ADVANCES IN OCEANOGRAPHY AND LIMNOLOGY

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SUPPLEMENTARY MATERIAL

Historical colonisation patterns of *Dolichospermum lemmermannii* (Cyanobacteria) in a deep lake south of the Alps

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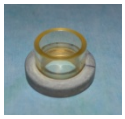
		AKINETES COUNTING PROCEDURE Laboratory operations	Weight of fresh sediment after laboratory operation
#1		2 g of fresh sediment sample were put in a 15 mL plastic tube (the exact weight of the sediment is measured with an analytical balance)	2 g
#2		The sample was exposed to cold and hot acid and basic treatment (10% HCl, 10% KOH, 10% HF)	2 g
#3		The resulting sample was diluted to 100 mL and fixed with Lugol's solution. The sample was stored in the dark at 4°C	2 g
#4		5 mL of fixed sample (3) were diluted in 95 mL of tap water	0.1 g
#5		10 mL of sample (4) were transferred to Utermohl counting chambers	0.01 g
#6		The whole bottom of a counting chamber was observed under an inverted microscope at 40x (Zeiss Axiovert 135)	0.01 g

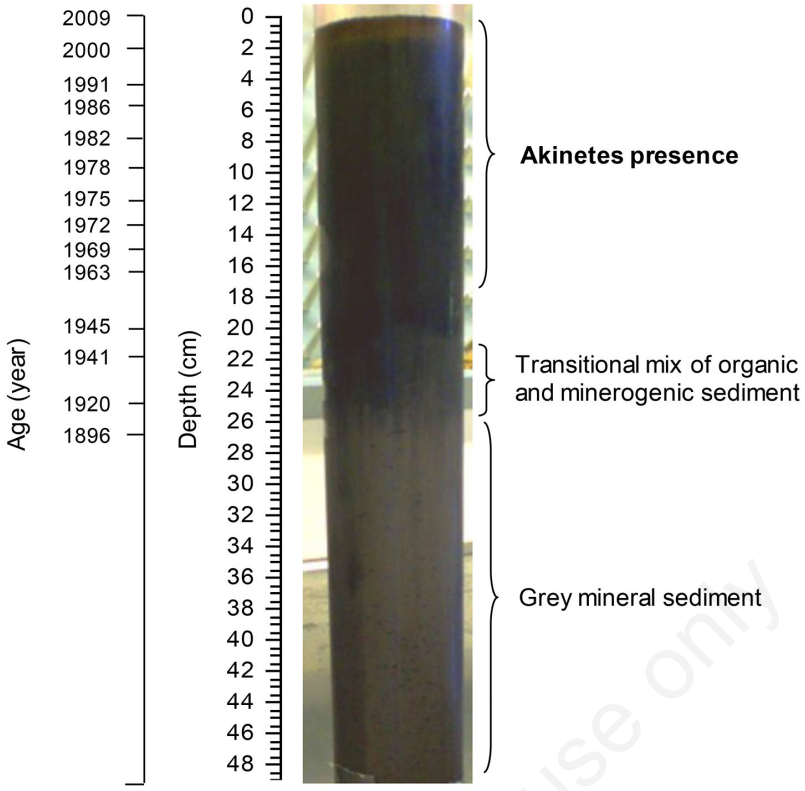
The final counting provides the number of akinetes in 0.01 g wet weight (ww). The number of cells N is expressed in cells/grams ww: N = cells/0.01 = cells x 100.*
This value is transformed in no. of akinetes/grams dry weight (dw) multiplying the result by a coefficient obtained from % H_2O in the respective sediment layer.

*The computation will take into consideration the exact weight of the fresh sediment measured with an analytical balance.

Supplementary Fig. 1. Laboratory procedure used to estimate the density of akinetes in the core sediment layers. The scheme reports the principal laboratory steps performed to obtain samples of akinetes free of most of the organic and inorganic sediment.

		AKINETES GERMINATION PROCEDURE **Laboratory operations**	**Weight of fresh sediment after laboratory operations**
#1		4 g of fresh sediment sample was diluted in ASM-1 medium (the exact weight of the sediment is measured with an analytical balance)	4 g
#2		A total volume of 60 mL was made by adding culture media ASM-1 containing actidione (cycloheximide, 250 mg L^{-1}) as an inhibitor of eukaryotic growth. The flasks were incubated at 20°C under continuous irradiance with day light florescent lamps at 85 µmol m^{-2} s^{-1}.	4 g
#3		After 16 or 21 days, half of the total volume (30 mL, with the germinated filaments of Nostocales) was diluted to 100 mL and fixed with Lugol's solution. Samples were stored in the dark at 4°C	2 g
#4		2.5 mL of 100 mL fixed sample (3) were diluted in 97.5 mL of tap water	0.05 g
#5		10 mL of this 100 mL solution (4) were transferred to Utermohl counting chambers	0.005 g
#6		The whole bottom of a counting chamber was observed under an inverted microscope at 40x (Zeiss Axiovert 135)	0.005 g
		The final counting provides the number of cells in 0.005 g wet weight (ww). The number of cells N is expressed in cells/grams ww: N= cells/0.005 = cells x 200.* This value is transformed in no. of cells/grams dry weight (dw) multiplying the result by a coefficient obtained from % H_2O in the respective sediment layer.	
		*The computation will take into consideration the exact weight of the fresh sediment measured with an analytical balance.	

Supplementary Fig. 2. Laboratory procedure used to estimate the density of cells germinated from viable akinetes isolated from the core sediment layers. The procedure allows to obtain living populations of *Dolichospermum*.



Supplementary Fig. 3. The core sediment collected in 2009. The 2014 core (where the sub-fossil akinetes were determined) and the 2009 core showed a well resolved and comparable pattern of water content. Excluding the 5 years gap between the two cores, the figure shows the approximate extent of the sediment layer where akinetes were found. In the last ~50 years (akinetes presence), the organic content approximately doubled due to the combined increase in lake productivity and decreasing input of minerogenic materials in relation to the construction of hydropower plants within the lake watershed (Milan *et al*., 2015).

REFERENCES

Milan M, Bigler C, Salmaso N, Guella G, Tolotti M (2015). Multiproxy reconstruction of a large and deep subalpine lake's ecological history since the Middle Ages. J. Great Lakes Res. (In Press).