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Recent Observation of a Proliferation of *Ranunculus trichophyllus* Chaix. in High-altitude Lakes of the Mount Everest Region

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Abstract

Large populations of the threadleaf water-crowfoot, *Ranunculus trichophyllus*, were discovered in several high-altitude lakes in the Himalayas of Nepal in which the species did not occur as recently as 1987. One of the study lakes, at 4760 m, is the highest altitude from which an aquatic angiosperm has been reported. A canonical discriminant analysis suggests that the key environmental factors differing between vegetated and non-vegetated lakes among a chain of five interconnected water bodies are length of the ice-free season, conductivity, non-volatile suspended solids, and bicarbonate. We believe, however, that an increased length of the ice-free season is likely the controlling factor in the recent invasion by *Ranunculus trichophyllus*. Its range expansion is likely a signal of a warming alpine climate in our study area.

Introduction

Evidence is accumulating in many regions of warming climate and biological responses to this environmental change, including latitudinal and altitudinal shifts of the distributions of certain animals and plants, as well as phenological changes (IPCC, 2001; Poff et al., 2002; Walther et al., 2002). In the Himalayan region, surface warming of the atmosphere has been shown over the past three decades (Shrestha et al., 1999; WWF Nepal Program, 2005, and references therein), but there have been few documented biological responses (Lami et al., 1998).

The presence and species richness of biodiversity in alpine aquatic environments are limited by various environmental factors, including harsh conditions associated with a cool and short growing season, the persistence of ice cover, high-UV exposure, low concentration of ions and nutrients, and disturbance by events of mass erosion and glacial outburst (Ward, 1994; Ormerod et al., 1994; Lami et al., 1998; Suren and Ormerod, 1998; Lacoul, 2004). Moreover, the colonization of high-alpine aquatic habitats is made difficult because of infrequent visits by faunal vectors of dispersal (Ward, 1994).

Previous surveys of high-altitude lakes in the Himalayas of Nepal, with fieldwork occurring as recently as 1987, did not report any aquatic angiosperm plants above ca. 4000 m (Löffler, 1969; Scott, 1989). There is, however, paleolimnological evidence that aquatic plants may have occurred in lakes above 4000 m during recent Holocene warm periods, including the Medieval warming that ended around 450 years ago (Lami et al., 1998).

Within this context, we report the remarkable observation of abundant populations of a submerged aquatic angiosperm, the threadleaf water-crowfoot (*Ranunculus trichophyllus* Chaix. var. *trichophyllus*), in alpine lakes as high as 4760 m. This species was found flowering and setting seed during the ice-free season in several lakes. We also discuss the environmental factors that may be influencing the altitudinal limit for this species, and the possible influence of recent climate warming.

Methods

STUDY AREA AND LAKES

Glaciers and glacial lakes are abundant and widespread in high-altitude regions of Nepal, particularly in the altitudinal range of 4000–

5600 m, where water bodies are ice free for 3–4 months each year (Hutchinson, 1937; Löffler, 1969; Manca et al., 1998). Our study lakes are part of a chain of water bodies impounded by a lateral moraine of the Ngojumba glacier on the southern slopes of Mount Cho Oyu, close to Mount Sagarmatha (Everest). The lakes occur within an altitudinal range of 4690 m to 4980 m, and their water sources are meltwater of glaciers and snow plus seasonal rainfall. The region has a monsoon climate, with an annual rainfall of 950–1,050 mm, 80% falling during the monsoon of June to September, and a monthly minimum temperature of -7.7°C (January) and maximum of 16.2°C (August) (Tartari et al., 1998a). Lakes in the study area are ice covered for 5–9 months of the year. The watershed surface is mostly exposed bedrock, plus glacial debris of gneiss, granite, and some calcareous metasedimentary rock (Bortolami, 1998), with vegetated patches of shrubby juniper, rhododendron, forbs, and graminoids. Lake sediment is silt-clay in deeper water and sand-silt in the littoral zone (Tartari et al., 1998b). The lakes are devoid of fish. Additional data for the study lakes are provided in Table 1.

WATER SAMPLING AND ANALYSIS

The study lakes were accessed by trekking and were sampled during the growing season (pre-monsoon and monsoon) of each of 1999 and 2000. A composite of five water samples was collected from the littoral zone in nalgene bottles pre-cleaned with distilled water and rinsed several times with lake water. Analyses were made according to standard methods (A.P.H.A., 1995), unless otherwise noted.

