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Historical abundance and morphology of *Didymosphenia* species in Naknek Lake, Alaska

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Since the 1980s, nuisance blooms of *Didymosphenia geminata* (Lyngbye) M. Schmidt have been documented in sites that are warmer and more mesotrophic than historical records indicate. While the invasion of *D. geminata* in New Zealand is well documented, it is less clear whether nuisance blooms in North America are a new phenomenon. In order to test the hypothesis that *D. geminata* blooms have increased in recent years, we examined the historical record of this species in sediments of Naknek Lake, in Katmai National Park, Alaska. Chronological control was established by relating the presence of two ash layers to known volcanic eruptions. We identified two species of *Didymosphenia* within the sediment record: *D. geminata* and *D. clavaherculis* (Ehrenberg) Metzeltin et Lange-Bertalot. This is the first published record of *D. clavaherculis* in North America. We found no statistically significant change in the numerical presence of *D. geminata* or *D. clavaherculis,* as a group, in Naknek Lake between the years 1218 and 2003. While there has been no sudden, or recent, increase in abundance of *Didymosphenia* in Naknek Lake, morphological features of *D. geminata* populations in Naknek Lake are distinct compared to morphological features of *D. geminata* in streams containing nuisance blooms from sites in North America and New Zealand. Variance in the morphology of *Didymosphenia* cells may help determine relationships between distinct sub-populations and establish the history of habitat invasion.

Key words: diatom, *Didymosphenia geminata, Didymosphenia clavaherculis*, morphology, counting, bloom, stream, lake, invasion, history, Alaska

Introduction

Until recently, *Didymosphenia geminata* (Lyngbye) M. Schmidt was thought to be limited to cold, low-nutrient, well-oxygenated waters in northern latitudes. Nuisance blooms

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of this diatom, however, have been documented in warmer and more mesotrophic sites since the 1980s (NOGA 2003, KAWECKA and SANECKI 2003, SUBAKOV-SIMIĆ and CVIJAN 2004). *Didymosphenia geminata* is of concern in stream ecosystems because of its capacity to form thick masses, impacting biological and physical stream conditions. Further, this species is reported in streams and rivers across the United States (KUMAR et al. 2009). This diatom has demonstrated its invasive ability, evidenced by the presence of *D. geminata* in South Island of New Zealand in 2004 (KILROY et al. 2007). Although some reports assert that the nuisance blooms of *D. geminata* in North America (KUMAR et al. 2009) and Europe (KAWECKA and SANECKI 2003) are of recent occurrence, there are few to no historical data to establish the historical abundance of *D. geminata*. The purpose of this study is to examine the historical record of *Didymosphenia* in order to test the hypothesis that blooms have increased in recent years. This paper documents the record of *Didymosphenia* over 800 years in the sediments of Naknek Lake, Alaska.

Didymosphenia geminata was first recorded from the Faroe Islands in 1819 (LYNGBYE 1819). Other records in the early literature also mention the presence of *D. geminata*, including a reference to large masses in the Kanchou region of China (SKVORTZOW 1935). It is possible that extensive blooms are a normal part of this diatom's life history, but few data exist to quantify stream growth habits. Recognition of the patterns of *D. geminata* growth is needed to understand the current blooms in North America (KUMAR et al. 2009) and Europe (BELTRAMI et al. 2008) and the expansion to New Zealand. While the invasion of *D. geminata* in New Zealand is well documented, it is less clear whether nuisance blooms in North America are a new phenomenon.

Although several species of *Didymosphenia* are known from lakes (SKVORTZOW and MEYER 1928, KOCIOLEK et al. 2000), *D. geminata* is most often recorded in streams and rivers (SHEATH et al. 1986, LAPIERRIERE et al. 1989, MILLER et al. 1992, SHEATH et al. 1996, ELLWOOD and WHITTON 2007) where it appears to reach its greatest biomass (KILROY et al. 2007). Determining the history of diatoms in streams and rivers, however, is more problematic than in lakes, as streams are high flow systems that typically do not leave a continuous sedimentary record that can be interpreted (SMOL 2002). Even so, reconstruction of environmental change in rivers by examining stream diatoms deposited in the sediments of lakes has been shown to be successful (SMOL 2002). In sites where streams or rivers flow into lakes, records of historical change in river systems may be archived in lake sediments. For example, the relative abundance of stream diatoms [*Hannaea arcus* (Ehrenberg) Patrick and *Meridion circulare* (Greville) Agardh] found in lake sediments was used to reconstruct historical river discharge in the high arctic (LUDLAM et al. 1996, ANTONIADES and DOUGLAS 2002). Because *Didymosphenia* reaches its greatest abundance in streams and rivers, we propose that the concentration of cells in lake sediments is directly related to the concentration of cells in stream inflows. The presence of diatom cells preserved in lake sediments and the accurate dating of those sediments provide that opportunity to examine changes in cell abundance over time.

