

Taxonomy and ecology of epilithic diatoms in Irish lakes

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DECLARATION

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SUMMARY

Benthic algae are significant primary producers and important in biogeochemical cycling in shallow lakes in particular, but epilithic diatom ecology is poorly understood compared to phytoplankton and higher trophic levels. I explored diatom taxonomy and ecology to investigate the suitability of epilithic diatoms for determining the biological condition of Irish lakes. I examined intraspecific variation in populations of two widespread diatom genera to understand the implication of diatom species-complexes for biodiversity and environmental assessments.

Diatom composition in 220 samples from the epilithon of 91 limnologically diverse reference and impacted lakes was analysed, with a replicated spring and summer analysis in my study. Multivariate techniques enabled six characteristic groups of diatom assemblages and their relationship with key abiotic environmental variables to be identified. It revealed that physicochemical gradients of alkalinity-pH, total phosphorus and true colour explained the greatest amount of variation in diatom assemblages in both seasons.

Lake classifications are successful when diatom metrics reliably reflect the response of assemblages to environmental stressor gradients. The Lake Trophic Diatom Index was validated for Water Framework Directive classifications after reference condition was assessed and a highly significant relationship with total phosphorus was identified. This represents an improvement on a previous study that found diatom indices to be poor indicators of trophic condition in Irish lakes. A key finding relative to comparable international studies was that the diatom-phosphorus relationship was not confounded by factors such as lake type. The clearest seasonal pattern that emerged was a consistently low variability in index scores in high status lakes, with variability

increasing with declining trophic condition thereafter. Nevertheless, averaging seasonal metric scores provided broadly equivalent levels of explained variation relative to that reported for other phototrophic indices.

The distribution, morphology and ultrastructure of *Brachysira* and *Encyonopsis* in a large number of river and lake populations was assessed. Examination of original type material for morphologically challenging *Encyonopsis* provided new information and taxonomic uncertainty in *E. angusta* was resolved by a proposed synonymy with *E. krammeri*. Four new diatom species were described, and greater morphological diversity was discovered. Further validation of nomenclatural types and molecular characterisation is required before many cosmopolitan morphospecies can be formally named. My analyses provided support for the concept of taxonomic redundancy within diatom complexes given the adequate performance of the diatom metric and comparability of species trophic optima when assessed with weighted averaging. However, I also found evidence for differences in ecological niches amongst new and established species that suggested potential for improvements in precision in environmental indices are also achievable.

My study provided a rare example of a contemporary taxonomic investigation for a group of freshwater algae in Irish waters. The broader implication of my findings is that algal diversity in Ireland is actually quite poorly documented. As such, my research provides further opportunities to understand patterns of diatom diversity in the near future. The final chapter of this thesis summarises and discusses the role of diatoms and benthic algal indicators for lake phytobenthos classifications, with suggestions for future research areas.

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ABBREVIATIONS

ANOVA – Analysis of Variance

CCA – Canonical Correspondence Analysis

DI-TP – Diatom inferred total phosphorus

EQR – Ecological Quality Ratio

IndVal – Indicator Values according to Dufrêne and Legendre (1998)

LM – Light microscopy

LTDI – Lake Trophic Diatom Index

pCCA – partial Canonical Correspondence Analysis

SEM – Scanning electron microscopy

TCD – Trinity College Dublin

WA – Weighted average

WFD – Water Framework Directive

TERMS

Acidobiontic – occurs at pH ≤ 5.5

Acidophilous – occurs at pH ~ 7 with a wider distribution at pH < 7

Alkalibiontic – occurs at a pH > 7

Alkaliphilous – occurs at a pH ~ 7 with a wider distribution at pH > 7

Cryptic species - defined as two or more distinct species that were classified as a single one due to morphological similarity.

Epilithon – represents the community of organisms growing on hard surfaces in the phytobenthos.

Eulittoral – as defined in Cantonati & Lowe (2014) representing a more specific region in the littoral zone where diatom sampling was undertaken.

Holotype - single specimen designated as the type in the original publication.

Intraspecific: Variation occurring between individuals of the same species.

Isotype – any duplicate of the holotype.

Littoral – shoreline area extending to a depth at which 1% of incoming surface light remains.

Morphodeme - a population of organisms characterized by a shared morphology.

Morphotype - any of a group of different types of individuals of the same species in a population.

Nomenclature - a set of formal rules typically accepted to govern the naming of species and groups of species.

Phytobenthos – operational name to represent all of the photosynthesising microbes and algae in the littoral zone predominately.

Pseudocryptic species – a more controversial concept that defines species that are challenging but not impossible to separate morphologically.

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TABLE OF CONTENTS

<i>DECLARATION</i>	<i>ii</i>
<i>SUMMARY</i>	<i>iii</i>
<i>ACKNOWLEDGEMENTS</i>	<i>v</i>
<i>ABBREVIATIONS</i>	<i>vi</i>
<i>TERMS</i>	<i>vii</i>
<i>TABLE OF CONTENTS</i>	<i>viii</i>
<i>LIST OF FIGURES</i>	<i>xiii</i>
<i>LIST OF TABLES</i>	<i>xxi</i>
Chapter 1 - Introduction	1
1.1. OVERVIEW	1
1.1.1. WHAT ARE DIATOMS?	1
1.1.2. DIATOM-ENVIRONMENTAL RELATIONSHIPS	3
1.1.3. DIATOM MONITORING AND THE WATER FRAMEWORK DIRECTIVE (WFD)	5
1.1.4. DIATOM CLASSIFICATION IN IRELAND	11
1.2. RESEARCH OBJECTIVES	14
1.3. THESIS CONTRIBUTION	15
Chapter 2 - Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish lakes	18
2.1. ABSTRACT	18
2.2. INTRODUCTION	19
2.3. MATERIALS AND METHODS	24
2.3.1. STUDY LAKES AND DIATOM SAMPLING	24
2.3.2. ENVIRONMENTAL DATA	25
2.3.3. DIATOM IDENTIFICATION	28

