Development of a diatom-based multimetric index for acid mine drainage impacted depressional wetlands

Electronic Supplemental Material:

Table S1 List of metric categories and candidate metrics used in the development of four MMIs for reference and disturbed depressional wetlands in the Mpumalanga Highveld region.

Category/Candidate metric	Description and source
Diversity	Stevenson et al. (2013)
Hurlbert's evenness (EHurlbert)	Evenness of taxa Wang et al. (2005)
Species richness (S)	No. of taxa in samples (richness)
Shannon diversity (H)	Evenness and richness of taxa
% dominance	Relative abundance of the most commonly observed taxon in a sample
Similarity to reference sites	Stevenson et al. (2013)
% reference taxa	Taxa characteristic of reference sites based on relative abundance of reference taxa to total no. of taxa in the sample
% tolerant taxa	Taxa characteristic of impaired sites by mining based on relative abundance of tolerant taxa to total no. of taxa in the sample
% reference individuals	Relative abundance of reference individuals characteristic of reference sites based on relative abundance of all individuals (valves) typical of reference sites to total no. of individuals in the sample
% tolerant individuals	Relative abundance of tolerant individuals characteristic of impaired sites by mining based on relative abundance of all individuals (valves) typical of disturbed sites to total no. of individuals in the sample Wang et al. (2005)
% similarity to reference sites	Bray-Curtis similarity in taxa composition to all reference sites

% reference taxa found in reference sites that occurred in impaired sites	Relative abundance of taxa found in reference sites that occurred in impaired sites
% reference individuals found in reference sites that occurred in impaired sites	Relative abundance of individuals (valves) found in reference sites that occurred in impaired sites
No. of distinct reference taxa	No. of taxa found predominantly in reference sites not in impaired sites
Functional group	Passy (2007a, b); Rimet and Bouchez (2012)
Mobile % taxa and individuals	Relative abundance of taxa and individuals which are free moving e.g. some species vertically migrate into the sediments to acquire nutrients
Teratology % taxa and individuals	Relative abundance of taxa and individuals having deformed frustules, often caused by metals and trace elements
Pioneer % taxa and individuals	Relative abundance of taxa and individuals which colonise bare substrates faster than other species
Adnate % taxa and individuals	Relative abundance of taxa and individuals which grow parallel to the substrate, attached by their valve face
Pad (attached to substrate) % taxa and individuals	Relative abundance of taxa and individuals which grow upright to substrate, attached by a mucilage pad
Stalk (attached to substrate) % taxa and individuals	Relative abundance of taxa and individuals which grow upright to substrate, attached by a stalk
Colonial % taxa and individuals	Relative abundance of taxa and individuals that can form colonies of multiple cells, possessing tall stature characteristics or if comprised of just a few cells, having short stature characteristics; can form various types of colonies e.g., filamentous-, ribbon- and rosette colonies.
Non-colonial % taxa and individuals	Relative abundance of taxa and individuals that are unicellular
Tube-living % taxa and individuals	Relative abundance of taxa and individuals which live in mucous substance within which they can move freely
Filamentous % taxa and individuals	Relative abundance of taxa and individuals that form long visible chains of attached cells; forms protrude above the biofilm extending into the water column

Rosette % taxa and individuals	Relative abundance of taxa and individuals which produce a short stalk, forming colonies which are similar to a fan/rosette shape
Ribbon % taxa and individuals	Relative abundance of taxa and individuals attached to one another either by interlocking spines or by a layer of mucous on their valve face, forming long, ribbon-like colonies
High profile guild % taxa and individuals	Relative abundance of taxa and individuals of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, pedunculate and colonial centrics
Low profile guild % taxa and individuals	Relative abundance of taxa and individuals of short stature, including prostrate, adnate, small erect, solitary centrics, slow-moving species
Motile guild % taxa and individuals	Relative abundance of taxa and individuals of fast-moving species
Planktonic guild % taxa and individuals	Relative abundance of taxa and individuals which are solitary or colonial centrics, pennates
Taxonomic composition	Wang et al. (2005)
% taxa in genera	Relative abundance of taxa within each genus based on relative abundance of no. of taxa in the genus to total no. of taxa in the sample
% individuals in genera	Relative abundance of individuals within the genus based on relative abundance of all individuals in the genus to total no. of individuals in the sample

Table S2 Mean, standard deviation (SD), minimum (min) and maximum (max) values of physical and chemical variables of reference (Ref) and disturbed (Dist) sites used to develop the four MMIs: MM1-1 using all 15 reference sites; MMI-2 using 4 reed depressional reference sites; MMI-3 using 6 salt depressional reference sites; and MMI- 4 using 5 open-water and grass depressional reference sites. All 19 disturbed sites were used to develop each MMI.