Differences in valve morphology of *D. geminata* cells from different regions of Europe and Asia have been noted (ANTOINE and BENSON-EVANS 1983, STOERMER et al. 1986, METZELTIN and LANGE-BERTALOT 1995) and these morphological differences are thought to reflect distinct sub-populations. Although differences in the valve margins of diatoms are easily recognized visually, until recently few tools have existed to evaluate statistically sig-

nificant differences in shape (STOERMER et al. 1986). Diatoms, in particular, are a group of organisms whose species concepts and boundaries are based largely on differences in valve shape (STOERMER et al. 1986). Qualitative evaluation of valve morphology of cells of *Didymosphenia* may provide insights into the nature of the relatedness of populations and the history of range expansion and invasion into new habitats. Furthermore, preliminary evaluation of molecular markers in the internal transcribed spacer (ITS) regions of the 18S rRNA gene shows a distinction between populations of *D. geminata* from different geographic regions (CARY et al. 2008). These results not only indicate a marked separation between the European and North American populations of *D. geminata*, but the close affiliation of North American and New Zealand populations. The second objective of this paper is to examine the morphology of *D. geminata* cells preserved in sediments dating to 800 years B.P. and compare valve shape to modern, bloom-forming populations of *D. geminata* from around the world.

Materials and methods

Naknek Lake is located in Katmai National Park, Alaska, near the base of the Alaskan Peninsula (latitude 58°40'N and longitude 156°12'W) (Fig. 1). The lake is 64 km long and up to 13 km wide and has a maximum depth of 173 m (LAPERRIERE 1997). The lake is fed

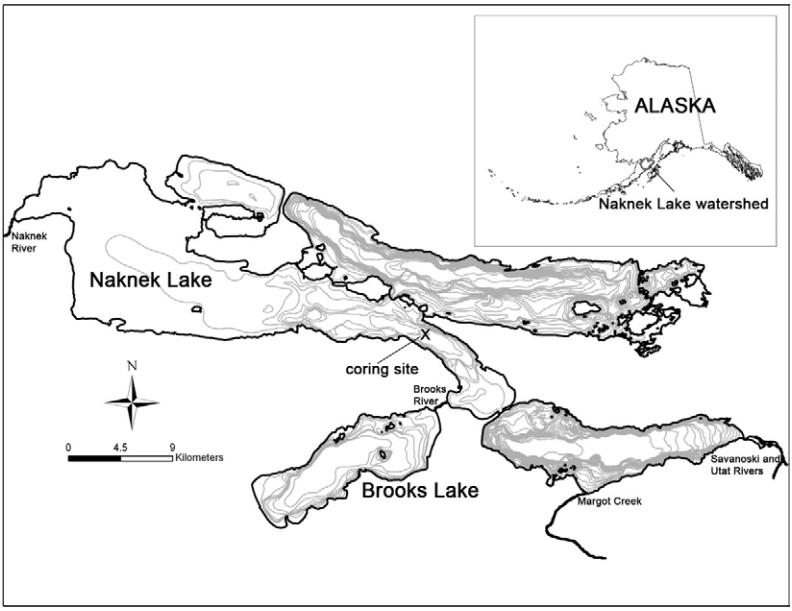


Fig. 1. Map showing Alaska (inset) with the Naknek Lake Watershed in Katmai National Park. Bathymetric map of Naknek and Brooks Lakes with contours at 10 m intervals. The site where the sediment core was taken is marked by an »X«. Brooks Lake flows into Naknek Lake via Brooks River, providing much of the clear water inflow. The Savanoski and Utak Rivers and Margot Creek are the major inflows to the southern arm of Naknek Lake. The outlet of Naknek Lake is the Naknek River. Map courtesy of the National Park Service, based on 1963 data.

both by glacial meltwater and clearwater streams such as the major inflowing tributary, Brooks River. Naknek Lake drains west into Bristol Bay through the Naknek River. Naknek Lake is bounded by terminal moraines that were deposited by glaciers that flowed from east to west during the last glaciation. Based on dating of the terminal moraines, Naknek Lake is estimated to have formed approximately 14,000 years BP. The lake today receives a large input of silt particles from active glaciers in its watershed. The sediments are grey in color and suspended silt particles prevent light penetration in much of the lake (LAPERRIERE and EDMUNDSON 2000). Nutrient concentrations are low and the lakes are considered oligotrophic (GOLDMAN 1960, LAPERRIERE and JONES 2002).

