For midsummer, the lowest total phosphorus (18 μg L^{-1}) and chlorophyll-*a* concentrations (12 μg L^{-1}) were recorded by Deevey (1940) in the 1930s, and based on guidelines established by Frink and Norvell (1984), the lake would be classified as mesotrophic at that time (Tables 1, 2). By the 1970s, summer concentrations of total phosphorus (37–58 μg L^{-1}) and chlorophyll-*a* (30–50 μg L^{-1}) had more than doubled, and total nitrogen concentrations and Secchi disk depths ranged between 660 and 1150 μg L^{-1} and 1.5–2.2 m, respectively (Siver et al. 1996), indicating eutrophic conditions that persisted into the early 1990s (Canavan and Siver 1995).

Based on a model developed by Siver and Marsicano (1996) that utilized scaled chrysophytes to infer trophic status, the condition of Bantam Lake in 1991 scored as one of the most eutrophic waterbodies in the state. As part of this study, we used this inference model to estimate shifts in the trophic status of the lake over the length of the core, covering the time period 1857–1991 (Table 2). Based on these findings, the lake was oligotrophic to early mesotrophic between 1857 and 1926, mesotrophic from 1926 to 1946, and eutrophic since at least 1964.

The planktic diatom flora found in the lower sections of the core and prior to the turn of the twentieth century was comprised of *Discostella stelligera* (Cleve and Grunow) Houk and Klee, *Lindavia bodanica* (Eulenstein ex Grunow) Nakov, Guillary, Julius, Theriot and Alverson, *Tabellaria flocculosa* (Roth) Kützing, and *A. ambigua* (Fig. 2). The relative abundances of *D. stelligera*, *L. bodanica* and *T. flocculosa* declined after that point and became minor components of the flora after 1964. Concurrent with these changes were increases in the importance of *Fragilaria crotonensis* Kitton, *Asterionella formosa* Hassall, and after 1964, of both *Stephanodiscus parvus* Stoermer & Håkansson, and *Stephanodiscus minutulus* (Kützing) Cleve and Möller (Fig. 2). The relative abundance of *A. ambigua* was more variable, increasing after the turn of the twentieth century and reaching a peak around 1926, followed by a decline in the 1930s and 1940s, and then increasing once again to a second peak in the early 1980s.

Based on a combination of planktic diatom and scaled chrysophyte species, three significantly different communities, each with at least 70% similarity in the Bray–Curtis measurement, were revealed along the length of the core in an nMDS analysis (Fig. 3). The differences in community structure tracked shifts