2.3.4.	STATISTICAL ANALYSIS.....	28
2.4.	RESULTS.....	31
2.4.1.	PHYSICOCHEMICAL AND ENVIRONMENTAL GRADIENTS	31
2.4.2.	EPILITHIC DIATOM ASSEMBLAGES.....	38
2.4.3.	DIATOM COMPOSITIONAL GROUPS	40
2.4.4.	DIATOM ENVIRONMENT RELATIONSHIPS	41
2.4.5.	WFD CLASSIFICATION	45
2.4.6.	DIATOM CLASSIFICATION & ECOLOGICAL QUALITY RATIO (EQR).....	48
2.5.	DISCUSSION	53
2.5.1.	OVERVIEW	53
2.5.2.	LAKE ENVIRONMENTAL VARIATION.....	53
2.5.3.	DIATOM COMPOSITION	54
2.5.4.	DIATOM ENVIRONMENT RESPONSE.....	59
2.5.5.	LAKE REFERENCE CONDITIONS.....	60
2.5.6.	DIATOM METRIC CLASSIFICATION	62
2.5.7.	FUTURE DEVELOPMENTS	65
2.5.8.	CONCLUSIONS	67
2.6.	ACKNOWLEDGEMENTS.....	68
2.7.	REFERENCES.....	69
	APPENDIX A.....	89
	APPENDIX B.....	124
	APPENDIX C.....	136
	APPENDIX D	137
	APPENDIX E	138
	APPENDIX F	141
	APPENDIX G	144
	APPENDIX H	147

APPENDIX I.....	149

Chapter 3 - A review of the genus *Brachysira* in Ireland with the description of *Brachysira praegeri* and *Brachysira conamarae*, new

raphid diatoms (Bacillariophyceae) from high status waterbodies 150

3.1. ABSTRACT	150
3.2. INTRODUCTION	151
3.3. METHODOLOGY	153
3.3.1. MATERIALS AND METHODS	153
3.4. RESULTS	159
3.4.1. <i>BRACHYSIRA</i> DISTRIBUTION.....	159
3.4.2. DIATOM MORPHOLOGY	164
3.4.3. DIATOM MORPHOLOGY	179
3.5. DISCUSSION.....	196
3.6. ACKNOWLEDGEMENTS	201
3.7. REFERENCES.....	202

Chapter 4 - Taxonomy, ecology and analysis of type material of some small *Encyonopsis* with description of new species in Ireland..... 212

4.1. ABSTRACT	212
4.2. INTRODUCTION	213
4.3. MATERIALS AND METHODS.....	216
4.3.1. COLLECTION AND PREPARATION OF SAMPLES.....	216
4.3.2. MICROSCOPIC EXAMINATION.....	216
4.3.3. MORPHOLOGICAL ANALYSIS.....	219
4.3.4. ENVIRONMENTAL ANALYSIS.....	219
4.3.5. TAXON DESCRIPTION & TYPE SPECIMENS	220

4.4.	RESULTS	221
4.4.1.	TAXONOMIC RESULTS	233
4.5.	DISCUSSION	274
4.6.	ACKNOWLEDGEMENTS	281
4.7.	REFERENCES.....	283

Chapter 5 - Conclusions and Recommendations 291

5.1.	OVERVIEW	291
5.2.	MAIN CONCLUSIONS.....	293
5.3.	DISCUSSION	297
5.3.1.	DIATOM-ENVIRONMENT RELATIONSHIP	297
5.3.2.	DIATOM TAXONOMIC RESOLUTION AND INDICATOR VALUE	303
5.4.	RECOMMENDATIONS	311
	APPENDIX J.....	314

REFERENCES 315

LIST OF FIGURES

Figure 1.1 Epilithic diatoms were sampled in a shallow tray with a toothbrush by scrubbing the top surface of five cobble to boulder sized stones.....	7
Figure 1.2 Distribution in Ireland of the 91 core study lakes where diatom sampling was done.....	8
Figure 1.3 Lake types and periphyton communities varied across the network of sampled waterbodies. Calcifying marl lakes in karstic and limestone regions were sampled (i-ii) and these often had thick calcareous deposits of cyanobacteria. Nevertheless, diatoms were well represented on the top surfaces (iii) while in nutrient impacted lakes <i>Cladophora glomerata</i> was rarely as extensive as noted here (iv).....	9
Figure 1.4 Low and moderate alkalinity littoral zones also varied in condition (i-iv) with some exposed shorelines with deep photic zones (ii) contrasting with littoral areas where sedimentation (iii) or opportunistic Zygnematales cover was high, but diatoms were sufficiently abundant in all of these habitats.....	10
Figure 2.1 Geographic location of the 91 study lakes in Ireland.....	27
Figure 2.2 Mean and 95% confidence interval of lake physicochemical variables for the three a priori lake types in spring (sp) and summer (su) seasons, with results of Bonferroni corrected tests of significance between lake type, season, and their interaction indicated where significant.....	37
Figure 2.3 Principal Component Analysis (PCA) ordination of lake physicochemistry in the 91 study lakes, displaying PCA axes 1-2 with physicochemical variables displayed as black arrows and significant supplementary variables represented passively in the ordination as grey arrows. Codes and abbreviations used for all variables are provided in Table1-2 and a biplot of PCA axes 1-3 is provided in Appendix 3, Fig. 3.1.....	38
Figure 2.4 Canonical Correlation Analysis (CCA) triplots for spring and summer seasons showing the distribution of lakes along the first two axes based on the relationships between diatom taxa and significant environmental variables. Groups resulting from cluster analysis are fitted to convex hulls and lakes coloured with blue or red circles identify candidate reference lakes discussed in the text.....	44
Figure 2.5 Scatterplot of the Lake Trophic Diatom Index (LTDI) for the compiled candidate reference lakes. Grey points represent LTDI scores and the series is ordered by increasing median LTDI value identified with darker circles. Lakes are identified as low (LA), moderate (MA) and high (HA) alkalinity. Where results of paleolimnology are available then reference condition when validated is indicated with a (Y) or alternatively with (N). A vertical line at	