Cores were obtained from Naknek Lake in 2003 using both a hammer core and piston core. The coring location, at a water depth of 61 m, is shown in figure 1. The tops of the hammer cores were extruded at 1 cm intervals in the field in order to preserve the integrity of recent sediments. The top of the second hammer core, the middle of the first hammer core, and the bottom of the piston core were combined to make a total composite core of 452 cm in length. The cores were correlated at the two major tephras encountered with 1.3 m included from the first hammer core, 1.94 m from the second hammer core, and 1.28 m from the piston core. Sediments were sectioned, freeze dried, and stored at the University of Alaska Fairbanks.

The cores were dated based on the 1912 Katmai ash layer and the »brown ash« layer. The age of the brown ash was determined by AMS dating of terrestrial macrofossils from cores from nearby lakes, and determined to be 1570 +/– 30 yr cal BP. Analyses for loss on ignition (LOI) and percent biogenic silica (opal) were made at the University of Alaska Fairbanks. Subsamples from 38 sections of the core were sent to Institute of Arctic and Alpine Research (INSTAAR) for diatom preparation and analysis.

Dried sediment from each sample was weighed to the nearest milligram to obtain approximately 1000 mg of dry sediment. The samples were hydrated with 15 mL of deionized water for 12 hours in 50 mL centrifuge tubes. Organic material was oxidized using 15 mL of 30% H_2O_2 in a digestion over 6 days (RENBERG 1990). Following the digestion, deionized water was added to bring the total volume to 50 mL. The samples were allowed to settle for 8 hours, decanted, and rinsed with deionized water 6 times to remove H_2O_2. The cleaned sediments were well mixed by shaking and 0.500 mL was placed on glass cover slips. Five replicate cover slips were made for each of the 38 samples. The cover slips were allowed to dry and were mounted on glass microslides using a high refractive mounting medium (Zrax). Permanent slides and cleaned material are archived in the University of Colorado INSTAAR Diatom Database (Accession 10753–10790).

Ideally, a calibration between cell abundance in a river inflow and the sediment records should be able to be established. In this study, however, we were not able to obtain modern collections from the river inflow to determine the relation between river and sediment cell abundances. Reconstruction of the river abundance based on the lake sediments is based on an assumption of constant sedimentation rate within Naknek Lake. Although studies of streams mention the presence of *D. geminata* (PATRICK and FREESE 1961, FOGED 1981, LAPERRIERE et al. 1989, HEIN 1990) it is not noted to be of great abundance. Other limnological surveys in the area do not mention the presence of cells (GOLDMAN 1960, OSWOOD 1989, LAPERRIERE 1997, LAPERRIERE and EDMUNDSON 2000, LAPERRIERE and JONES 2002) in lakes or streams. The inflow (Brooks River) to Naknek Lake is one of the most frequent

sites of visitors to the area (J. SCHERER, National Park Service, personal comm.). The core was collected in 2003, and four years later in 2007, reports of masses of *Didymosphenia* were made on Brooks Falls (D. BOGAN, University of Alaska, Anchorage, personal comm.). However, we are not able to evaluate the correlation between river and sediment abundance.

We systematically examined the entirety of each of 185 slides for *Didymosphenia* cells using the light microscope (Olympus Vanox, Zeiss Universal, and Nikon Optiphot) at low magnification (40 times to 200 times). A distinction between *D. geminata* and *D. clavaherculis* (Ehrenberg) Metzeltin et Lange-Bertalot was not made for all specimens because fragments of cells were included in the counts. We were not able conclusively to determine species level determinations of *Didymosphenia* on valve fragments. Where whole valves were located, their images were recorded and several morphological measurements were made. For each slide, *Cymbella mexicana* (Ehrenberg) Cleve cells were also counted. *Cymbella* and *Didymosphenia* are both members of the family *Cymbellaceae*. They share many characteristics such as asymmetry about the apical axis and similar apical pore fields (KOCIOLEK and STOERMER 1988). Because of these shared traits, as well as the fact that they have been seen to grow in similar places and produce a profuse amount of stalk, it has been proposed that the number of *C. mexicana* present will co-vary with the number of *Didymosphenia* cells.