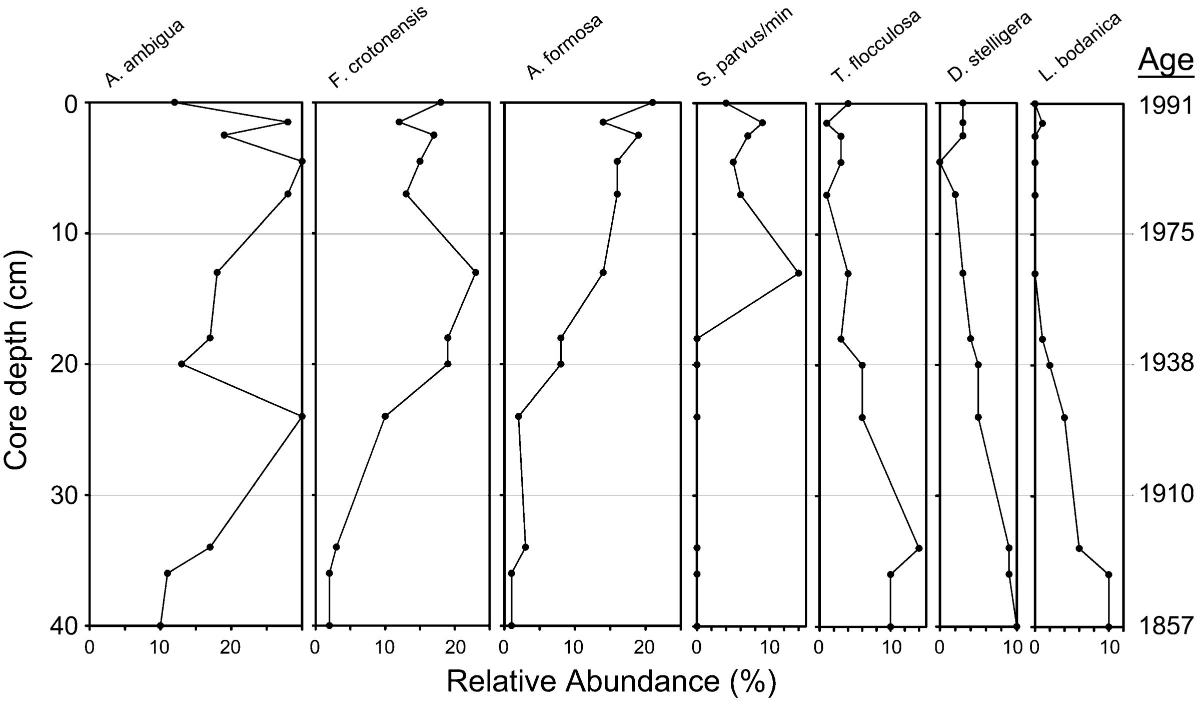

Fig. 2 Stratigraphies of planktic diatoms in Bantam Lake, Connecticut, over ~ 130 years from approximately 1857–1991, including profiles for *Aulacoseira ambigua*, *Fragilaria crotonensis*, *Asterionella formosa*, *Stephanodiscus parvus*, *S. minutulus*, *Tabellaria flocculosa*, *Discostella stelligera* and *Lindavia bodanica*

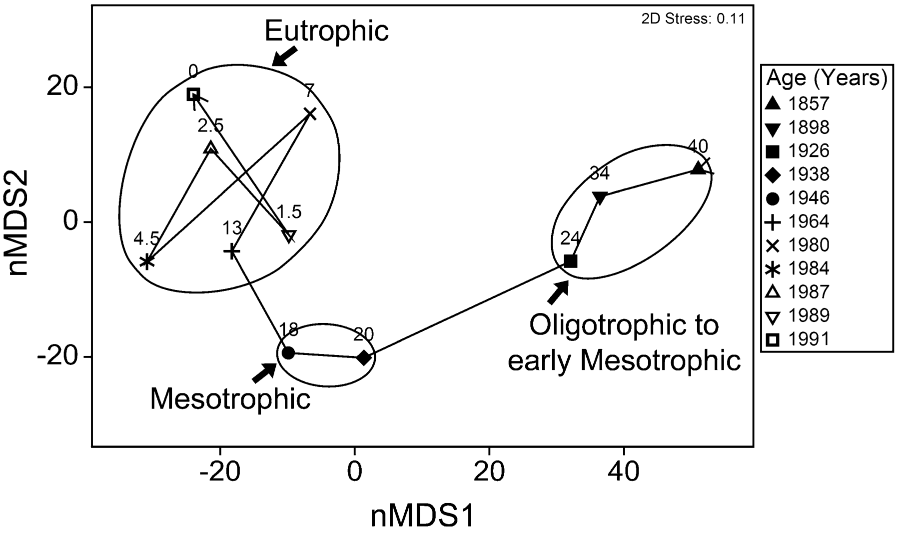

Fig. 3 Result of a 2-dimensional non-metric multidimensional scaling (nMDS) analysis indicating the ordination of 11 strata from a sediment core taken from Bantam Lake, Connecticut, based on a Bray–Curtis similarity measure using the relative abundances of scaled chrysophytes and planktic diatoms. The line connects sites chronologically over ~ 130 years, and the different trophic state conditions and core depths associated with each sample are indicated on the figure

in lake trophic status. Prior to 1926, the communities were similar to each other, very different from later communities, and corresponded with oligotrophic to early mesotrophic conditions. Community structure was also similar between 1964 and 1991, under eutrophic conditions, and very different from communities that characterized earlier time periods. Mesotrophic time periods supported communities that were intermediate between those found under oligotrophic and eutrophic conditions.