Water temperature was measured at midday in the epilimnion using a mercury thermometer, and conductivity with a temperature-corrected Fisher (C-33) probe. Local ice-free data were collected from local yak herders and hoteliers, who keep track of this information. Transparency was determined with a 20-cm Secchi disc, averaged over two measurements. Field pH was measured with color-sensitive pH strips (Merck; range 5.5–9.0; graduation 0.5 pH unit) and with a Hannah pH meter (HI 9214). Conductivity and pH were re-analyzed in the laboratory to verify the field results. Water samples for later analysis were divided into sub-samples of 50 or 100 mL and preserved according to the specific determination to be performed (A.P.H.A., 1995), as follows: (a) chilling in an icebox for subsequent analysis of calcium, magnesium, sodium, potassium, chloride, sulfate, and phosphorus, and (b) acidification with 40% H_2SO_4 added in the field to

TABLE 1

Physical-chemical environmental characteristics (mean \pm SE) of the study lakes. *F*-values are from an ANOVA, with significance $P < 0.05$. Values followed by a different letter differ according to a test of honest significant difference ($P < 0.05$).

Environmental variable	(1) TsoMengma	(2) Longponga	(3) DudhPokhari	(4) Donang	(5) Ngojumba	<i>F</i> -value
Altitude (m)	4680 ^a	4715 ^b	4750 ^c	4970 ^d	4980 ^e	—
Surface area (ha)	3.4 ^a	23.8 ^b	44.1 ^c	65.0 ^d	28.0 ^e	—
Maximum depth (m)	1.3 ^a	25.3 ^b	45.3 ^c	37.7 ^d	35.3 ^e	—
Ice-free (days)	184 \pm 3 ^a	190 \pm 3 ^a	191 \pm 3 ^a	153 \pm 2 ^b	153 \pm 3 ^b	51.2
Conductivity (μ S cm ⁻¹)	45.0 \pm 2.0 ^a	43.9 \pm 1.6 ^a	41.8 \pm 1.1 ^a	32.8 \pm 0.8 ^b	33.1 \pm 0.8 ^b	33.8
pH (pH unit)	7.3 \pm 0.1 ^a	7.2 \pm 0.1 ^{ab}	7.2 \pm 0.1 ^b	7.0 \pm 0.1 ^c	7.0 \pm 0.1 ^c	16.1
Secchi transparency (m)	2.6 \pm 0.1 ^a	3.8 \pm 0.2 ^b	3.7 \pm 0.2 ^b	3.6 \pm 0.2 ^b	3.8 \pm 0.2 ^b	12.6
Temperature (°C)	8.8 \pm 0.9 ^a	9.3 \pm 1.0 ^a	9.2 \pm 1.02 ^a	4.3 \pm 0.5 ^b	4.1 \pm 0.5 ^b	12.3
Ca ⁺⁺ (mg L ⁻¹)	5.6 \pm 0.2 ^a	5.5 \pm 0.3 ^a	5.8 \pm 0.28 ^a	4.1 \pm 0.4 ^b	4.3 \pm 0.5 ^b	5.6
Mg ⁺⁺ (mg L ⁻¹)	0.43 \pm 0.01 ^a	0.42 \pm 0.01 ^a	0.4 \pm 0.01 ^a	0.36 \pm 0.02 ^b	0.39 \pm 0.02 ^a	1.3
Na ⁺ (mg L ⁻¹)	1.2 \pm 0.1 ^a	1.1 \pm 0.1 ^a	0.8 \pm 0.03 ^{ab}	0.66 \pm 0.05 ^b	0.78 \pm 0.04 ^a	10.6
K ⁺ (mg L ⁻¹)	0.55 \pm 0.02 ^a	0.57 \pm 0.03 ^a	0.6 \pm 0.02 ^a	0.49 \pm 0.03 ^a	0.47 \pm 0.04 ^b	2.3
HCO ₃ (mg L ⁻¹)	19.4 \pm 1.5 ^a	18.5 \pm 0.5 ^a	18.0 \pm 0.5 ^a	14.4 \pm 0.7 ^b	15.4 \pm 0.7 ^b	6.0
Cl (mg L ⁻¹)	0.15 \pm 0.01	0.14 \pm 0.01	0.2 \pm 0.1	0.23 \pm 0.02	0.19 \pm 0.06	1.0 ^{ns}
SO ₄ (mg L ⁻¹)	5.5 \pm 0.1 ^a	5.6 \pm 0.1	3.6 \pm 0.1 ^b	3.6 \pm 0.1 ^b	3.5 \pm 0.1 ^b	151.7
Total suspended solids (mg L ⁻¹)	2.1 \pm 0.3 ^a	1.9 \pm 0.3 ^a	1.6 \pm 0.3 ^a	1.1 \pm 0.2 ^b	1.1 \pm 0.1 ^b	3.9
Volatile suspended solids (mg L ⁻¹)	0.40 \pm 0.1	0.37 \pm 0.05	0.37 \pm 0.02	0.40 \pm 0.1	0.37 \pm 0.04	0.1 ^{ns}
Nonvolatile suspended solids (mg L ⁻¹)	1.7 \pm 0.2 ^a	1.6 \pm 0.3 ^a	1.3 \pm 0.3 ^a	0.73 \pm 0.1 ^b	0.70 \pm 0.1 ^b	5.8
Total nitrogen (μ g L ⁻¹)	227 \pm 7.3	194 \pm 12.8	226 \pm 6.4	193 \pm 25.9	203 \pm 19	1.1 ^{ns}
Dissolved nitrogen (μ g L ⁻¹)	162 \pm 4.3	148 \pm 9.2	166 \pm 2.8	153 \pm 12.3	141 \pm 15	1.1 ^{ns}
Total phosphorus (μ g L ⁻¹)	8.1 \pm 0.2	7.3 \pm 0.2	7.9 \pm 0.3	7.3 \pm 0.4	7.8 \pm 0.4	1.2 ^{ns}
Dissolved phosphorus (μ g L ⁻¹)	4.2 \pm 0.2 ^a	3.9 \pm 0.2 ^a	4.2 \pm 0.2 ^a	3.3 \pm 0.3 ^b	3.6 \pm 0.3 ^{ab}	2.7
Total Chlorophyll (μ g L ⁻¹)	0.7 \pm 0.1 ^{ad}	1.5 \pm 0.1 ^b	1.6 \pm 0.1 ^b	0.9 \pm 0.2 ^{cd}	0.9 \pm 0.2 ^{acd}	10.9