Electronic images of cells were captured using an Olympus Vanox microscope equipped with a 63 times oil immersion lens (1.4 NA) and a 3.3 M Q Imaging camera and software. We were able to differentiate between the various *Cymbella* forms at low magnification because the striae density in *C. mexicana* have a unique light refraction that makes the cells appear light blue under low magnification. The calibration and measuring capabilities of the imaging software were used to measure six key dimensions of *Didymosphenia* specimens from Naknek Lake: length, width, footpole, footpole-constriction, headpole, and headpole-constriction (Figs. 2–9). Images were obtained of *D. geminata* populations during blooms, from sites archived in the INSTAAR Diatom Database including Matapédia River, Québec (10752), Popo Agie River, Wyoming (10745), Blue River, Colorado (10610) and Waiau River, New Zealand (10580). Measurements of the six morphological features were made for 15 individuals from each population.

Relationships between diatom morphology and sites were explored using principal components analysis (PCA) and analysis of variance (ANOVA). All ordination and statistical analyses were run in R software using default functions in the vegan package of R (R Development Core Team 2006).

Results

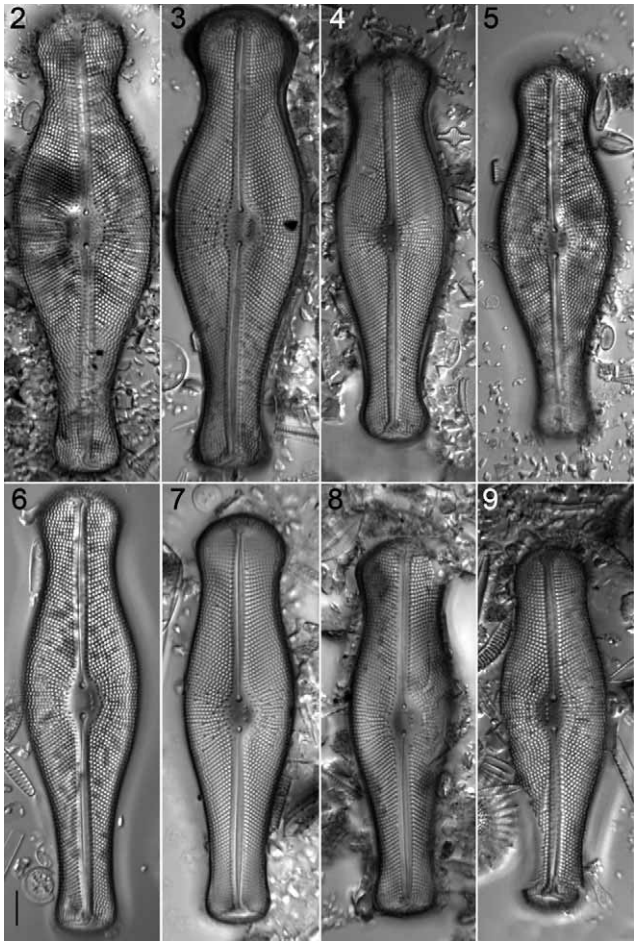
Our analysis was based on enumeration of all *Didymosphenia* valves and valve fragments observed within the five duplicate slides from selected strata in the sediment record. During our examination, however, we noted two species of *Didymosphenia* were present in Naknek Lake (Tab. 1). We identified *D. geminata* (Figs. 2–5) and propose that the second species belongs to *D. clavaherculis* (Ehrenberg) Metzeltin et Lange-Bertalot (Figs. 6–9), which most noticeably differs in the ratio of the width of the central valve to the headpole width. This is the first known record of the *D. clavaherculis* in North America. Both *D.*

Tab. 1. Comparison of morphological features of *D. clavaherculis*, three morphotypes of *D. geminata* based on METZELTIN and LANGE-BERTALOT (1995), and four complete *D. clavaherculis* specimens in Naknek Lake. Note that *D. geminata* var. *stricta* M. Schmidt is a later homonym of *D. clavaherculis*. *Didymosphenia clavaherculis* was described from diatomites (»infusorial earths«) of Ireland (EHRENBERG 1842).