Characteristics and growth of *Aulacoseira ambigua* in Bantam Lake

Valves of *Aulacoseira ambigua* in the Bantam Lake core possess an unornamented valve face, striae consisting of large areolae that spiral to the right on the mantle, distinctive spines, and a well-defined collum (Fig. 4). Cells are connected into long filaments with linking spines that are short, of even length, terminate each mantle costae, are triangular to heart-shaped, and interlock to form a zipper-like connection between frustules (e.g. black arrow on Fig. 4c). Separating spines, which are very rare, are slightly longer than linking spines, of even-length, and pointed. The ringleiste is hollow and yields a distinctive indentation when viewed with light microscopy, enhancing identification of specimens. Valves possess one to a few elongated rimoportulae (white arrows on Fig. 4c), each of which terminates a stria next to the collum (arrow on Fig. 4d).

Remains of *A. ambigua* were found throughout the Bantam Lake core, with relative abundances ranging between 10 and 30% (Table 3). The 2600 specimens measured in this study ranged in length from 4.1 to 19.6 µm, with a mean of 11.2 µm, and in diameter from 3.3 to 17.8 µm, with a mean of 8.5 µm (Table 3). The length to width ratio (L:W) ranged from 0.5 to 3.2, with an overall mean of 1.4. Specimens with lower L:W ratios were generally shallow valves with a wide diameter. The mean minimum diameter of the 60 smallest valves was 4.5 µm, and the mean maximum diameter of the 60 largest valves was 15.4 µm. These valve metrics indicate that during cell division, valve diameter decreases to approximately one third of its size at auxospore germination.

Of the 13 strata analyzed, the mean valve diameter of *A. ambigua* ranged from 7.6 to 9.7 µm (Table 3). Despite differences in mean diameter among strata, the ranges in diameter between the largest and smallest valves were similar. Significant differences in valve diameter (ANOVA, $p < 0.05$) were documented for