Variables	MMI-1				MMI-2				MMI-3				MMI-4							
	Ref (n = 15)				Ref (n = 4)				Ref (n = 6)				Ref (n = 5)				Dist (n = 19)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Cond (mS m^{-1})	612	933	16	3700	158	91	84	280	1378	1194	425	3700	57	75	16	190	337	336	28	1340
pH	8.77	1.16	6.32	10.47	7.65	1.39	6.32	9.35	9.61	0.44	9.21	10.47	8.65	1.01	6.97	9.66	8.37	0.98	6.69	9.84
Turbidity (NTU)	65.1	50	4.2	192	20.2	19.7	4.2	48.6	115.3	37.6	100	192	40.9	27.7	7.8	78.1	206.8	367.1	1.8	999
NH_3[a]	0.32	0.27	0.06	1.1	0.46	0.43	0.19	1.1	0.36	0.23	0.12	0.69	0.16	0.09	0.06	0.29	1.1	3.17	0.03	14
TN[a]	6.63	7.05	1.9	32	3.5	1.17	1.9	4.6	10.55	10.8	3	32	4.44	0.91	3.2	5.6	5.12	7.73	0.25	32
TP[a]	2.68	3.49	0.03	11	0.29	0.31	0.03	0.64	6.4	2.97	3.5	11	0.13	0.11	0.03	0.28	1.15	1.97	0.03	7.5
PO_4^{2-a}	2.31	3.11	0.03	9	0.06	0.07	0.03	0.17	5.68	2.5	3.3	9	0.06	0.05	0.03	0.14	0.61	1.33	0.03	5.7
Ca^{2+a}	17.3	9.8	7.3	46	17.3	4.3	11	20	19.7	13.1	12	46	14.4	10.6	7.3	33	177.6	246.9	7.2	929
Mg^{2+a}	15.2	14.7	5	63	18	6.7	9.8	26	10.8	8.8	5	27	18.2	25.1	5.6	63	268.8	634.5	9.4	2700
Na^{+a}	1548.7	2670.2	14	10700	293	191.1	133	555	3596.7	3597.8	1040	10700	95.8	154.1	14	370	444.4	583.7	13	2510
K^{+a}	76.5	106.8	3.4	372	14.1	5.2	7.2	19	175.3	121	41	372	7.7	3	3.4	11	31.2	35.9	1.7	151
Cl^{-a}	1455.5	2548.7	15	10200	358.5	252.4	149	698	3358.8	3487.1	823	10200	49.2	51.5	15	139	218.9	409.7	11	1830
SO_4^{2-a}	528.2	983.4	4.3	3720	10.6	10.3	4.3	26	1291	1317.8	284	3720	27	26.9	9.8	73	1740.6	2988.2	13	12300
F^{-a}	1.5	1.1	0.3	4	0.7	0.2	0.5	0.9	2.7	0.8	2	4	0.6	0.3	0.3	1	3.7	6.1	0.2	20
DOC[a]	91.8	104.3	24	457	36.5	15.2	24	55	156.7	152.7	45	457	58.2	26.1	27	98	43.2	70.1	0.25	300
Alkalinity[a]	959	1563	26	6430	242	67	182	335	2067	2188	777	6430	204	321	26	776	381	588	44	2023
Al[a]	0.03	0.06	0.01	0.2	0.01	0.00	0.01	0.01	0.05	0.08	0.01	0.2	0.04	0.08	0.01	0.19	0.91	2.77	0.01	12
Fe[a]	0.15	0.27	0.01	0.92	0.22	0.33	0.02	0.71	0.02	0.03	0.01	0.09	0.24	0.39	0.01	0.92	1.26	4.56	0.01	20
Mn[a]	0.05	0.07	0.01	0.23	0.08	0.07	0.01	0.14	0.02	0.03	0.01	0.08	0.06	0.1	0.01	0.23	0.13	0.34	0.01	1.5