Feature	Comparison of features of *D. clavaherculis* to those of *D. geminata*	*D. clavaherculis* from Naknek Lake. N = 4
1) General valve outline	Valve outline symmetrical, no cymbelloid type bend in the apical axis, as in *D. geminata.*	Slight asymmetry (Fig. 9)
2) Size	Generally larger size range (L = 90–180 µm, W = 34–45 µm)	Size range (L = 111–118, W = 22–30)
3) Midvalve to footpole outline	Shape of outline from midvalve to footpole less concave. The footpole is relatively broader. Ratio of the central valve to headpole is 1.1 – 1.3 (much lower than in *D. geminata*).	Shape of outline from midvalve to footpole less concave. The footpole is relatively broader. Ratio of the central valve to headpole is 1.0 – 1.1
4) Central area	Central area thin and narrowly elliptic to lanceolate	Not as described
5) Striae	Striae number 7–9 in 10 µm, as compared to 8–10 in *D. geminata*. Striae more strongly diverge toward the headpole and footpole than in other *Didymosphenia* species.	Striae number 8–9 in 10 µm. Striae not as described.
6) Number of stigmata	Range 2–7 stigmata per valve. Specimens from Spitzbergen were found to possess 1–2 stigmata, while specimens from Angara River (Russia) and Lake Koko Nor (Tibet) consistently possessed 4–7 stigmata. (D. Metzeltin pers. comm.).	Stigmata number 2–3 per valve.

geminata and *D. clavaherculis* were found in the core, although only a few complete specimens of *D. clavaherculis* were present. We included fragments of cells in our analysis, which precluded identification to the species level. Because of the small number of *D. clavaherculis* valves, we grouped both species together to examine abundance of *Didymosphenia* in Naknek Lake over the past 800 years.

The total abundance of *D. geminata* and *D. clavaherculis* in the sediment record that we analyzed ranged from 5–30 valves mg^{-1} sediment, while the total abundance of *C. mexicana* ranged from 5–300 valves mg^{-1} sediment (Fig. 10). *Didymosphenia* is present throughout the sediment record with no trends in the total abundance between the years 1218 and 2003, based on comparison of the sample means and standard deviations. The biogenic silica in the sediments, the source of which is considered to be the cell walls of diatoms, reached it greatest percent of the total near 1900. While the peak value in opal represents a peak in diatom concentration in the sediments, and therefore diatom biomass, there was no concurrent increase in *Didymosphenia* cells. Similarly, the minor changes measured in organic matter, measured as percent loss on ignition, does not appear to be related



Figs. 2–9. Light micrographs of *Didymosphenia*. Figs. 2–5: *Didymosphenia geminata*. Figs. 6–9: *Didymosphenia clavaherculis*. Scale bar in Fig. 6 is equal to 10 µm and applies to all images.

to a change in concentration of *Didymosphenia* or *C. mexicana*, at least not within the measurements of those changes that we had the ability to obtain. We found no statistically significant relationship between *Didymosphenia* and any of the sediment variables. On the other hand, we found a statistically significant relation between valve concentration of *Didymosphenia* and *C. mexicana* ($r^2 = 0.09$, $p < 0.01$) (Fig. 11). Therefore, cells of *Didymosphenia* and *C. mexicana* responded in a similar manner to one another.

We observed differences in shape between *D. geminata* in Naknek Lake in other regions of the world. These differences caused us to ask how the morphologically different forms in Naknek Lake compare to the variations seen in other geographical areas, namely the Matapedia River, Québec, Popo Agie River, Wyoming, Blue River, Colorado and Waiau River, New Zealand where nuisance blooms of *D. geminata* occur (KILROY 2008, SIMARD and SIMONEAU 2008, SPAULDING et al. 2008). Specifically, we were interested in determining if a »nuisance form« could be identified.