LTDI = 20 indicates the expected value of the LTDI used for WFD classification. The blue vertical line at LTDI = 28 indicates the position of the high-good status class boundary. Three lakes where reference condition is in doubt are indicated in red.....47

Figure 2.6 Relationships between mean lake total phosphorus concentrations and Ecological Quality Ratio (EQR) with 95% confidence limits for low (LA), moderate (MA) and high (HA) alkalinity lake types in spring and summer. Outlier lakes are identified.....51

Figure 2.7 Regression of spring and summer LTDI metric values with fitted confidence (grey) and prediction (red) intervals, and a one to one line (black) for comparison. Candidate reference lakes are indicated in dark blue and red.....52

Figure C.1 PCA plots of axis-1 and axis-2 of 91 studied lakes with lakes grouped by Low (LA), Moderate (MA) and High (HA) alkalinity types in convex hulls along PC-1 and categorised by total phosphorus indicating a trophic gradient along PC-2. Labelled samples refer to candidate reference lakes or waterbodies discussed in the text.....136

Figure C.2 PCA plots of axis-1 and axis-3 of 91 studied lakes with physiochemical parameters displayed as black arrows and significant supplementary variables represented passively in the ordination as grey arrows.....136

Figure D.1 Correlation matrix of explanatory variables with significant Pearson correlations ($p < 0.05$) coloured blue (positive) or red (negative) and circles filled conditional on the strength of the association between the variables. The meaning of environmental codes is explained in the introduction and in Appendix 1.....137

Figure E.1 Non-hierarchical Clustering (PAM) of Diatom Community (Bray-Curtis) 6-Cluster.....138

Figure E.2 Non-hierarchical Clustering (PAM) of Diatom Community (Bray-Curtis) 7-Cluster.....139

Figure E.3 Partitioning around Medoids (PAM) of diatom community (Bray-Curtis) 6-Cluster.....140

Figure E.4 Partitioning around Medoids (PAM) of diatom community (Bray-Curtis) 7-Cluster.....140

Figure G.1 The distribution of physicochemical and landuse variables amongst the six clusters. The inner line is the median, the box margins represent the 25th and 75th percentile with bars extending to the 5th and 95th percentiles. Where notches overlap between boxes then medians are assumed to be statistically non-significant ($p < 0.05$). 145

Figure G.2 The distribution of diatom diversity and LTDI metric scores in the six clusters. The inner line is the median, the box margins represent the 25th and 75th percentile with

bars extending to the 5th and 95th percentiles. Where notches overlap between boxes then medians are assumed to be statistically non-significant ($p < 0.05$)	146
Figure H.1 Relationships of Ecological Quality Ratio and total phosphorus in low (LA), moderate (MA) and high (HA) alkalinity lakes in spring for lakes with a restricted range of total phosphorus ≤ 0.05 mg/L-P.....	147
Figure H.2 Relationships of Ecological Quality Ratio and total phosphorus in low (LA), moderate (MA) and high (HA) alkalinity lakes in summer for lakes with a restricted range of total phosphorus ≤ 0.05 mg/L-P.....	148
Figure I.1 Histograms showing the distribution of Ecological Quality Ratios (EQR) for epilithic diatom samples for three lake alkalinity types based on an expected value of the LTDI = 20.....	149
Figure 3.1 Ireland. (a) Map showing the location of the sampling sites and the distribution of the primary acidic bedrock geology. The regions and lakes indicated are referred to in the text. (b) <i>Brachysira</i> species as % of all diatom species at sites. (c) number of <i>Brachysira</i> species at sites. Light grey refers to river sites and dark grey refers to lakes in (b) and (c). Rivers and lakes are represented by light grey and grey dots, respectively.	160
Figure 3.2 The distribution of the more abundant <i>Brachysira</i> taxa in Irish lakes and rivers. Rivers and lakes are represented by light grey and grey dots, respectively.	162
Figures 3.3-3.24 <i>Brachysira praegeri</i> sp. nov. Figs 3–18. LM. Fig. 8. Holotype specimen. Figs 7–10. Range of valves from the holotype population (TCD0018137) L. Nambrackmore. Figs 3–6, 11–17. Valves showing morphological range and size variation in other populations. Fig. 18. A teratological valve giving the abnormal impression of rostrate ends. Figs 19–24. SEM. Fig. 19. External view. Fig. 20. Internal view. Figs 21–24. Variation in valve outline and occurrence of papillae in the central area. Scale bar in Fig. 18 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm	169
Figures 3.25 - 3.33 <i>Brachysira conamarae</i> sp. nov. Figs 25–26. LM. Fig. 25. Holotype specimen (TCD0018138). Figs 27–33. SEM. Fig. 27. External view of an elongate valve with slightly more prominent axial area. Figs 28–30. Variation in valve morphology apparent for the holotype population with varying pattern of occurrence of papillae over the valve face. Fig. 31. Oblique valve in tilt illustrating ultrastructural features. Fig. 32. Detail of the centre of the frustule with papillae over the entire valve face. Fig. 33. Detail of the valve end and T-shape distal raphe endings. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm	178

