Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information: Analytical Methods

The influence of pH on dissolved organic matter fluorescence in inland waters

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Supplementary Table 1: The mean, standard deviation (std dev), and percent standard deviation (% std dev) expressed as a percentage of the mean of 10 replicate readings of four test samples at ambient pH, providing insight into the background variability attributable to instrumental fluctuations.

Lake		pH	Replicate	Peak A	Peak C	Peak M	Peak T	Peak B
Ängbybadet	Ambient	7.6	Mean	1.43	0.69	0.76	0.59	0.35
			1 Stdev	0.12	0.01	0.03	0.05	0.07
			1 stdev %	**1**	**1**	**2**	**3**	**2**
Krycksjön	Ambient	7.7	Mean	2.84	1.23	1.55	1.06	0.67
			1x Stdev	0.04	0.02	0.03	0.04	0.02
			1 stdev %	**2**	**1**	**2**	**3**	**2**
Nordic Reservoir	Ambient	5.9	Mean	1.43	0.82	0.65	0.60	0.44
		5.9	1x Stdev	0.02	0.01	0.01	0.01	0.01
			1 stdev %	**2**	**2**	**2**	**2**	**2**
Strandsjön	Ambient	7.1	Mean	3.02	1.29	1.39	0.92	0.68
		7.1	1x Stdev	0.29	0.03	0.07	0.09	0.09
			1 stdev %	**10**	**2**	**5**	**10**	**13**

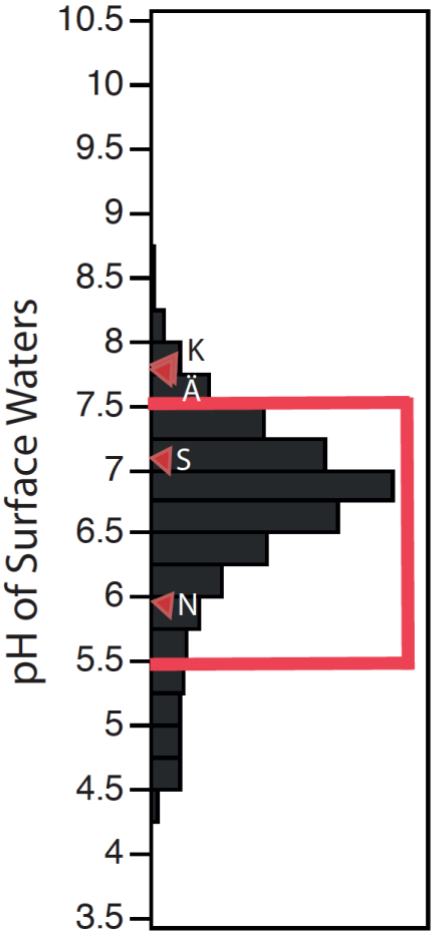
Table 1 p values from ionic strength experiment. All values are compared to ionic strength = 0, pH = 6.5 (typical conditions for humic standard). All values that are the same colour as the reference block are not statistically different ($p > 0.05$).

Supplementary Table 2. Optical characteristics of ambient water samples for all four test samples.