[a] mg L^{-1}. Alkalinity as $CaCO_3$

Table S3 Indicator species analysis results showing taxa to be significantly ($P < 0.05$) present in reference (Ref) and disturbed (Dist) sites. Taxa typical of reference and disturbed sites were used to calculate the following metrics for each MMI: number of distinct reference taxa, and % reference and % tolerant taxa and individuals.

	MMI-1		MMI-2		MMI-3		MMI-4	
Taxon	Ref	Dist	Ref	Dist	Ref	Dist	Ref	Dist
Achnanthidium minutissimum (Kützing) Czarnecki							X	
Encyonema mesianum (Cholnoky) D.G.Mann							X	
Eunotia bilunaris (Ehrenberg) Schaarschmidt	X						X	
Craticula buderi (Hustedt) Lange-Bertalot			X			X		
Ctenophora pulchella (Ralfs ex Kützing) D.M.Williams & Round		X		X		X		X
Encyonopsis minuta Krammer & E.Reichardt		X						
Eolimna minima (Grunow) Lange-Bertalot			X					
Fragilaria fasciculata (C.Agardh) Lange-Bertalot		X		X		X		X
Fragilaria nanana Lange-Bertalot		X						
Gomphonema parvulum Kützing			X					
Halamphora coffeiformis (C.Agardh) Levkov				X		X		X
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski		X						
Navicula adamantiformis Archibald				X		X		X
Nitzschia acidoclinata Lange-Bertalot							X	
Nitzschia archibaldii Lange-Bertalot			X					
Nitzschia etoshensis Cholnoky					X			
Nitzschia frustulum (Kützing) Grunow		X				X		X
Nitzschia gracilis Hantzsch						X		X
Nitzschia lacuum Lange-Bertalot					X			
Nitzschia liebethruthii Rabenhorst					X			
Nitzschia nana Grunow		X						
Stauroneis phoenicenteron (Nitzsch) Ehrenberg			X					
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst	X						X	
Gomphonema auritum A.Braun ex Kützing	X						X	
Gomphonema spec. aff. *stonei* Reichardt ssp.			X					
Pinnularia spec. aff. *tirolensis* (Metzeltin & Krammer) Krammer			X					
Rhopalodia gibberula (Ehrenberg) Otto Müller	X				X			

Table S4a Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1							MMI-2							MMI-3						
	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Diversity																					
Hurlbert's evenness (EHurlbert)	0.1	0.5	0.2	0.5	0.61	0.544	0.38	0.5	0.7	0.2	0.5	2.11	0.035	0.32	0.2	0.5	0.2	0.5	0.26	0.799	0.34
Species richness (S)	8.0	16.0	12.0	19.0	1.67	0.096	0.44	20.0	31.0	12.0	19.0	1.71	0.088	0.36	8.0	11.5	12.0	19.0	2.93	0.003	0.43
Shannon diversity (H)	0.3	1.7	0.7	1.6	0.24	0.808	0.43	1.7	2.5	0.7	1.6	2.07	0.039	0.36	0.7	1.3	0.7	1.6	1.08	0.279	0.38
% dominance	21.4	45.4	26.0	60.4	0.21	0.835	0.42	23.2	26.1	26.0	60.4	2.43	0.015	0.41	33.0	56.1	26.0	60.4	0.48	0.633	0.37
Similarity to reference sites																					
% reference taxa	0.0	7.7	0.0	0.0	3.79	< 0.001	1.61	16.7	23.6	0.0	10.3	3.63	< 0.001	2.12	16.7	19.4	0.0	0.0	3.85	< 0.001	1.55
% tolerant taxa	0.0	0.0	0.0	11.8	3.96	< 0.001	1.11	0.0	0.0	0.0	0.6	2.61	0.009	0.84	0.0	0.0	0.0	10.3	2.91	0.004	1.04
% reference individuals	0.0	1.2	0.0	0.0	3.77	< 0.001	2.15	34.6	40.9	0.0	1.0	3.63	< 0.001	2.27	4.0	38.1	0.0	0.0	3.72	< 0.001	2.05
% tolerant individuals	0.0	0.0	0.0	3.2	3.57	< 0.001	2.02	0.0	0.0	0.0	5.9	2.61	0.009	1.60	0.0	0.0	0.0	2.4	2.91	0.004	1.89
% similarity to reference sites	10.4	22.6	2.8	11.7	3.83	< 0.001	0.48	41.8	55.2	4.1	9.3	3.08	0.002	0.92	30.0	42.0	0.2	4.7	3.37	< 0.001	1.02
% reference taxa found in reference sites that occurred in impaired sites	55.6	81.8	46.7	64.4	2.65	0.008	0.19	69.4	85.4	34.3	59.3	2.52	0.012	0.19	75.0	82.1	12.5	29.6	3.60	< 0.001	0.54
% reference individuals found in reference sites that occurred in impaired sites	53.0	95.8	12.4	84.0	2.72	0.006	0.26	89.4	93.8	12.4	76.6	1.95	0.051	0.30	87.0	95.0	6.6	27.8	3.05	0.002	0.66
No. of distinct reference taxa	0.0	1.0	0.0	0.0	3.73	< 0.001	1.63	5.0	6.5	0.0	2.0	3.63	< 0.001	2.17	2.0	3.0	0.0	0.0	3.79	< 0.001	1.40