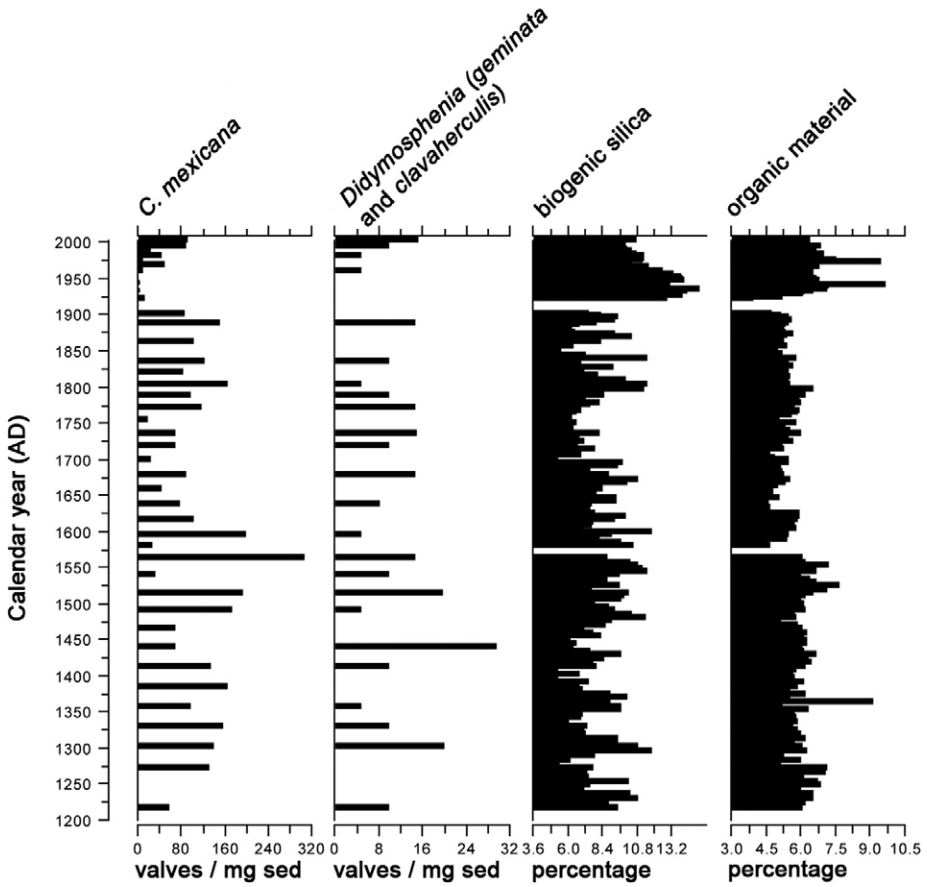


Fig. 10. Graph showing *Cymbella mexicana* abundance (valves mg^{-1} sediment), *Didymosphenia* abundance (valves mg^{-1} sediment), percent biogenic silica and percent organic matter (g cc^{-1}) measured as loss on ignition at 550 °C against calendar year from 1218 to 2003 A.D.

Results of measurement of six morphological dimensions of *D. geminata* specimens from Naknek Lake including length, width, footpole, footpole-constriction, headpole, and headpole-constriction are shown in table 2. We excluded specimens of *D. clavaherculis* from the analysis. The mean valve length from the Naknek Lake population was less than the mean of all the other sites, except for Blue River, Colorado. In contrast, the standard deviation of valve length from Naknek Lake specimens was the greatest of all sites. We found more consistency among the morphological measurements of *D. geminata* from Québec, Wyoming, New Zealand, and Colorado compared with *D. geminata* in Naknek Lake as demonstrated by mean and standard deviation (Tab. 2). A correlation matrix showed that valve length and headpole width were highly correlated with footpole width in all samples (Tab. 3). Therefore, footpole width was removed from further analysis.

Principal components analysis (PCA) of the remaining variables (Fig. 12) shows that the first two principal axes are significant, as indicated by a broken stick model (Fig. 13), and account for 82% of the total variance. While cell width, length and headpole width

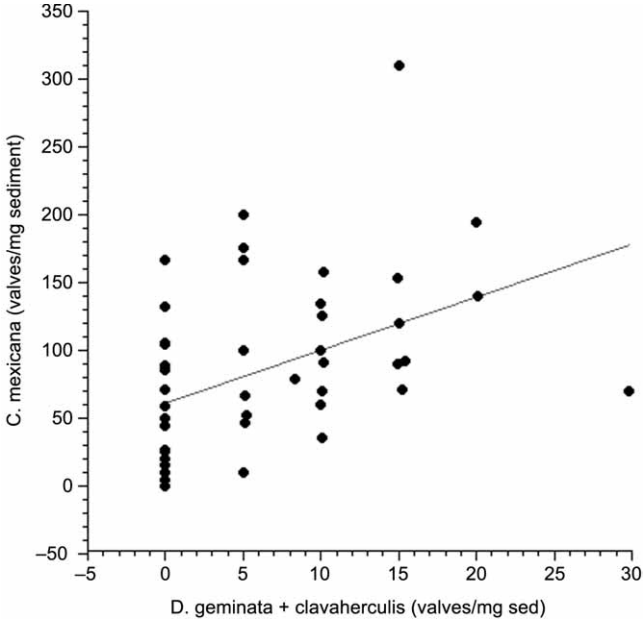


Fig. 11. Graph showing relationship between *Didymosphenia* and *C. mexicana* valves / mg sediment). The linear regression of valve concentration was statistically significant ($r^2 = 0.09$, $p < 0.01$).