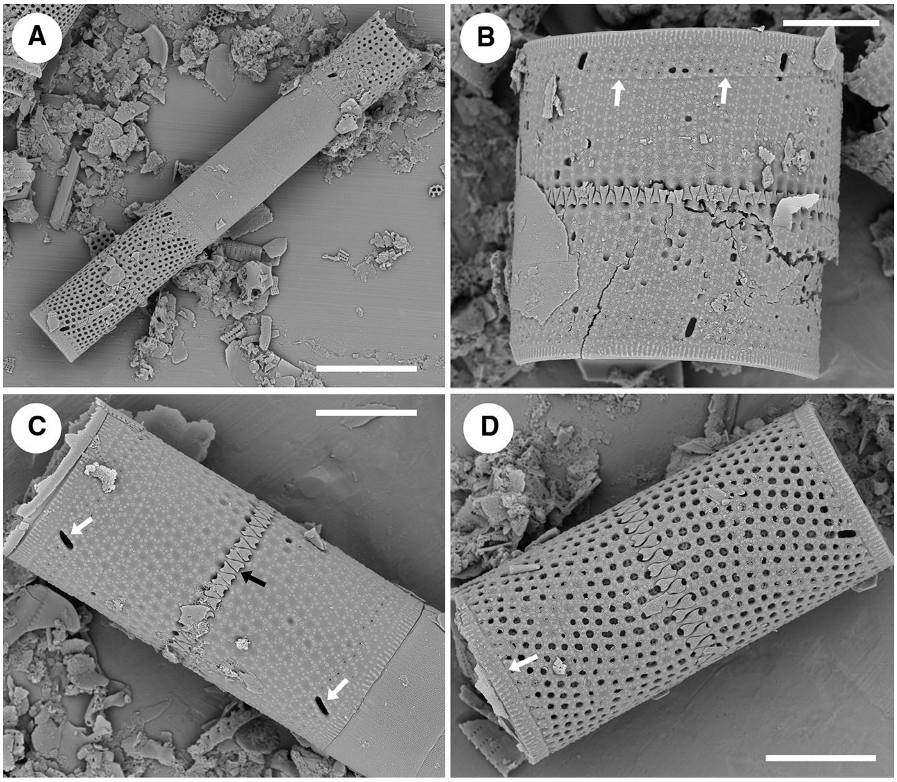

Fig. 4 Scanning electron micrographs of *Aulacoseira ambigua* specimens from a sediment core taken from Bantam Lake, Connecticut. **a** A portion of a narrow filament where the girdle bands are still attached to the end cell. **b** Two wide valves from adjoining cells. Note the short connecting spines, the two elongated rimoportulae on the top valve, and one rimoportula on the bottom valve. A step can be seen on the upper valve (two arrows). **c**, **d** Valves from adjoining cells. Note the large areolae aligned in spiral rows, rows of connecting spines (black arrow on C), elongated rimoportulae (white arrows on C), and the short collum (white arrow on D). Scale bars = 5 µm

populations from different strata, and a clear trend in the mean diameter was observed over the length of the core (Figs. 5, 6). Mean diameters of valves from strata in the top portion of the core (0–14 cm; e.g. Fig. 5a) were significantly smaller than those in strata from 18 to 32 cm (e.g. Fig. 5b), which in turn were significantly smaller than those in strata near the core bottom, between 34 and 42 cm (e.g. Fig. 5c). Mean diameter was highest between 1857 (40–42 cm) and the turn of the century (34–36 cm), with values between 9.4 and 9.7 µm, which declined to approximately 8.9 µm between 1910 (30–32 cm) and 1946 (18–19 cm), and then experienced a large drop between 1946 and 1964 (13–14 cm), to 7.6 µm. The mean diameter remained low and relatively constant after 1964 (Table 3; Fig. 6). The total decline in mean diameter between the bottom and top of the core was approximately 2 µm. The significant differences in mean diameter between the three time intervals (pre-1900, post-1964 and the time between these periods) corresponded to the major shifts in planktic community structure based on diatom and scaled chrysophyte species (Fig. 3). As a result, the mean diameter of *A. ambigua* valves was highly correlated with the site scores of nMDS axis 1 ($r^2 = 0.77$; $p < 0.001$; Fig. 7b), which in turn, also tracked inferred shifts in trophic status. In contrast, the relative percentages of *A. ambigua* valves were not correlated with position of the strata on axis 1 of the nMDS ($r^2 = 0.03$; $p = 0.288$; Fig. 7a).

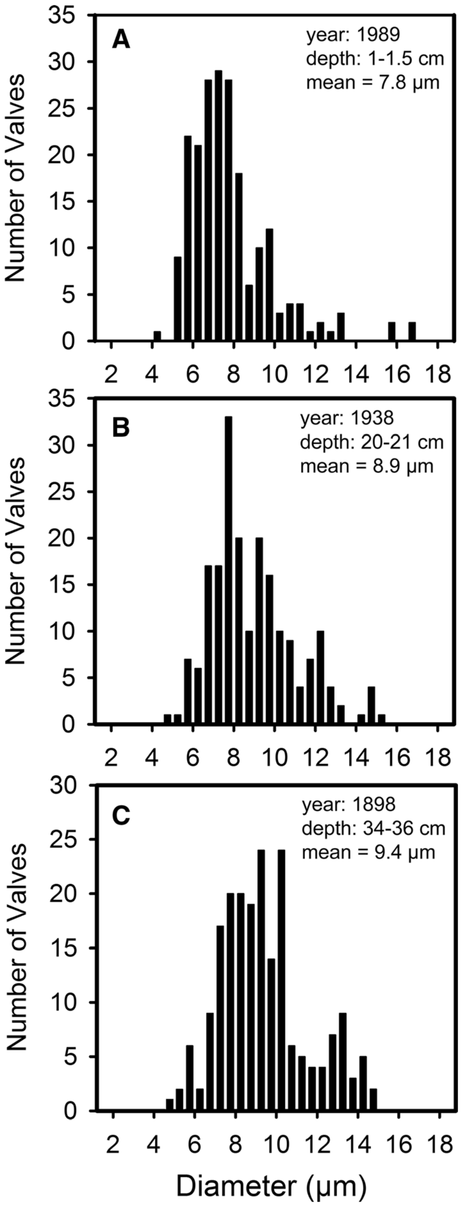

Fig. 5 Histograms depicting the size distributions of *Aulacoseira ambigua* valves from depths of 1–1.5 cm **a**, 20–21 cm **b**, and 34–36 cm **c** in a sediment core from Bantam Lake, Connecticut, representing the years 1989, 1938, and 1898, respectively