Table S4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1							MMI-2							MMI-3						
	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Functional group																					
Mobile % taxa	70.0	94.4	17.1	85.2	2.71	0.007	0.17	91.7	94.7	17.1	85.2	2.39	0.017	0.20	88.2	96.9	17.1	85.2	2.64	0.008	0.19
Mobile % individuals	55.0	98.6	27.0	91.6	2.03	0.042	0.17	92.8	98.3	27.0	91.6	1.22	0.224	0.19	96.6	99.5	27.0	91.6	2.39	0.017	0.18
Pioneer % taxa	0.0	2.8	0.0	3.7	0.39	0.699	1.01	0.0	2.8	0.0	3.7	0.86	0.389	0.99	0.0	0.0	0.0	3.7	1.64	0.101	1.16
Pioneer % individuals	0.0	0.8	0.0	0.6	0.18	0.86	1.68	0.0	5.2	0.0	0.6	0.21	0.838	1.82	0.0	0.0	0.0	0.6	2.10	0.036	2.07
Adnate % taxa	0.0	10.0	0.0	6.9	0.68	0.499	0.59	2.8	6.7	0.0	6.9	0.81	0.417	0.59	10.0	14.6	0.0	6.9	2.32	0.020	0.53
Adnate % individuals	0.0	9.6	0.0	3.8	0.43	0.664	1.47	2.0	4.5	0.0	3.8	0.28	0.776	1.57	9.6	23.7	0.0	3.8	2.10	0.036	1.31
Pad (attached to substrate) % taxa	0.0	0.0	0.0	11.8	3.38	< 0.001	1.04	0.0	4.7	0.0	11.8	2.27	0.023	0.80	0.0	0.0	0.0	11.8	3.27	0.001	1.00
Pad (attached to substrate) % indiv.	0.0	0.0	0.0	8.2	2.96	0.003	1.71	0.0	1.0	0.0	8.2	1.79	0.074	1.46	0.0	0.0	0.0	8.2	3.27	0.001	1.58
Stalk (attached to substrate) % taxa	0.0	20.0	0.0	11.1	1.25	0.211	0.99	19.4	21.1	0.0	11.1	2.27	0.023	0.80	0.0	0.0	0.0	11.1	2.27	0.023	1.06
Stalk (attached to substrate) %indiv.	0.0	9.4	0.0	4.2	0.04	0.972	1.38	8.4	13.9	0.0	4.2	0.89	0.372	1.40	0.0	0.0	0.0	4.2	2.94	0.003	1.75
Colonial % taxa	0.0	5.9	0.0	10.7	0.99	0.321	0.93	0.0	1.9	0.0	10.7	2.03	0.042	0.71	0.0	0.0	0.0	10.7	2.43	0.015	0.78
Colonial % individuals	0.0	0.6	0.0	5.8	2.14	0.032	1.87	0.0	0.7	0.0	5.8	1.79	0.073	1.49	0.0	0.0	0.0	5.8	2.37	0.018	1.56
Non-colonial % taxa	60.0	94.1	81.0	89.3	0.99	0.321	0.10	91.7	98.1	81.0	89.3	2.03	0.042	0.07	90.9	100.0	81.0	89.3	2.43	0.015	0.07
Non-colonial % individuals	34.0	99.4	49.8	94.2	2.14	0.032	0.16	98.6	99.3	49.8	94.2	1.79	0.073	0.13	96.6	100.0	49.8	94.2	2.37	0.018	0.12
Ribbon % taxa	0.0	0.0	0.0	5.9	1.40	0.163	1.08	0.0	1.9	0.0	5.9	1.51	0.131	0.90	0.0	0.0	0.0	5.9	2.73	0.006	1.10
Ribbon % individuals	0.0	0.0	0.0	2.0	2.16	0.031	1.79	0.0	0.5	0.0	2.0	1.39	0.165	1.53	0.0	0.0	0.0	2.0	2.73	0.006	1.67
High profile guild % taxa	0.0	13.9	0.0	13.8	0.04	0.972	0.94	10.0	19.5	0.0	13.8	0.77	0.441	0.61	0.0	0.0	0.0	13.8	3.46	< 0.001	0.94
High profile guild % individuals	0.0	6.0	0.0	4.2	1.01	0.313	1.52	6.6	8.3	0.0	4.2	1.01	0.310	1.05	0.0	0.0	0.0	4.2	3.46	< 0.001	1.45