achieve pH $<$ 2 for analysis of total and dissolved nitrogen (samples were neutralized with NaOH prior to digestion). The analytical methods used in the laboratory were:

- calcium, magnesium, sodium, and potassium by flame atomic absorption spectrophotometry;
- bicarbonate by carbonate-hydroxide titration;
- chloride by an argentometric spectrophotometry;
- sulfate by a gravimetric method based on BaSO₄;
- total suspended solids (TSS), non-volatile suspended solids (NVSS), and volatile suspended solids (VSS) by weighing after passing through a 1.5 μ m Whatman 934 AH filter;
- total chlorophyll by filtration through Whatman GF/C filters (particle retention 1.2 μ m) in the field, storage with desiccant (silica gel), and analysis by fluorometry in the laboratory (Knowlton, 1984; Sartory and Grobbelaar, 1986) the filtrate from chlorophyll processing was used for analysis of dissolved nutrients (DN and DP), while non-filtered water was used for total nutrients (TN and TP; see below);
- phosphorus (total and dissolved) analyzed after the methodology of Prepas and Rigler (1982);
- nitrogen (total and dissolved; nitrate plus ammonium) determined by second derivative analysis of persulfate oxidized samples (Crumpton et al., 1992).

PLANT SAMPLING

Lakes supporting aquatic plants were surveyed monthly during the ice-free season of both study years, using 10 randomly placed quadrats of 50 cm \times 50 cm within the vegetated littoral zone. Biomass of *Ranunculus*, the only plant species present in the lakes, was collected, dried at 105°C, and weighed.

DATA ANALYSIS

Differences of variables among lakes were examined using one-way analysis of variance, with post-hoc comparison of means using

Tukey's HSD test. To achieve homogeneity of variance, the data for environmental variables (with the exception of pH) were ln-transformed. The data matrices were also analyzed using a canonical discriminant analysis (CDA). CDA is used to discriminate among four or more groups with multivariate factors; it recognizes linear combinations of discriminating variables, which maximize differences between groups and allows for interaction between factors. In running the CDA, we progressively eliminated ineffective factors (step-wise forward method). The statistical analyses were done using STATISTICA 5.1 for Windows (StatSoft, 1997).