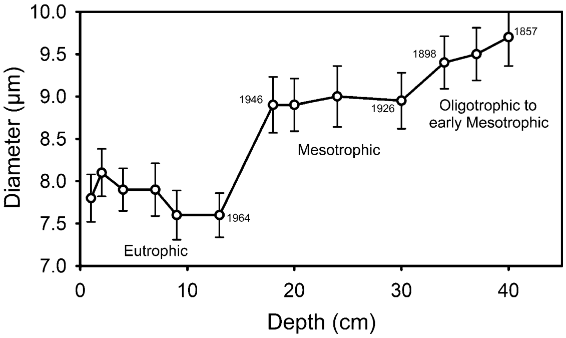

Fig. 6 The change in mean diameter ± SE of *Aulacoseira ambigua* specimens versus depth in a sediment core from Bantam Lake, Connecticut. Shifts in trophic conditions inferred from scaled chrysophytes and key dates are noted

We estimated the number of additional cell divisions necessary to decrease the mean valve diameter from 9.7 µm (the value for 1857) to 8.9 µm (middle of the core), and eventually to 7.6 µm (top of the core) (Table 4). Assuming a decline in diameter of 0.3 µm per division, 5 and 14 additional cell divisions would be needed to decrease the mean valve diameter of the population from 9.7 µm, to 8.9 µm or 7.6 µm, respectively. The number of divisions more than

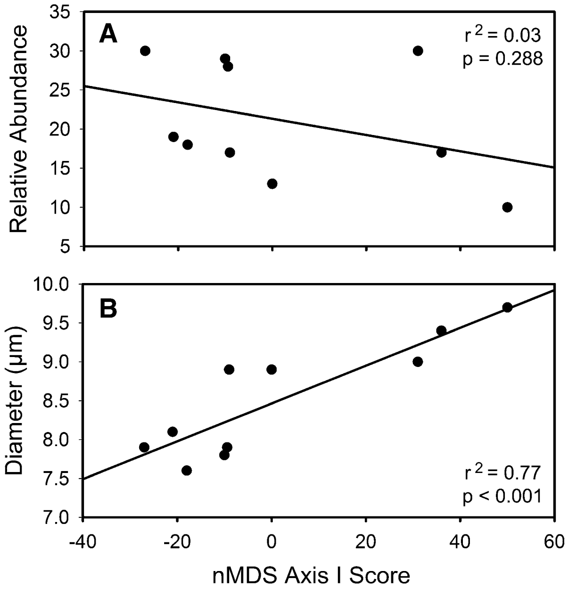

Fig. 7 Relative abundance **a** and mean valve diameter **b** versus sample scores on axis 1 of the nMDS analysis

doubles with smaller decreases in diameter per cell division (Table 4).

Discussion

The practice of using shifts in the relative abundances of species, especially of diatoms, to infer past environmental change in lakes has been an effective and powerful tool in paleolimnological investigations (Smol and Stoermer 2010). Changes in relative abundances of diatoms have been especially useful for tracking shifts in trophic state conditions (Hall and Smol 2010; Whitmore et al. 2015), acidic deposition (Chen et al. 2008; Finkelstein et al. 2014), alterations in land use (Siver et al. 1999), and climate (Saros et al. 2012; Boeff et al. 2016). There are, however, situations where relying on relative abundances makes it difficult to reconstruct past environmental conditions and can even be misleading. For example, if the relative percentage of a diatom taxon declines over time in a core from a given site, it does not necessarily mean that the growth of that organism's population also declined. It is possible that the growth of that population remained constant, or actually increased, but to a lesser degree than that of other species, resulting in a decline in its relative importance. If indeed the overall growth of the taxon's population did decline, then it should be coupled with an increase in its mean valve size. If, however, the mean size of valves declined, despite a decrease in its relative abundance, then the taxon's population likely experienced an increase in growth, but to a lesser extent than that of other species. Likewise, if the same taxon was also found at a second site that experienced the same environmental change, but harbored a different suite of diatom species, the relative abundance of the taxon of interest may be found to have increased. These seemingly contradictory trends in relative abundance, recorded for the same taxon at two sites that experienced the same environmental change, could lead to erroneous and contradictory conclusions about the degree of environmental change that occurred over time between the two sites. Under such a scenario, and as clearly demonstrated in our findings, coupling