Table S4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1							MMI-2							MMI-3						
	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Functional group																					
Low profile guild % taxa	0.0	13.6	5.3	17.6	1.53	0.127	0.47	5.6	15.9	5.3	17.6	0.93	0.351	0.41	0.0	10.9	5.3	17.6	1.43	0.152	0.47
Low profile guild % indiv.	0.0	26.0	0.6	34.6	0.10	0.917	0.87	4.4	13.2	0.6	34.6	0.85	0.394	0.91	0.0	23.0	0.6	34.6	0.38	0.703	0.87
Motile guild % taxa	0.0	63.9	35.3	58.8	0.64	0.521	0.36	50.0	62.5	35.3	58.8	0.57	0.570	0.22	75.0	79.2	35.3	58.8	3.37	< 0.001	0.27
Motile guild % individuals	0.0	65.2	4.0	26.8	0.17	0.862	0.73	66.2	70.0	4.0	26.8	1.66	0.096	0.65	13.2	74.6	4.0	26.8	1.40	0.162	0.67
Planktonic guild % taxa	0.0	5.6	0.0	6.7	1.20	0.23	0.80	0.0	5.3	0.0	6.7	1.34	0.180	0.71	0.0	3.2	0.0	6.7	1.21	0.225	0.77
Planktonic guild % individuals	0.0	1.0	0.0	1.2	0.80	0.423	1.79	0.0	1.3	0.0	1.2	0.28	0.776	1.69	0.0	0.5	0.0	1.2	1.31	0.190	1.84
Taxonomic composition																					
% Achnanthidium taxa	0.0	5.6	0.0	6.3	0.40	0.689	0.75	5.0	5.6	0.0	6.3	0.08	0.935	0.70	0.0	0.0	0.0	6.3	1.64	0.102	0.90
% Achnanthidium individuals	0.0	1.4	0.0	1.2	0.14	0.889	1.65	1.4	5.9	0.0	1.2	0.77	0.440	1.77	0.0	0.0	0.0	1.2	2.51	0.012	2.02
% Cocconeis taxa	0.0	0.0	0.0	2.9	1.74	0.081	1.41	0.0	0.0	0.0	2.9	1.31	0.191	1.15	0.0	0.0	0.0	2.9	2.03	0.043	1.32
% Cocconeis individuals	0.0	0.0	0.0	0.2	1.62	0.106	3.53	0.0	0.0	0.0	0.2	1.10	0.271	3.37	0.0	0.0	0.0	0.2	2.03	0.042	3.57
% Craticula taxa	0.0	5.6	0.0	3.7	0.96	0.336	0.99	2.8	4.7	0.0	3.7	0.69	0.487	1.08	8.3	10.9	0.0	3.7	2.63	0.009	0.91
% Craticula individuals	0.0	0.8	0.0	0.4	0.74	0.462	1.51	2.0	5.1	0.0	0.4	2.13	0.034	1.44	0.2	0.7	0.0	0.4	0.74	0.462	1.71
% Ctenophora taxa	0.0	0.0	0.0	2.9	2.90	0.004	1.64	0.0	0.0	0.0	2.9	1.71	0.088	1.22	0.0	0.0	0.0	2.9	2.03	0.042	1.31
% Ctenophora individuals	0.0	0.0	0.0	0.2	2.90	0.004	2.44	0.0	0.0	0.0	0.2	1.71	0.088	1.94	0.0	0.0	0.0	0.2	1.05	0.294	1.59
% Cyclotella taxa	0.0	0.0	0.0	2.9	1.39	0.164	1.18	0.0	1.4	0.0	2.9	0.66	0.507	1.02	0.0	0.0	0.0	2.9	0.72	0.471	1.08
% Cyclotella individuals	0.0	0.0	0.0	0.2	1.03	0.303	2.02	0.0	0.4	0.0	0.2	0.21	0.836	1.85	0.0	0.0	0.0	0.2	1.05	0.294	1.59
% Encyonopsis taxa	0.0	0.0	0.0	3.3	2.90	0.004	1.79	0.0	0.0	0.0	3.3	1.31	0.191	1.28	0.0	0.0	0.0	3.3	2.03	0.043	1.45
% Encyonopsis individuals	0.0	0.0	0.0	0.2	2.90	0.004	3.48	0.0	0.0	0.0	0.2	1.01	0.312	2.81	0.0	0.0	0.0	0.2	2.03	0.042	2.96