Results

Ranunculus trichophyllus was abundant on silty and sandy littoral substrates of Lakes Tso-Mengma (4680 m), Longponga (4715 m), and Dudh Pokhari (4750). It did not occur in Lakes Donang (4970 m) or Ngojumba (4980 m). The biomass of *Ranunculus* was highly seasonal (Fig. 1). It was present in open-water areas of inflow and outflow streams prior to complete ice-melt of the lakes; an April sampling of vegetated habitat in Tso-Mengma averaged 4.6 g m⁻², Longponga 7.6 g m⁻², and Dudh Pokhari 8.2 g m⁻² (Tso-Mengma was significantly less than the other two lakes; $p < 0.05$). Biomass increased rapidly to a seasonal maximum in late July and early August, to an average of 21.5 g m⁻² in Tso-Mengma, 35.9 g m⁻² in Longponga, and 44.6 g m⁻² in Dudh Pokhari (Tso-Mengma significantly less; $p < 0.05$). During the peak season, the average cover of *Ranunculus trichophyllus* was 10–15% in the littoral zones of the study lakes, to a maximum depth of about 4.5 m. The biomass declined during September to November, to an average of 17.9 g m⁻² in Tso-Mengma, 28.4 g m⁻² in Longponga, and 32.2 g m⁻² in Dudh Pokhari (Tso-Mengma significantly less; $p < 0.05$). Icing of the lake surface began in November, ending our seasonal observations.

Environmental data for the study lakes are presented in Table 1. In synopsis, the lake water chemistry is characterized by circumneutrality (pH ca. 7.2), oligotrophy (dissolved phosphorus 3–5 μ g L⁻¹), hard

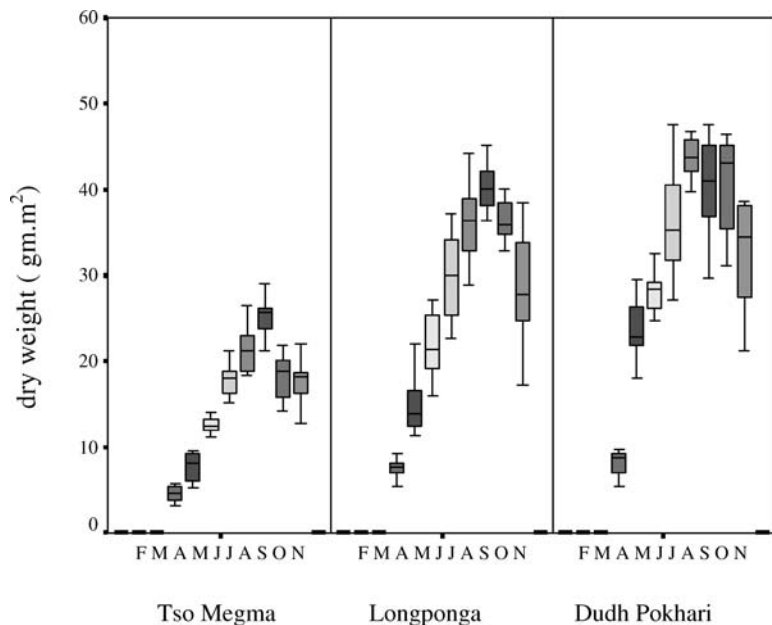


FIGURE 1. Monthly biomass (dry weight g m^{-2}) of *Ranunculus trichophyllus* in three high-alpine lakes. The central line indicates the median value of the data; boxes denote 25th to 75th percentile, and whiskers represent 10th and 90th percentile.

water (calcium $3\text{--}7 \text{ mg L}^{-1}$), and moderate transparency (Secchi depth $2\text{--}5 \text{ m}$) because of suspended glacial flour (total suspended solids $1.1\text{--}2.1 \text{ mg L}^{-1}$). The CDA identified four critical environmental variables (ice-free days, conductivity, non-volatile suspended solids, and bicarbonate) and two canonical functions that discriminated significantly between lakes with or without macrophytes ($P < 0.05$ by chi-square test) and that together explained 99% of the differences. Axis 1 of the CDA (Fig. 2; Table 2) was strongly dominant, accounting for 97% of the cumulative variance (eigenvalue 12.44) and clearly discriminating Lakes Donang and Ngojumba (without *Ranunculus*; right-hand side of the discriminant function) from the other three lakes (left-hand side). Axis 2 is much weaker, accounting for 2% of the variance (eigenvalue 0.36), and it did not help to discriminate lakes on the basis of the presence of *Ranunculus*.