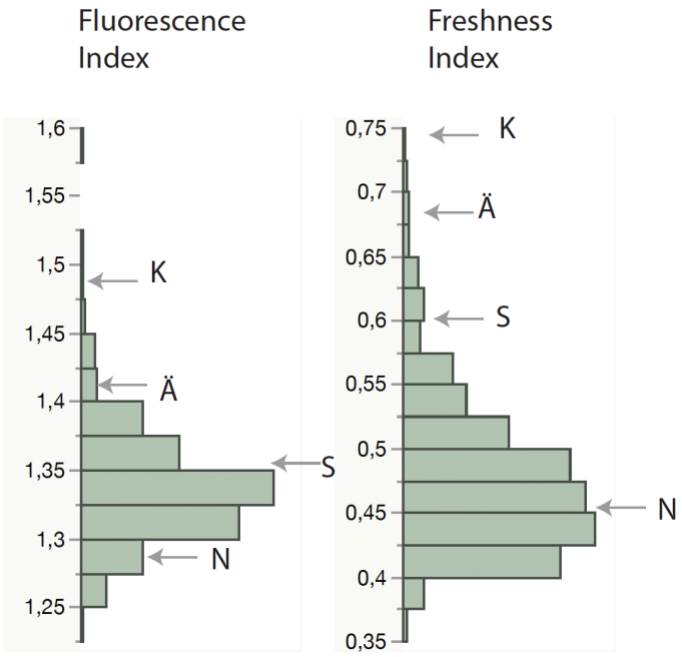
	Ängbybadet		Krycksjön		Strandsjön		Nordic Reservoir		
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	*n*
Fluorescence Index	1,42	0,00	1,49	0,00	1,35	0,00	1,28	0,00	10
Freshness Index	0,68	0,00	0,76	0,00	0,61	0,00	0,45	0,00	10
Humification Index	5,3	0,0	5,2	0,0	6,3	0,1	5,3	0,1	10
a254 (m-)	66,5	7,4	126,8	0,1	154,1	0,1	108,2	0,1	10
SUVA	1,9		2,1		2,6		3,1		1

Supplementary Table 2. Ionic strength results from the paired comparison of means student's t-test to identify pH ranges that were most stable across a gradient of ionic strengths using the commercially available Nordic Reservoir. All values were compared to the reference sample with ionic strength of 0 M, and pH of 6.5 (shaded in dark green), since the solution would typically be made into 0 M Milli-Q or deionized water. All values that are the same color (green) as the reference block (pH 6.5) are not statistically different ($p > 0.05$), and considered highly stable.