Table S4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1							MMI-2							MMI-3						
	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
% Eolimna taxa								2.8	3.3	0.0	0.0	1.90	0.057	1.52							
% Eolimna individuals								1.4	24.4	0.0	0.0	3.20	0.001	2.61							
% Epithemia taxa								0.0	1.4	0.0	0.0	0.53	0.598	1.78							
% Epithemia individuals								0.0	0.1	0.0	0.0	0.66	0.509	3.22							
% Fistulifera taxa															0.0	3.2	0.0	0.0	1.65	0.098	1.91
% Fistulifera individuals															0.0	1.8	0.0	0.0	1.76	0.078	2.34
% Fragilaria taxa								0.0	1.4	0.0	0.0	0.43	0.670	1.46							
% Fragilaria individuals								0.0	0.1	0.0	0.0	0.13	0.898	2.24							
% Gomphonema taxa	0.0	13.9	0.0	3.4	1.36	0.174	1.41	10.0	15.3	0.0	3.4	2.38	0.017	1.21	0.0	0.0	0.0	3.4	2.20	0.028	1.81
% Gomphonema individuals	0.0	2.8	0.0	0.4	1.41	0.158	1.80	5.2	7.8	0.0	0.4	2.95	0.003	1.60	0.0	0.0	0.0	0.4	2.20	0.028	2.14
% Halamphora taxa	0.0	5.0	0.0	4.8	0.66	0.508	0.74	2.8	3.3	0.0	4.8	0.65	0.515	0.75	0.0	7.3	0.0	4.8	1.60	0.110	0.73
% Halamphora individuals	0.0	6.2	0.0	1.6	0.64	0.52	1.61	1.4	4.5	0.0	1.6	0.85	0.393	1.72	0.0	22.8	0.0	1.6	1.50	0.134	1.45
% Mayamaea taxa								0.0	3.3	0.0	0.0	1.94	0.053	2.16	0.0	3.2	0.0	0.0	1.51	0.132	2.14
% Mayamaea individuals								0.0	0.2	0.0	0.0	1.80	0.071	4.07	0.0	0.3	0.0	0.0	1.40	0.163	3.43
% Navicula taxa	0.0	7.7	0.0	10.5	0.80	0.425	0.58	5.0	6.7	0.0	10.5	1.18	0.239	0.55	6.3	12.2	0.0	10.5	1.27	0.203	0.50
% Navicula individuals	0.0	3.0	0.0	1.8	0.28	0.781	1.87	0.8	1.2	0.0	1.8	0.24	0.807	1.90	3.0	5.0	0.0	1.8	1.50	0.135	1.66
% Nitzschia taxa	0.0	30.6	13.3	29.4	0.00	1.000	0.41	19.2	30.6	13.3	29.4	0.41	0.685	0.29	27.3	37.7	13.3	29.4	1.94	0.052	0.33
% Nitzschia individuals	0.0	26.4	1.8	12.8	0.23	0.822	0.99	24.4	32.5	1.8	12.8	1.14	0.256	0.93	6.4	40.6	1.8	12.8	1.31	0.192	0.92
% Pinnularia taxa								0.0	1.4	0.0	0.0	0.90	0.369	1.87							
% Pinnularia individuals								0.0	0.1	0.0	0.0	0.90	0.368	2.38							