Table 4 Estimated numbers of cell divisions needed to decrease the mean valve diameter of a population of *Aulacoseira ambigua* cells from 9.7 µm to either 8.9 µm or 7.6 µm

Decrease in diameter per cell division (µm)	Number of cell divisions needed to decrease the mean valve diameter of the population from 9.7 µm to:	
	Mean of 8.9 µm	Mean of 7.6 µm
0.1	16	42
0.15	11	28
0.2	8	21
0.25	7	17
0.3	5	14

The model assumes that all cells in a population divide simultaneously, and with the same decrease in valve diameter. Decreases of 0.1 µm, 0.15 µm, 0.2 µm, 0.25 µm and 0.3 µm in diameter per cell division are assumed information on mean valve size with inferences based on relative abundance estimates, can help decipher the overall impact of past environmental change.

In previous work on Connecticut lakes, *A. ambigua* was reported in waterbodies with trophic state conditions ranging from oligotrophic to hypereutrophic, but with highest concentrations in eutrophic lakes (Siver and Kling 1997; Siver 1999). In Bantam Lake, the lowest relative abundance of *A. ambigua* was recorded in the deepest sample from the core (1857), corresponding to a time when species more commonly associated with oligotrophic waterbodies, such as *Discostella stelligera*, *Lindavia bodanica*, and *Tabellaria flocculosa* (Siver 1999; Siver and Hamilton 2011), were most abundant. Over the next 70 years the relative abundance of *A. ambigua* tripled, largely as a result of a decline in the oligotrophic taxa, indicating an increase in trophic status. The mean valve diameter of *A. ambigua* also declined significantly during that time period, supporting the hypothesis that the lake became more eutrophic, as inferred from the model using scaled chrysophyte remains. Over the ensuing 40 years the relative abundance of *A. ambigua* declined by half, concurrent with a rise in relative abundance of other eutrophic species, such as *Fragilaria crotonensis*, *Stephanodiscus parvus*, and *Stephanodiscus minutulus*. Data on mean valve diameter, however, indicate that growth of *A. ambigua* did not decline during that time period, but instead continued to increase as the lake became even more eutrophic. Between 1964 and 1991, increases in the relative abundance of *A. ambigua* and declines in its mean valve diameter are in agreement, and confirm the increase in trophic status inferred from scaled chrysophytes and observed through direct water chemistry and Secchi depth measurements. In summary, including data on mean valve diameter helped to explain discrepancies based solely on relative abundance data, and further clarified the importance of *A. ambigua* in Bantam Lake over time.

Aulacoseira ambigua was present throughout the Bantam Lake core, indicating that this species has been a common element of the planktic community for more than 150 years. Unpublished monitoring reports we made over multiple years just prior to when the core was retrieved from Bantam Lake indicate that growth of *A. ambigua* begins during spring overturn, continues throughout the summer, and ends after fall overturn. Although it is unknown if the same growth pattern occurred prior to the time when monitoring was being done, it was shown to be the case during a monitoring effort in 2019 (AER 2020), implying that populations of *A. ambigua* are sustained over an approximate seven-month period each year. Although we do not know if cells grow more or less at any point during the seven-month period, remains of the population become part of the sediment record and can be used to estimate population dynamics over time.