Tab. 2. Mean, standard deviation and number of *D. geminata* valves measured for valve length, width, footpole width, footpole constriction, headpole width, and headpole constriction (all in mm) for Naknek Lake, Matapédia River (Québec), Popo Agie River (Wyoming), Blue River (Colorado) and Waiau River (New Zealand).

	Length	Width	Footpole	Footpole constriction	Headpole	Headpole constriction
Naknek, n = 14						
mean	123.3	37.6	19.8	16.8	27.2	22.3
std dev	10.1	4.2	1.9	1.3	3.5	2.4
Matapédia, n = 15						
mean	126.6	40.5	22.5	16.9	30.0	21.4
std dev	4.5	1.4	0.9	1.1	1.0	0.9
Popo Agie, n = 15						
mean	146.7	44.6	24.3	18.2	31.6	22.4
std dev	4.1	1.0	0.9	1.2	1.1	1.8
Blue, n = 15						
mean	118.9	42.0	20.6	15.8	28.7	20.7
std dev	6.1	1.8	1.0	1.6	1.1	2.0
Waiau, n = 15						
mean	126.8	40.4	22.5	17.7	29.1	22.2
std dev	2.2	1.6	0.9	1.8	1.8	1.7

Tab. 3. Correlation matrix of measurements of morphological features. Because length and footpole measures were highly correlated (0.8), the footpole measure was removed from the principal components analysis.

	Length	Width	Footpole	Footpole constriction	Headpole	Headpole constriction
Length	1.0					
Width	0.6	1.0				
Footpole	0.8	0.7	1.0			
Footpole-constriction	0.5	0.4	0.6	1.0		
Headpole	0.7	0.7	0.8	0.5	1.0	
Headpole-constriction	0.2	0.1	0.4	0.7	0.4	1.0

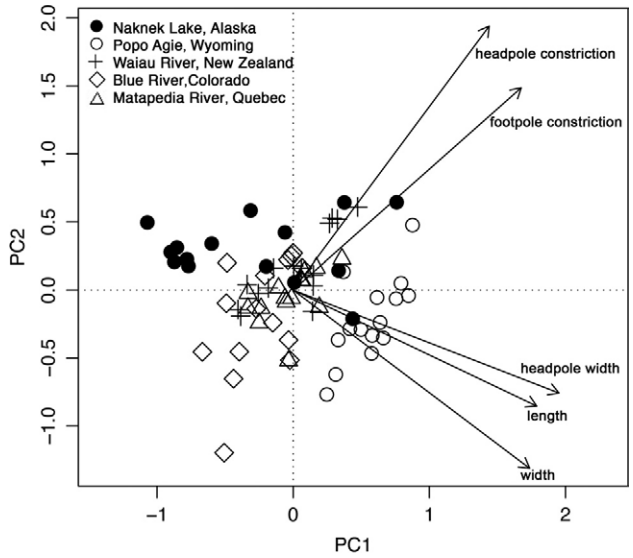


Fig. 12. Principal components analysis (PCA) of valve morphology of five populations of *D. geminata*. Each point represents a single specimen with five measures (valve length, width, headpole, footpole constriction, and headpole constriction) in samples from Naknek Lake, Wyoming, New Zealand, Colorado and Québec. Origin of the biplot represents the average value for each variable. Variables increase in the direction of the arrow and the length of the arrows represent strength of that variable. The angles between arrows are approximate representation of correlation between variables.

co-vary with one another, the constriction of the footpole and headpole co-vary with one another. The Naknek Lake specimens have the greatest variation in morphology when compared to populations from other sites. The specimens from Blue River are also variable, but not to the extent of the Naknek Lake population. Specimens from Popo Agie, Waiau and Matapedia rivers are all more closely clustered to one another. Results of an