Figures 3.34–3.43 <i>Brachysira styriaca</i> and <i>Brachysira zellensis</i> . Figs 34–40. <i>B. styriaca</i> , LM. Fig. 41. <i>B. styriaca</i> , SEM. Fig. 42. <i>B. zellensis</i> , LM. Fig. 43. <i>B. zellensis</i> , SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	180
Figures 3.44–3.51 <i>Brachysira liliana</i> . Figs 44–47. LM. Figs 48–51. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	180
Figures 3.52–3.60 <i>Brachysira vitrea</i> . Figs 52–56. LM. Figs 57–60. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.	181
Figures 3.61–3.80 <i>Brachysira neglectissima</i> . Figs 61–70. LM. Figs 71–80. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	183
Figures 3.81–3.98 <i>Brachysira brebissonii</i> and <i>Brachysira intermedia</i> . Figs 81–91. <i>B. brebissonii</i> , LM. Figs 92–94. <i>B. intermedia</i> , LM. Fig. 95. SEM, <i>B. intermedia</i> . Figs 96–98. SEM, <i>B. brebissonii</i> . Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	185
Figures 3.99–3.110 <i>Brachysira</i> sp. (aff. <i>B. intermedia</i>). Figs 99–105. LM. Figs 106–110. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	186
Figures 3.111–3.135 <i>Brachysira garrensis</i> . Figs 111–130 LM. Fig. 135–SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.	188
Figures 3.136–3.172 <i>Brachysira microcephala</i> morphotype BMIC-1. Figs 136–165. LM. Figs 166–172. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	190
Figures 3.173–3.205 <i>Brachysira microcephala</i> morphotype BMIC-2. Figs 173–197. LM. Figs. 198–205. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	191
Figures 3.206–3.239 <i>Brachysira microcephala</i> morphotype BMIC-3. Figs 206–229. LM. Figs 230–239. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	193
Figures 3.240–3.252 <i>Brachysira microcephala</i> morphotype BMIC-4. Figs 240–246. LM. Figs 247–252. SEM. Scale bar = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	195
Figure 4.1 Ireland. Map illustrating locations where <i>Encyonopsis</i> was present (+) or absent (-) at ≥ 1% abundance in lake and rivers. Named waterbodies refer to the location of type lakes or rivers referred to in the text.	218
Figure 4.2 SEM of <i>Encyonopsis</i> external valve view indicating location where measurement of valve length (a) valve width (b) and counting of dorsal (c) and ventral (d) striae and areolae density (e) was determined.....	219

Figures 4.3–4.27 <i>Encyonopsis carraensis</i> sp. nov. Figs 3–20. LM. Fig. 11. Holotype specimen. Figs 3–20. Valves showing morphological range and size variation in populations. Figs 21–27. SEM. Fig. 21. External view. Figs 22–23. Internal view. Figs 24–27. Variation in valve outline. Scale bar in Fig. 20 = 10 µm for all LM figures. Scale bar in all SEM micrographs = 1 µm.....	237
Figure 4.28 Lough Carra, Co. Mayo. Shoreline at Pleasure Island (a) the type locality of <i>Encyonopsis carraensis</i> with an extensive deposit of submerged (b) and exposed (c) marl precipitating cyanobacteria over boulders.....	239
Figures 4.29–4.71 <i>Encyonopsis hibernica</i> sp. nov. Figs 29–60. LM. Fig. 44. Holotype specimen. Figs 29–60. Valves showing morphological range and size variation in several populations. Figs 61–71. SEM. Fig. 61. External view. Fig. 62. Internal view. Figs 63–71. Variation in valve outline. Scale bar in Fig. 60 = 10 µm for all LM figures except holotype specimen. Scale bar in all SEM micrographs = 1 µm and = 2 µm in Figs 65–70.....	241
Figure 4.72 Lough Inchicronan, Co. Clare (a) the type locality of <i>Encyonopsis hibernica</i> showing the shallow littoral (b) and closeup of epilithic habitat (c) with associated macroscopic colonies of <i>Chaetophora</i> F.Schrank and <i>Dichothrix</i> G.Zanardini ex É.Bornet & C.Flahault, also with some minor calcification.....	243
Figures 4.73–4.98 <i>Encyonopsis alpina</i> Krammer & Lange-Bertalot Typus: Prap. 5b IOK. Individuals from type material Typus: Prap. 5b IOK. Figs 73–94. LM. Figs 73–94. Valves showing morphological variability and variation in size in type population. Figs 95–98. SEM. Fig. 95. External view. Fig. 96. External view of tilted frustule illustrating the arrangement of the intact cingulum, composed of two bands, with copulae tapered near the middle and having a single visible row of pores. Figs 98. Internal view. Scale bar in Fig. 94 = 10 µm for all LM figures. Scale bar in all SEM micrographs = 2 µm.....	244
Figures 4.99–4.138 <i>Encyonopsis alpina</i> Krammer & Lange-Bertalot. Individuals from Ireland. Figs 99–131. LM. Figs 99–131. Valves showing morphological and variability in size for populations from Irish lakes. Figs 132–138. SEM. Figs 132–135. External view. Figs 136–138. Internal view. Scale bar in Fig. 131 = 10 µm for all LM figures. Scale bar in all SEM micrographs = 1 µm.....	245
Figures 4.139–4.177 <i>Encyonopsis</i> Typus: Prap. 77A IOK. Figs 139–177. LM. Figs 139–151. <i>E. angusta</i> Krammer & Lange-Bertalot. Figs 152–173. <i>Encyonopsis krammeri</i> Reichardt. Figs 174–177. <i>E. alpina</i> Krammer & Lange-Bertalot. Scale bar in Fig. 151 = 10 µm for all LM figures.....	247

Figures 4.178–4.200 *Encyonopsis tavorana* Krammer Typus: Prap. 517B IOK. Figs 178–192. LM. Figs 178–192. Valves showing range of morphological variability and variation in type population. Figs 193–200. SEM. Fig. 193. External view. Fig. 194. Internal view. Figs 195–199. SEM showing variation in outline and apices in type population. Fig. 200. Close up of valve at pole showing form of distal raphe ending and shape of areolae. Scale bar in Fig. 189 = 10 μ m for LM figs 178–189 and in Fig. 192 = 10 μ m for LM figs 190–192. Scale bar in all SEM micrographs = 1 μ m.....251