Among the environmental variables used by the CDA, the number of ice-free days correlated most strongly with Axis 1 ($r=0.81$; it also had the highest F statistic of 51.9 (4, 45); $p < 0.001$), followed by conductivity ($r=0.71$; $F=26.4$ (8, 88); $p < 0.001$), non-volatile suspended solids ($r=0.64$; $F=15.3$ (12, 114); $p < 0.001$), and bicarbonate ($r=0.62$; $F=12.9$ (16, 128); $p < 0.001$). In the stepwise addition of variables in successive order, the Wilk's lambda was minimized from 0.17, 0.09, 0.07, and 0.05, respectively, suggesting the high significance of the discriminatory power of the model. Among the three lakes supporting *Ranunculus*, the average length of the ice-free season was 195 ± 15 days (mean \pm SE), significantly longer ($p < 0.05$) than the non-vegetated lakes (158 ± 15 days). The conductivity of lakes with *Ranunculus* ($43.6 \mu\text{S cm}^{-1}$) was higher than those without ($30.8 \mu\text{S cm}^{-1}$), as was the concentration of bicarbonate (18.6 mg L^{-1} versus 14.9 mg L^{-1}).

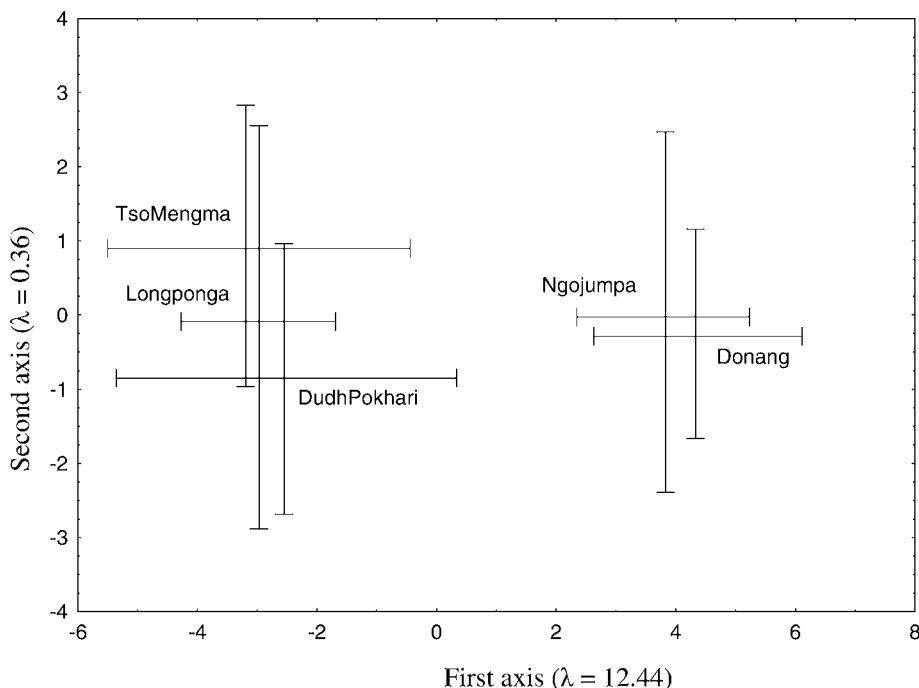


FIGURE 2. Arrangement of scores (crossing points represent mean, with range bars the 95% confidence interval) for the first two canonical functions by canonical discriminant analysis (CDA). The three lakes on the left-hand side support *Ranunculus trichophyllus* and those on the right do not.

Discussion

We observed abundant populations of *Ranunculus trichophyllus* in extremely high-altitude lakes (to 4780 m), at elevations much higher than previously reported for this species (to 3600 m elsewhere in the Himalayas; Zhengyi et al., 1990). Moreover, these populations occur at higher elevations than any published altitudinal record (known to us) for an aquatic angiosperm. Other extreme records of plants in alpine lakes include Lake Titicaca, Peru-Bolivia, at 3900 m with 23 vascular species (Dejoux, 1994), and observations of *Callitriche palustris*, *Isoetes bolanderi*, *Myriophyllum exalbescens*, *Nuphar luteum*, and *Potamogeton alpinus* in glacier-fed lakes at 3000–3355 m in western North America (Welsh et al., 1993; Hart and Cox, 1995). There are, however, more extreme altitudinal records for non-angiosperm macrophytes, including *Chara* species in Lake Puma Yumco in Tibet at 5030 m (Mitamura et al., 2003).