Table S4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1							MMI-2							MMI-3						
	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
% Planothidium taxa								0.0	0.2	0.0	0.0	0.62	0.538	1.68							
% Planothidium individuals								0.0	1.4	0.0	0.0	0.35	0.725	1.57							
% Pseudostaurosira taxa								0.0	1.4	0.0	0.0	0.96	0.340	2.05							
% Pseudostaurosira individuals								0.0	0.2	0.0	0.0	0.96	0.340	2.92							
% Rhopalodia taxa															0.0	6.1	0.0	0.0	2.78	0.005	2.17
% Rhopalodia individuals															0.0	0.9	0.0	0.0	2.82	0.005	3.27
% Sellaphora taxa								0.0	1.0	0.0	0.0	1.77	0.076	2.66							
% Sellaphora individuals								0.0	3.9	0.0	0.0	1.34	0.180	1.36							
% Stauroneis taxa								0.0	1.4	0.0	0.0	2.20	0.028	3.35							
% Stauroneis individuals								0.0	0.1	0.0	0.0	2.20	0.028	4.27							

Table S4b Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

	MMI-4				|Z|	P	CV
	Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median			
Diversity							
Hurlbert's evenness (EHurlbert)	0.1	0.4	0.2	0.5	0.89	0.374	0.41
Species richness (S)	9.0	11.0	12.0	19.0	1.64	0.102	0.41
Shannon diversity (H)	0.3	0.9	0.7	1.6	1.10	0.271	0.45
% dominance	21.4	79.8	26.0	60.4	1.17	0.241	0.39
Similarity to reference sites							
% reference taxa	13.6	23.1	0.0	3.4	3.34	< 0.001	1.36
% tolerant taxa	0.0	0.0	0.0	11.8	2.68	0.007	0.93
% reference individuals	24.6	82.4	0.0	0.6	2.83	0.005	1.32
% tolerant individuals	0.0	0.0	0.0	2.6	2.61	0.009	1.88
% similarity to reference sites	24.3	66.4	0.8	3.7	3.02	0.003	1.14
% reference taxa found in reference sites that occurred in impaired sites	55.6	61.5	18.2	31.6	3.31	< 0.001	0.45
% reference individuals found in reference sites that occurred in impaired sites	53.0	95.8	6.0	47.2	2.67	0.008	0.62
No. of distinct reference taxa	2.0	4.0	0.0	1.0	3.31	< 0.001	1.15
Functional group							
Mobile % taxa	70.0	88.5	17.1	85.2	0.50	0.619	0.20
Mobile % individuals	55.0	96.6	27.0	91.6	0.43	0.67	0.20
Pioneer % taxa	3.8	9.1	0.0	3.7	1.75	0.08	0.85
Pioneer % individuals	0.8	79.8	0.0	0.6	2.47	0.014	1.37
Adnate % taxa	0.0	9.1	0.0	6.9	0.50	0.619	0.66
Adnate % individuals	0.0	0.4	0.0	3.8	1.71	0.088	1.71
Pad (attached to substrate) % taxa	0.0	7.7	0.0	11.8	1.25	0.213	0.78
Pad (attached to substrate) % individuals	0.0	3.0	0.0	8.2	0.82	0.413	1.36
Stalk (attached to substrate) % taxa	27.3	30.0	0.0	11.1	3.09	0.002	0.95
Stalk (attached to substrate) % individuals	25.4	94.0	0.0	4.2	2.56	0.01	1.16
Colonial % taxa	9.1	22.2	0.0	10.7	2.45	0.014	0.71