Figures 4.201–4.245 *Encyonopsis* aff. *tavorana*. Figs 201–236. LM. Figs 201–236. Valves showing morphological variability for a size diminution series. Figs 237–245. SEM. Figs 237 & 239–243. External view. Figs 238 & 244–245. Internal view. Scale bar in Fig. 236 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....252

Figures 4.246–4.287 *Encyonopsis* sp. 1 Figs 246–287. LM. Figs 246–281. Valves showing morphological range and size variation in populations. Figs 282–287. SEM. Figs 282–285. External view. Figs 286–287. Internal view. Scale bar in Fig. 257 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....253

Figures 4.288–4.319 *Encyonopsis minuta* Krammer & Reichardt Figs 288–319. LM. Figs 288–311. Valves showing morphological range and size variation in populations with clearly capitate apices. Figs 312–319. SEM. Figs 312–315. External view. Figs 316–318. Internal view. Fig. 319. Internal view of raphe fissure with clear intermissio visible. Scale bar in Fig. 311 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....255

Figures 4.320–4.352 *Encyonopsis minuta* Krammer & Reichardt Figs 320–345. LM. Figs 320–345. Illustrating morphology of populations with a size reduction series. Figs 346–352. SEM. Figs 346–350. External view with valves maintaining bluntly rounded poles. Figs 351–352. Internal view showing distinctive intermissio at central area. Scale bar in Fig. 345 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....256

Figures 4.353–4.396 *Encyonopsis* sp. 2 aff. *minuta* Figs 353–385. LM. Figs 353–385. Valves showing morphological range and size reduction in populations. Figs 386–396. SEM. Figs 386–395. External valve view with narrow rostrate apices. Fig 396. Internal view lacking a clear intermission at central area. Scale bar in Fig. 385 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....260

Figures 4.397–4.404 *Encyonopsis horticola* Van de Vijver & Compère Figs 397–402. LM. Figs 397–402. Valves showing morphological range of a single population in L. Gur, Co. Limerick. Figs 403–404. SEM. Fig 403. External view. Fig. 404. Internal view. Scale bar in Fig. 402 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.....261

Figures 4.405–4.416 *Encyonopsis* sp. 3 Figs 405–412. LM. Figs 405–412. Valves showing morphological range and size variation. Figs 413–416. SEM. Figs 413 & 415. External view. Figs 414 & 416. Internal view. Scale bar in Fig. 412 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm 262

Figures 4.417–4.448 *Encyonopsis krammeri* Reichardt. Figs 417–448. LM. Figs 417–440. Valves showing morphological range and size reduction series in Irish lakes. Figs 441–448. SEM. Figs 441 & 443–446. External view. Figs 442 & 447. Internal view. Fig. 448 Girdle view. Scale bar in Fig. 440 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm ...263

Figures 4.449–4.480 *Encyonopsis krammeri* Reichardt. Figs 449–472. LM. Figs 449–454. Valves illustrating the dorsiventral form and narrow rostrate poles for the most commonly encountered form. Figs 455–460. A population with more protracted subcapitate poles. Figs 461–472. A more lanceolate form corresponding to *E. angusta* Krammer & Lange-Bertalot with more clearly capitate rounded ends. Figs 473–480. SEM. Figs 473–478. External view of frustules with differing outline and apices but with equivalent ultrastructural morphology. Figs 479–480. Internal view with thickened silica between helictoglossa and end of apices. Scale bar in Fig. 472 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm ...264

Figures 4.481–4.510 *Encyonopsis neerlandica* Van de Vijver, Verweij, Van der Wal & Mertens. Figs 481–501. LM. Figs 481–501. Valves showing morphological range and size reduction series in populations from Irish lakes. Figs 502–510. SEM. Figs 502 & 504–506. External view showing variation in size and shape of apices. Figs 507–510. Internal view. Figs 507–509. Variation in valve outline and shape of apices. Fig. 510. Detail of valve apex and helictoglossa. Scale bar in Fig. 490 = 10 μm for LM figures 481–490. Scale bar in Fig. 501 = 10 μm for LM figures 491–501. Scale bar in all SEM micrographs = 1 μm 265

Figures 4.511–4.529 *Encyonopsis subminuta* agg. Figs 511–522. Valves showing morphological range and size variation in populations from Irish lakes. Figs 523–529. SEM. Figs 523–527. External and internal view of *Encyonopsis subminuta* Krammer & Reichardt. Fig. 528–529. Internal and external of *E. aff. subminuta*. Scale bar in Fig. 522 = 10 μm for LM figures. Scale bar in all SEM micrographs = 1 μm 267

Figure 4.530 Box plots of the distribution of valve characters measured with SEM for *Encyonopsis*. Only diatom frustules having data for all measured characters is plotted. Boxes represent the 25–75% quartiles (excluding outliers) with the median shown as a horizontal bold line. Whiskers represent the upper and lower data points less than 1.5 times the box height and outliers are individual points outside the whiskers. A. Boxplot comparing the distribution of valve length data. B. Comparison of valve width. C. Comparison of valve length to breadth ratio. D. Comparison of ventral stria density. All data are measured using

populations of *Encyonopsis* from Ireland unless otherwise stated. *E. neerlandica* (ENER), *E. carraensis* (ECAR), *E. subminuta* (ESUB), *E. krammeri* (EKRA), *E. krammeri* capitata form in *E. alpina* type material (EKRA-alp), *E. tavirana* type material (ETAV-typ), *E. aff. tavirana* Irish populations (ETAV-IE), *Encyonopsis* sp. 1 (ESP1), *E. alpina* type material (EALP-typ), *E. alpina* Irish populations (EALP-IE), *E. minuta* (EMIN), *Encyonopsis* sp. 2 (ESP2), *E. horticola* (EHRT), *E. hibernica* (EHIB), *Encyonopsis* sp. 3 (ESP3).....269