IS	variable	pH													
		3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	p<0.05 (%)	
0.0000	A	0.0104	0.0231	0.0135	0.0410	0.0202	0.0714		0.0892	0.0664	0.0426	0.0131	0.0689	63.6	
	C	0.0088	0.0514	0.0301	0.1699	0.2887	0.0456		0.5943	0.8383	0.0994	0.9749	0.3675	27.3	
	M	0.0158	0.0204	0.0243	0.0784	0.6220	0.3107		0.0305	0.0836	0.1269	0.1471	0.0691	36.4	
	T	0.6218	0.0266	0.0243	0.5378	0.3994	0.4963		0.2094	0.0564	0.0989	0.0425	0.0523	27.3	
	B	0.0234	0.0131	0.0342	0.0157	0.1034	0.0879		0.5434	0.0703	0.8109	0.0733	0.0879	36.4	
	FI	0.8915	0.6036	0.8483	0.1310	0.3203	0.5434		0.3737	0.1302	0.9697	0.0491	0.0231	18.2	
	FRESH	0.0165	0.0180	0.0466	0.2639	0.1699	0.5921		0.2360	0.1337	0.8805	0.0420	0.0689	36.4	
	HIX	0.0088	0.0088	0.0181	0.0256	0.0632	0.6001		0.6743	0.7804	0.6639	0.6970	0.1611	36.4	35.2
0.0005	A	0.0042	0.0199	0.0181	0.0672	0.0403	0.0199	0.0000	0.0000	0.0231	0.0181	0.0482	0.0405	91.7	
	C	0.0088	0.0516	0.0231	0.0145	0.0157	0.0236	0.0256	0.0784	0.0564	0.1471	0.2341	0.1383	50.0	
	M	0.0468	0.0689	0.0919	0.2726	0.0653	0.0444	0.0405	0.0181	0.0527	0.0470	0.0180	0.0410	58.3	
	T	0.2319	0.0261	0.0188	0.1704	0.3027	0.5409	0.0993	0.0239	0.0348	0.1012	0.0466	0.0836	41.7	
	B	0.0256	0.0230	0.0131	0.0555	0.3419	0.622	0.1381	0.0597	0.0270	0.0272	0.0604	0.1879	41.7	
	FI	0.1621	0.2094	0.1597	0.0927	0.1235	0.0664	0.2120	0.0664	0.0228	0.1939	0.0342	0.0360	25.0	
	FRESH	0.0181	0.0135	0.0231	0.0555	0.0506	0.088	0.0886	0.0271	0.0243	0.0234	0.0119	0.0181	66.7	
	HIX	0.0088	0.0042	0.0155	0.4096	0.4323	0.2605	0.6074	0.6976	0.4839	0.4555	0.0478	0.3709	33.3	51.0
0.0010	A	0.0088	0.0148	0.0231	0.2408	0.1463	0.0957	0.0188	0.0148	0.0270	0.0590	0.0672	0.0345	58.3	
	C	0.0148	0.0256	0.1665	0.0185	0.0664	0.0913	0.0524	0.5844	0.8363	0.3664	0.1004	0.1488	25.0	
	M	0.0180	0.0188	0.0664	0.4442	0.0953	0.0188	0.0466	0.0703	0.0673	0.0516	0.0266	0.0266	50.0	
	T	0.6220	0.0148	0.0224	0.0226	0.0689	0.0994	0.7648	0.4963	0.0584	0.0506	0.0342	0.1018	33.3	
	B	0.0277	0.0270	0.0170	0.1337	0.2827	0.4302	0.0996	0.8280	0.6220	0.0834	0.9654	0.1374	25.0	
	FI	0.6220	0.5606	0.1524	0.1788	0.2033	0.1212	0.6036	0.1844	0.8408	0.0313	0.0345	0.0313	25.0	
	FRESH	0.0237	0.0212	0.0181	0.0879	0.0703	0.0666	0.0426	0.0403	0.0695	0.0075	0.0266	0.0180	66.7	
	HIX	0.0000	0.0042	0.0191	0.6220	0.2538	0.2725	0.6750	0.4183	0.0426	0.6348	0.8805	0.6840	33.3	39.6
0.0020	A	0.0160	0.0234	0.1278	0.1236	0.0555	0.0160	0.0234	0.0234	0.0221	0.1029	0.0571	0.0564	50.0	
	C	0.0148	0.0266	0.4691	0.0516	0.0188	0.0664	0.6421	0.1347	0.4323	0.1205	0.1012	0.0784	25.0	
	M	0.0309	0.0342	0.1524	0.3203	0.0426	0.0277	0.0175	0.0262	0.0221	0.0180	0.0604	0.0288	75.0	
	T	0.0784	0.0234	0.0529	0.0529	0.8176	0.2872	0.1012	0.0266	0.0348	0.0270	0.0478	0.0288	50.0	
	B	0.1844	0.0664	0.0345	0.0540	0.5576	0.1980	0.0719	0.1372	0.0689	0.0894	0.0270	0.1354	16.7	
	FI	0.4514	0.1488	0.0843	0.4620	0.4659	0.7022	0.0781	0.0523	0.0590	0.0527	0.0533	0.0309	8.3	
	FRESH	0.0188	0.0000	0.0646	0.0604	0.0266	0.0338	0.0234	0.0148	0.0131	0.0194	0.0181	0.0088	83.3	
	HIX	0.0042	0.0042	0.1122	0.1349	0.0158	0.0814	0.0148	0.1449	0.0750	0.0188	0.1593	0.0516	41.7	43.8
p<0.05 (%)		71.9	75.0	59.4	18.8	21.9	25.0	41.7	34.4	34.4	34.4	53.1	40.6		



Supplementary Figure 1. Distribution of the pH found in boreal lakes and streams spanning across Sweden (*n*=48,358), with the bold red lines indicating the range between the lower 10% (pH 5.5) and upper 90% (pH 7.5) of the distribution. Red arrows indicate ambient pH of the test samples; Ä: Ängbybadet, K: Krycksjön, S: Strandsjön and N: Nordic Reservoir).



Supplementary Figure 2. Distribution of fluorescence index and freshness index across 560 lakes in Sweden(Kothawala et al. 2014), with test samples used in pH experiment indicated with arrows; Ä: Ängbybadet, K: Krycksjön, S: Strandsjön and N: Nordic Reservoir.

References

Kothawala, D. N., C. A. Stedmon, R. A. Muller, G. A. Weyhenmeyer, S. J. Kohler, and L. J. Tranvik. 2014. Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. Glob Chang Biol **20**:1101-1114.