Table S4b (contd.) Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

	MMI-4				|Z|	P	CV
	Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median			
Functional group							
Colonial % individuals	0.6	3.6	0.0	5.8	0.18	0.859	1.54
Non-colonial % taxa	60.0	77.8	81.0	89.3	2.45	0.014	0.10
Non-colonial % individuals	34.0	96.4	49.8	94.2	0.18	0.859	0.18
Ribbon % taxa	0.0	9.1	0.0	5.9	1.50	0.134	0.82
Ribbon % individuals	0.0	0.6	0.0	2.0	0.14	0.886	1.44
High profile guild % taxa	36.4	50.0	0.0	13.8	3.09	0.002	0.77
High profile guild % individuals	1.4	7.8	0.0	4.2	0.89	0.374	1.40
Low profile guild % taxa	3.8	13.6	5.3	17.6	0.71	0.477	0.43
Low profile guild % individuals	0.8	79.8	0.6	34.6	1.39	0.166	0.78
Motile guild % taxa	0.0	30.8	35.3	58.8	2.95	0.003	0.37
Motile guild % individuals	0.0	3.0	4.0	26.8	2.67	0.008	0.90
Planktonic guild % taxa	0.0	7.7	0.0	6.7	0.07	0.943	0.72
Planktonic guild % individuals	0.0	1.6	0.0	1.2	0.07	0.943	1.60
Taxonomic composition							
% Achnanthidium taxa	3.8	9.1	0.0	6.3	0.93	0.355	0.68
% Achnanthidium individuals	0.8	79.8	0.0	1.2	2.38	0.017	1.35
% Cocconeis taxa	0.0	0.0	0.0	2.9	0.11	0.912	1.13
% Cocconeis individuals	0.0	0.0	0.0	0.2	0.04	0.971	2.97
% Craticula taxa	0.0	0.0	0.0	3.7	1.60	0.109	1.36
% Craticula individuals	0.0	0.0	0.0	0.4	1.20	0.229	1.84
% Ctenophora taxa	0.0	0.0	0.0	2.9	1.88	0.06	1.27
% Ctenophora individuals	0.0	0.0	0.0	0.2	1.88	0.06	1.99
% Cyclotella taxa	0.0	0.0	0.0	2.9	1.43	0.152	1.13
% Cyclotella individuals	0.0	0.0	0.0	0.2	1.10	0.27	1.57
% Encyonema taxa	0.0	3.8	0.0	0.0	2.15	0.031	2.28

Table S4b (contd.) Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

	MMI-4				|Z|	P	CV
	Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median			
Taxonomic composition							
% Encyonema individuals	0.0	0.2	0.0	0.0	2.15	0.031	4.59
% Encyonopsis taxa	0.0	0.0	0.0	3.3	1.88	0.06	1.40
% Encyonopsis individuals	0.0	0.0	0.0	0.2	1.88	0.06	2.89
% Eunotia taxa	0.0	4.5	0.0	0.0	2.15	0.031	2.10
% Eunotia individuals	0.0	0.2	0.0	0.0	1.90	0.058	3.20
% Gomphonema taxa	18.2	22.7	0.0	3.4	3.05	0.002	1.36
% Gomphonema individuals	0.8	4.8	0.0	0.4	2.66	0.008	1.99
% Halamphora taxa	0.0	4.5	0.0	4.8	0.14	0.886	0.82
% Halamphora individuals	0.0	0.4	0.0	1.6	1.11	0.267	1.89
% Navicula taxa	0.0	4.5	0.0	10.5	2.03	0.043	0.65
% Navicula individuals	0.0	0.2	0.0	1.8	2.03	0.042	2.06
% Nitzschia taxa	0.0	18.2	13.3	29.4	2.52	0.012	0.41
% Nitzschia individuals	0.0	2.0	1.8	12.8	2.92	0.004	1.24

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