Figure 4.531 Comparison of valve morphometry for *Encyonopsis* taxa. A. PCA plot showing positions of *Encyonopsis* taxa in relation to first and second principal component axes. B. Data fitted to convex hulls with the morphological variables measured in SEM displayed as arrows (inset). Taxon codes are equivalent to Figure 4.530.....270

Figure 4.532 Scatter plots of the distribution of *Encyonopsis* taxa in relation to A. Total phosphorus (TP) and B. Alkalinity. Vertical lines indicate the location of optima derived from weighted averages of species occurrences for TP (red) and alkalinity (blue). Taxon codes correspond to Fig. 530, with All (ENCY All) and Unidentified (ENCY unid.) *Encyonopsis* categories illustrated for comparison.....271

Figure 4.533 NMDS biplot of Bray-Curtis dissimilarity matrix of untransformed relative abundance of *Encyonopsis* in lake periphyton samples. Inset is a plot of lakes with significant environmental factors ($p < 0.05$) fitted *a posteriori*, including true colour (TC), chlorophyll a (chl a), total phosphorus (TP), total oxidised nitrogen (TON), silica (Si), total ammonia (NH₄), conductivity (cond), alkalinity (alk) and pH. Main plot shows the location of *Encyonopsis* species added as weighted averages of site scores with surface contour plots for TP (red curves) and Alkalinity (blue curves) gradients fitted *a posteriori*.....273

Figure J.1 Appendix 10 Light and scanning electron microscope images of unidentified diatoms from the survey of Irish lake epilithon sample (i-iv). *Naviculadicta* or *Nupela* sp. (i). *Amphora* sp. (ii). *Delicatophycus* sp. (iii). *Achnantheidium* sp. (iv).....314

LIST OF TABLES

Table 2.1 The range and mean (parenthesis) physicochemistry of the 91 studied lakes with seasonal data for low (LA), moderate (MA) and high alkalinity (HA) lake types where available.....	33
Table 2.2 Supplementary physical, spatial and catchment landuse variables with variable codes and the range and mean (parenthesis) provided in low (LA), moderate (MA) and high (HA) alkalinity lakes. The mean and range for reference and best-available lakes (Candidate-Reference) is also provided.....	35
Table 2.3 Results of partial canonical correspondence analysis (pCCA) comparing the pure and shared effect of two groups of environmental variables (physicochemical versus physical-spatial defined in the methods) that were found to be significant in the spring and summer ordinations illustrated in figure 2.4	43
Table 2.4 Results of regression analysis quantifying the strength and significance of the relationships between total phosphorus (TP) and Lake Trophic Diatom Index EQRs in all lakes (ALL) and the low (LA), moderate (MA) and high (HA) alkalinity lake groups in spring (SP) and summer (SU). Values in parenthesis are statistics for LA and MA lakes with potential outlier lakes removed prior to model fitting.....	50
Appendix A.1 Lake average seasonal physicochemical variables and diatom metric scores.....	89
Appendix A.2 Supplementary physical, spatial and morphological variables.....	110
Appendix A.3 CORINE catchment landcover in the study lakes.....	117
Appendix B A list of all common diatom taxa with abundance and indicator categories identified.....	124
Table 3.1 Comparison of morphometric data and selected features of relevance in published accounts for some of the more closely related <i>Brachysira</i> taxa referred to in the text.....	156
Table 3.2 Comparison of morphometric features of the common <i>Brachysira</i> in Ireland including new species observed during this study. Mean values are given in parenthesis under the range. *Dist.PRE indicates the distance between the proximal raphe endings.....	170
Table 3.3 The number of occurrences, maximum relative abundance, environmental optima and tolerance (pH) and range for some parameters for common <i>Brachysira</i> taxa (for samples >1.0% abundance) determined in this study.....	173

Table 4.1 Comparison of the morphology of type material and other comparable *Encyonopsis* taxa. Numbers in parenthesis adjacent to stria density indicate occasional extreme values and represent averages for valve length and width. The number in parenthesis in the comments indicates the number of SEM images measured. Estimates provided for dorsal stria density precedes ventral stria density. Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).....222

Table 4.2 The number of lake occurrences, maximum percentage abundance in diatom samples, range and weighted average optima (in parentheses) for physiochemical variable for all common *Encyonopsis* taxa in the study.....235

CHAPTER 1

Introduction

1.1. OVERVIEW

This thesis proposes to increase knowledge of diatom diversity in Irish waters. It also has an applied focus, to investigate the potential indicator value of epilithic diatoms for lake ecological status classifications in Ireland. A further emphasis is to provide a contemporary taxonomic evaluation of two widespread diatom indicator taxa. This introduction provides general context to the study with more specific detail given in the introduction of the individual chapters. It summarises the most relevant ecological and taxonomic issues in an environmental monitoring context before a brief outline of the chapters and research objectives is provided.

1.1.1. WHAT ARE DIATOMS?

Diatoms are a species rich monophyletic group of eukaryotic, unicellular heterokont algae that are predominately autotrophic. They are formally classified in the Division Chrysophyta, Class Bacillariophyceae, in the kingdom Chromista. Alternative classifications for lower level taxonomic organisation are available but an established system based on the classification of Round et al. divides diatoms into three classes; the Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid diatoms) and Bacillariophyceae (Round et al., 1990). Planktonic centric diatoms are not considered in my study and it is principally diatoms in epilithic habitats, or hard surfaces, that is the focus of this research.