Our findings indicate that the range in *A. ambigua* valve diameter from each core depth remained relatively constant since 1857. On average, valves declined in size from 15.4 µm to 4.5 µm, implying a decrease in diameter to about one third that of initial cells. Similar relative ranges in size decline have been documented for other centric diatoms, including *Coscinodiscus* Ehrenberg taxa (Round et al. 1990) and other *Aulacoseira* species (Jewson 1992; Houk 2003; Houk and Klee 2007). *Aulacoseira subarctica* (O. Muller) Haworth is another species commonly found in the plankton of Connecticut lakes (Siver and Kling 1997). In a detailed study of the life cycle of *A. subarctica* over multiple years, Jewson (1992) reported that initial cells had an average diameter of 14.8 ± 2 µm, and that cell division continued until the diameter reached approximately one-third that of initial cells, similar to what we documented for *A. ambigua*. Jewson (1992) further noted that gamete formation and subsequent sexual reproduction began when *A. subarctica* cells reached a diameter between 3.8 and 7.4 µm. If gamete formation in *A. ambigua* was initiated at approximately the same cell diameter as documented for *A. subarctica*, then each sediment stratum examined most likely archives remains of a complete life cycle.

In the study of *Aulacoseira giraffensis* from the Eocene Giraffe Pipe fossil locality, a mean decrease of 0.2 µm per division in valve diameter was reported (Siver et al. 2019), with estimates ranging from 0.1 µm to occasionally as high as 0.5 µm for larger cells. For *A. subarctica*, Jewson (1992) reported a reduction in diameter of 0.32 µm per division for large cells, with a gradual decrease as cells became narrower. Given these size reduction measurements for *Aulacoseira* species, we estimated the number of cell divisions needed to reduce the mean valve diameter of a population from values observed under oligotrophic conditions to those found under eutrophic conditions in Bantam Lake. Estimates of 14, 21 and 42 additional cell divisions would be needed, assuming size reductions of 0.3, 0.2 and 0.1 µm per division, respectively. This represents an increase in growth of between 33 and 42% under eutrophic, compared to oligotrophic, conditions, and further illustrates how mean diameter can yield valuable information to supplement data on relative abundance data.

Our estimates of the increased number of divisions needed to reduce the mean valve size assume that each cell in the population divides simultaneously, that is, the time between cell divisions is the same for all cells. Round et al. (1990) pointed out that for some diatoms, such as *Ellerbeckia* Crawford, smaller cells in a population may not divide as fast as larger cells. In that case, the mean valve diameter of the population would decrease less per division than if all cells divided at the same time. If the same is true for *A. ambigua*, then the numbers of cell divisions needed to decrease the mean valve diameter we projected are underestimated.

The use of statistics related to valve size could also be applied to inferring changes that occurred in ancient waterbodies whose deposits contain fossil remains, as Siver et al. (2019) did using remains of *A. giraffensis* in the 10-m section of core from the Eocene Giraffe Pipe fossil locality. Measurements of valve diameter revealed a cyclic pattern that reflects distinct periods of lower versus higher growth of *A. giraffensis*, which correlated with shifts in abundance of a diverse array of microfossil taxa. Although more analysis needs to be completed on the Giraffe Pipe core, the mean valve diameter metric adds another tool for evaluating ancient lake conditions.

In summary, using shifts in relative abundances of diatom species to infer environmental change will remain a central tool in paleolimnological research. Relying solely on changes in relative abundances, however, can yield potential deficiencies in reconstructing past environments. Including information on change in the mean size of key diatom species, especially common taxa found over the entire length or large sections of a core, can provide an independent means to support or reject conclusions based solely on relative abundances. In the case of Bantam Lake, changes in the mean diameter of *A. ambigua* valves implied continued increases in growth of populations for more than ~ 130 years, and independently tracked the eutrophication of the waterbody based on inferences using scaled chrysophytes, and later verified using chemical analyses. In a large-scale fertilization study of Kootenay Lake, British Columbia, where nitrogen and phosphorus were added to the lake to increase food resources for kokanee salmon, Yang et al. (1996) noted a decline in valve diameter of two *Cyclotella* species, *C. comensis* (*Lindavia comensis* (Grunow) Nakov, Guilory, Julius & Theriot) and *C.* cf. *pseudostelligera*. The increased growth in response to eutrophication, coupled with a concurrent decline in valve diameter, supports our results and indicates similar findings for other centric diatoms. We note that although valve diameter is the recommended metric to use for centric species, valve length is probably more appropriate for pennate forms, given that these diatoms decrease in length more than width with size reduction. In conclusion, the valve-diameter metric is an easy-to-measure and inexpensive tool that can effectively enhance paleolimnological investigations.

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