Ranunculus trichophyllus is a widespread species in cool-temperate, boreal, arctic-tundra, and alpine-tundra biomes, including high mountains of Asia, Europe, and North America (Zhengyi et al., 1990; Tolmecher, 1996; Kozhova and Izmetéva, 1998). It occurs in water bodies with chemistry comparable to our study lakes, particularly with respect to high concentrations of calcium (Dale and Miller, 1978; Carbiener et al., 1990), and it is known to form mats, to have rapid growth following the melt-out of surface ice, and to propagate well from rhizome fragments (Barrat-Segretain and Gudrun, 2000). Like allied species of aquatic *Ranunculus*, it is a pioneering aquatic angiosperm that can invade lakes within only a few years after deglaciation, and then expand in abundance over several decades (Birks, 2000).

It appears that the presence of *Ranunculus trichophyllus* in our high-altitude study lakes is a recent phenomenon, because aquatic plants were not reported from these water bodies by other, competent biologists, who examined them as recently as 1987 (Löfller, 1969; Scott, 1989). The apparently recent invasion of the study lakes by *R. trichophyllus* may be related to climatic warming that has occurred in the high Himalayas during the past several decades (Li and Tang, 1986; Kothyari and Shing, 1996; Shrestha et al., 1999), similar to that of other mountainous regions (Oerlemans, 1994; Liniger et al., 1998; Hall and Fagre, 2003). The Himalayan warming has resulted in such phenomena as the extensive retreat of glacial fronts (Kotlyakov and Lebedeva, 1998; Kulkarni et al., 2002) and an increased frequency of glacial-lake outbursts and associated catastrophic damage in lower altitudes (Ives, 1986; Vuichard and Zimmermann, 1986). It is likely that warming has also resulted in an increased length of the ice-free season of high-altitude lakes; although this has not yet been documented by scientists in the Himalayas, earlier melt-out of ice-covered lakes and rivers is well known from better-studied regions elsewhere (Magnuson et al., 2000; Quayle et al., 2002). It has also been suggested that the warming should result in changes in the distribution of plant species and community types in alpine and high-latitude environments (Grabherr et al., 1994; Walther et al., 2002).

The means by which *Ranunculus trichophyllus* colonized our high-altitude study lakes is not known, but aquatic birds may have been a vector. Various species of aquatic birds migrate through the study region to and from breeding grounds in northern Eurasia (Scott, 1989). These include species of geese and other waterfowl, which may forage in shallow aquatic habitat and pick up fragments or seeds of aquatic plants.

Our canonical discriminant analysis suggests that, of the environmental variables that differed between lakes supporting *Ranunculus trichophyllus* or not, the key differences appear to be associated with length of the ice-free season, conductivity, non-volatile suspended solids, and bicarbonate concentration. However, we be-

TABLE 2

Standardized coefficients of key environmental variables in four canonical discriminant functions.

	Canonical function			
	Axis 1	Axis 2	Axis 3	Axis 4
Ice-free days	-0.81	-0.74	0.7	0.16
Conductivity	-0.71	0.004	-0.12	0.84
Non-volatile suspended solids	-0.64	1.04	0.46	0.63
Bicarbonate	-0.62	1.14	-0.62	0.39
Eigenvalue	12.44	0.36	0.01	0.002
Cumulative variance	0.97	>0.99	>0.99	1.00

lieve that in our study region an increasing length of the ice-free season is the key factor allowing *R. trichophyllus* to colonize high-altitude lakes, and that any influences of the other factors are secondary reflections of the ice-free period, relative height within a chain of connected lakes, or amounts of suspended inorganic particulates (glacial “flour”) and dissolved basic cations, especially calcium (Drever, 1997; Hobbie et al., 2003). Moreover, we believe that the presence of *R. trichophyllus* is an indicator of a warming climate, and that it may become a successful colonizer of many high-altitude lakes in the region.

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