Several species concepts have been applied to diatoms and there are varied estimates of the number of taxa that are likely to exist as a consequence (Guiry, 2012; Mann and Vanormelingen, 2013; Medlin, 2018). Despite differences of opinion in the actual diversity, diatoms are reported to be the most species rich group of algae and is the basis for their use as ecological indicators in aquatic ecosystems for at least one hundred years (Mann, 1999; Stevenson et al., 2010).

Broad concepts were a feature of the earliest systems at the end of the 19th century in Grunow and later by Hustedt (1932-1933), Cleve-Euler (1953) and Patrick and Reimer (1966). Krammer and Lange-Bertalot's monographs "*Süßwasserflora von Mitteleuropa*" were the mainstay of all global diatom identifications for many decades and are still relied on today for diatom identifications. Narrower sometimes informal categories such as "Sippen" were used to indicate when complexes of taxa were present. A range of infraspecific taxonomic levels such as subspecies, varieties and formae have been used to classify variation in the past, but these ranks are less frequently used in more contemporary studies. Many diatom species concepts have been too broadly diagnosed in the past and have concealed significant diversity (Mann, 1999). More finely resolved taxonomy indicates that species may even be endemics, restricted to a single lake or catchment, although debate on protist biogeography has been vigorous (Fenchel et al., 2019; Finlay and Fenchel, 2004; Mann and Droop, 1996; Williams, 2011). Some diatoms are globally widespread, but not necessarily to the extent that all distributions can be considered to always be cosmopolitan (Bahls, 2018).

1.1.2. DIATOM-ENVIRONMENTAL RELATIONSHIPS

Diatoms are a major algal group in lentic habitats and are commonly used to assess ecological status in phytobenthos assessments. Diatom taxa have ecological niches and autecological optima that were recognised at an early stage to correlate with important stressor gradients (Van Dam et al., 1994). Diatom indicator values are established using a variety of numerical approaches with differing levels of complexity (Jansen and Oksanen, 2013; Potapova et al., 2004). A widespread approach in diatom metrics uses weighted averaging of species indicator values and the underlying assumptions here are fewer (Zelinka and Marvan, 1961). Diatoms are now well established and effective bioindicators, routinely used to monitor quality of aquatic habitats in many regions, but have a more limited tradition of use in Ireland (Dalton et al., 2009).

Factors including the use of broad taxonomic concepts, inadequate description or illustration of type specimens and a dispersed and occasionally inaccessible literature has led to a practical necessity to lump species together during routine analysis in applied monitoring uses. Indicator diatoms used in environmental assessment are known to comprise of multiple taxa in species-complexes that can differ in their ecological niches (Potapova and Hamilton, 2007; Poulíčková et al., 2017). While the artificial aggregates are convenient, poor discrimination can limit the use and effectiveness of metrics in some contexts (Jüttner et al., 2013). But the implications for diatom-based assessment remains to be fully resolved, especially in the case of oligotrophic lakes where diatom diversity is less well documented (Kelly and Ector, 2012; Lange-Bertalot and Metzeltin, 1996; Rondón and Catalan, 2017). However, greater resources are a consequence for monitoring programs that incorporate higher

levels of taxonomic resolution and there are limitations to what can be routinely achieved with light microscopy (Bennett et al., 2014; Kahlert et al., 2019). Recent advances in molecular technologies has highlighted the limitations of classical morphology in discriminating taxa, especially in the case of cryptic species (Mann et al., 2020; Pinseel et al., 2019).

The available evidence indicates that differences in regional diatom species pools can be problematic for diatom indices developed in one region are transferred for use in another area (Pajunen et al., 2020). Differences in the extent of realised niches and a range of other stochastic processes related to local environmental gradients can all influence the effectiveness of the adopted metrics. Despite examples of success elsewhere, diatoms are indicated to exhibit weak niche conservation, but this occurs at global environmental and climatic scales (Soininen et al., 2019). Comparison of published species optima indicated that models had little or no predictive power outside their original spatial setting (Juggins, 2013). Taxonomic re-evaluation of some major indicator groups is ongoing and has helped to provide much needed clarity for problematic taxa that often dominate epilithic communities (Kahlert et al., 2019; Trobajo et al., 2013; Wetzel et al., 2015). As taxonomic investigations of national floras are undertaken, species aggregates are dis-entangled and new taxa are discovered, an increase in the precision of metrics is anticipated. However, other studies highlight the redundancy in indicator values as low sensitivity to changes in phosphorus concentration in many diatoms suggests increased discrimination will have a more limited effect on lake classifications (Kelly et al., 2015; Tapolczai et al., 2016).

1.1.3. DIATOM MONITORING AND THE WATER FRAMEWORK DIRECTIVE (WFD)

Freshwater habitats and lake biodiversity are subject to an increasing number of new pressures, often in combination with the intensification of pre-existing ones (Reid et al., 2019). Shallow lakes and littoral zones in particular are especially vulnerable to the effect of these multiple stressors (Beklioglu et al., 2016; Cantonati and Lowe, 2014; Evtimova and Donohue, 2016). Periphyton communities and epilithic diatoms perform a variety of functions and as dominant primary producers have a major role in biogeochemical cycling in lake littoral zones. Acknowledging this significance phytobenthos has been specified for monitoring in the normative definitions of the WFD as one part of biological quality element “macrophytes and phytobenthos” (EU WFD, 2000).