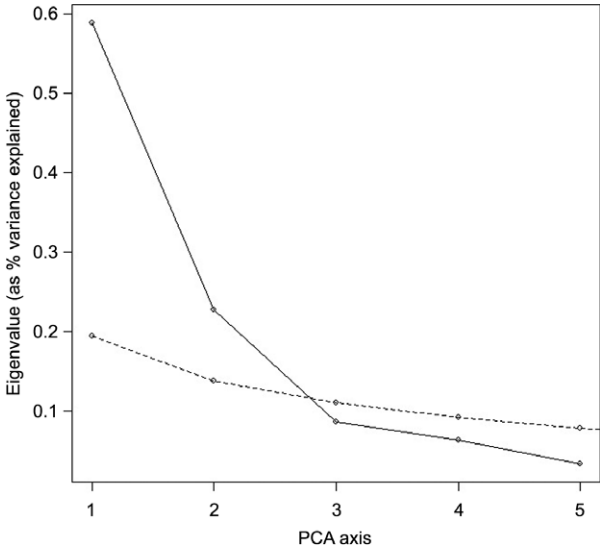


Fig. 13. Plot of PCA axes against eigenvalues for the calculated axes (solid line) and axes based on the Broken Stick model (dashed line). The values for axes 1 and 2 fall above the Broken Stick model and are therefore the only significant axes. Axes 1 and 2 account for 82% of the total variance.

analysis of variance (ANOVA) test show that the populations were significantly different in terms of valve length, width and headpole constriction (Tab. 4).

Tab. 4. Results of analysis of variance test of morphological differences between populations from Alaska, Québec, Wyoming, New Zealand, and Colorado. The populations were significantly different in terms of length, width and headpole constriction.

Measurement	F value	Level of significance (P > F)
Length	18.492	0.0001
Width	26.364	0.0001
Footpole constriction	0.0143	ns
Headpole	3.2800	0.1
Headpole constriction	19.855	0.0001

Discussion

We used the total abundance of *D. geminata* and *D. clavaherculis* valves in lake sediments to infer the historical pattern of valves in streams flowing into Naknek Lake. Because we were not able to obtain modern collections, however, our interpretation is based on the assumption that the relationship between river abundance and lake sediment concentration is constant over time. Ideally, a comparison of cell densities linked to a known

bloom of *D. geminata* could be linked to historical cell densities in the lake sediments. Further work to establish the relationship between river cell abundance and the sediment record is likely to be instructive. Such a comparison is needed to resolve whether large masses occurred in Brooks River prior to 2003, the time of coring.

Our results show that between AD 1218 and 2003, the concentrations of *D. geminata* and *D. clavaherculis* and *C. mexicana* remained relatively constant and responded in a similar manner. The abundance of these three diatom species did not appear to be correlated with percent loss on ignition or percent carbon/nitrogen ratio, $d^{15}N$ and $d^{13}C$ (FINNEY, unpublished data). The $d^{15}N$ ratio in sockeye salmon nursery lakes such as Naknek Lake, has been shown to reflect abundance of sockeye spawning in and around the lake (FINNEY et al. 2000). While recent work has suggested that the growth of *D. geminata* in rivers and streams is a new phenomenon in regions of North America (SPAULDING et al. 2008), we show that *Didymosphenia* abundance has not changed over time in Naknek Lake, Alaska. The presence of *D. geminata* in a high latitude site such as Naknek Lake is to be expected, based on historical records from Canada and Alaska (CLEVE 1894–1986, PATRICK and FREESE 1961, FOGED 1981, SHEATH et al. 1986, LAPIERRIERE et al. 1989, HEIN 1990, MILLER et al. 1992, SHEATH et al. 1996). It is interesting to note, however, that much of the work on diatoms from high latitudes has concerned analysis of sediment cores for paleolimnological reconstruction, and these studies do not report the presence of *Didymosphenia* (LUDLAM et al. 1996, ANTONIADES and DOUGLAS 2002). We believe the absence may be the result of using fixed counts of total diatom valves (usually 100–600 valves). Our results demonstrate that a method of counting using low magnification objectives and examining entire slides for large diatoms results is useful for 1) detecting and documenting large, rare species and 2) obtaining an adequate number of total cells for a representative count. Furthermore, this method may be more time-efficient than traditional microslide counts. In future work, inclusion of a greater number of specimens (microslides) identified and counted would allow for greater precision for each taxon to determine potential trends over time. Our results indicate that this approach to determining the historical abundance of *Didymosphenia* could be applied in lakes downstream of nuisance blooms. This approach could be repeated in regions in North America and Europe to quantify how, and whether, *D. geminata* is changing abundance and expanding its geographic range.

The variance in cell morphology within Naknek Lake is larger than in other *Didymosphenia geminata* populations that we examined from Québec, Wyoming, New Zealand, and Colorado. To date, we are not aware of published records of *D. clavaherculis* in North America, although HEIN (1990: Pl. 14, Fig. 1) shows an image of a specimen that could belong to this species. We suggest that in the examination of specimens from areas with invasive or nuisance blooms, morphological analysis combined with molecular markers would be a promising approach to determining if such nuisance blooms represent a new strain of *D. geminata*.

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