While several new assessment tools were developed in preparation for WFD lake monitoring in Ireland, phytobenthos classification has received more limited consideration (Donohue et al., 2009; Free et al., 2006). Irish lake studies have found good environmental responses in periphyton communities to environmental stressor gradients (Barbiero, 2000; DeNicola et al., 2006; Evtimova and Donohue, 2016) but a preliminary epilithic diatom study found diatom metrics to correlate poorly with lake trophic status (DeNicola et al., 2004). Paleolimnology and sediment diatom studies have indicated that diatoms are strongly related to lake physicochemical gradients but these integrate planktonic and littoral assemblages so do not address the need for a monitoring tool that can be used to classify lake phytobenthos (Chen et al., 2008; Leira et al., 2006). While diatoms sampled from eulittoral habitats have a proven applicability, contradictory accounts of the suitability of epiphytic versus epilithic substrate choices, the significance of physical disturbance in exposed littoral areas,

and the role of spatial and temporal processes remain nevertheless (Bennion et al., 2014; Poulíčková et al., 2004; Riato and Leira, 2020; Schönfelder et al., 2002; Snell and Irvine, 2013). King et al. reviewed the range of factors likely to affect diatom monitoring in lakes and seasonality was identified as a key uncertainty for classification (King et al., 2006). Since then, progress in implementing lake diatom monitoring has demonstrated the great variety of natural types and conditions that are distributed across Europe (Bennion et al., 2014; Gottschalk and Kahlert, 2012; Kelly et al., 2019). Acidity and nutrient gradients are often the most important gradients in controlling diatom distributions but the extent of these pressures on epilithic diatom assemblages in Irish lakes remains poorly known.

More recent developments have indicated that high-throughput sequencing DNA metabarcoding can be used for lake diatom biomonitoring, but with mixed success because DNA reference libraries remain incomplete (Bailet et al., 2019; Rivera et al., 2018). This could be a significant problem for the Irish diatom flora generally given potential for dispersal limitation amongst lake types in the Irish landscape. Studies examining the extent of mismatches between standard morphological analysis and diatom metabarcoding identify methodological issues in both approaches, but progress in the latter has been more rapid in river applications (Kelly et al., 2020). Most new species are still described using classical morphology and while lake diatom monitoring still relies on it for the most part, paleolimnological applications are entirely dependent on morphology. Therefore, of both these complementary approaches, a morphological approach in Ireland has multiple benefits and is preferred currently.

Chapter two of this thesis investigates the use of seasonal epilithic diatom assemblages to evaluate ecological status in a range of dystrophic to calcifying lakes in Ireland (Figure 1.1-1.4). It explores the natural and anthropogenic factors shaping assemblages before investigating the efficacy of the Lake Trophic Diatom Index to determine lake trophic status across three lake types. The study was replicated with a spring and summer analysis to explore the effect of seasonality on diatom responses in two seasons.



Figure 1.1 Epilithic diatoms were sampled in a shallow tray with a toothbrush by scrubbing the top surface of five cobble to boulder sized stones.

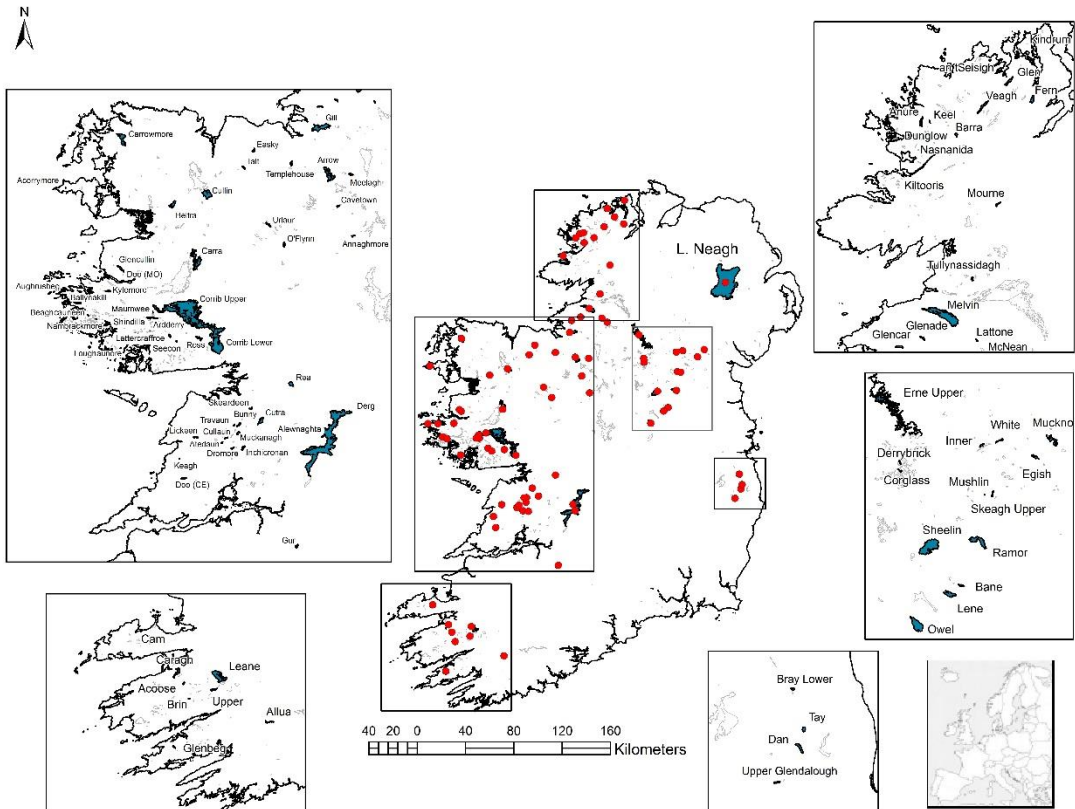


Figure 1.2 Distribution in Ireland of the 91 core study lakes where diatom sampling was done.



Figure 1.3 Lake types and periphyton communities varied across the network of sampled waterbodies. Calcifying marl lakes in karstic and limestone regions were sampled (i-ii) and these often had thick calcareous deposits of cyanobacteria. Nevertheless, diatoms were well represented on the top surfaces (iii) while in nutrient impacted lakes *Cladophora glomerata* was rarely as extensive as noted here (iv).

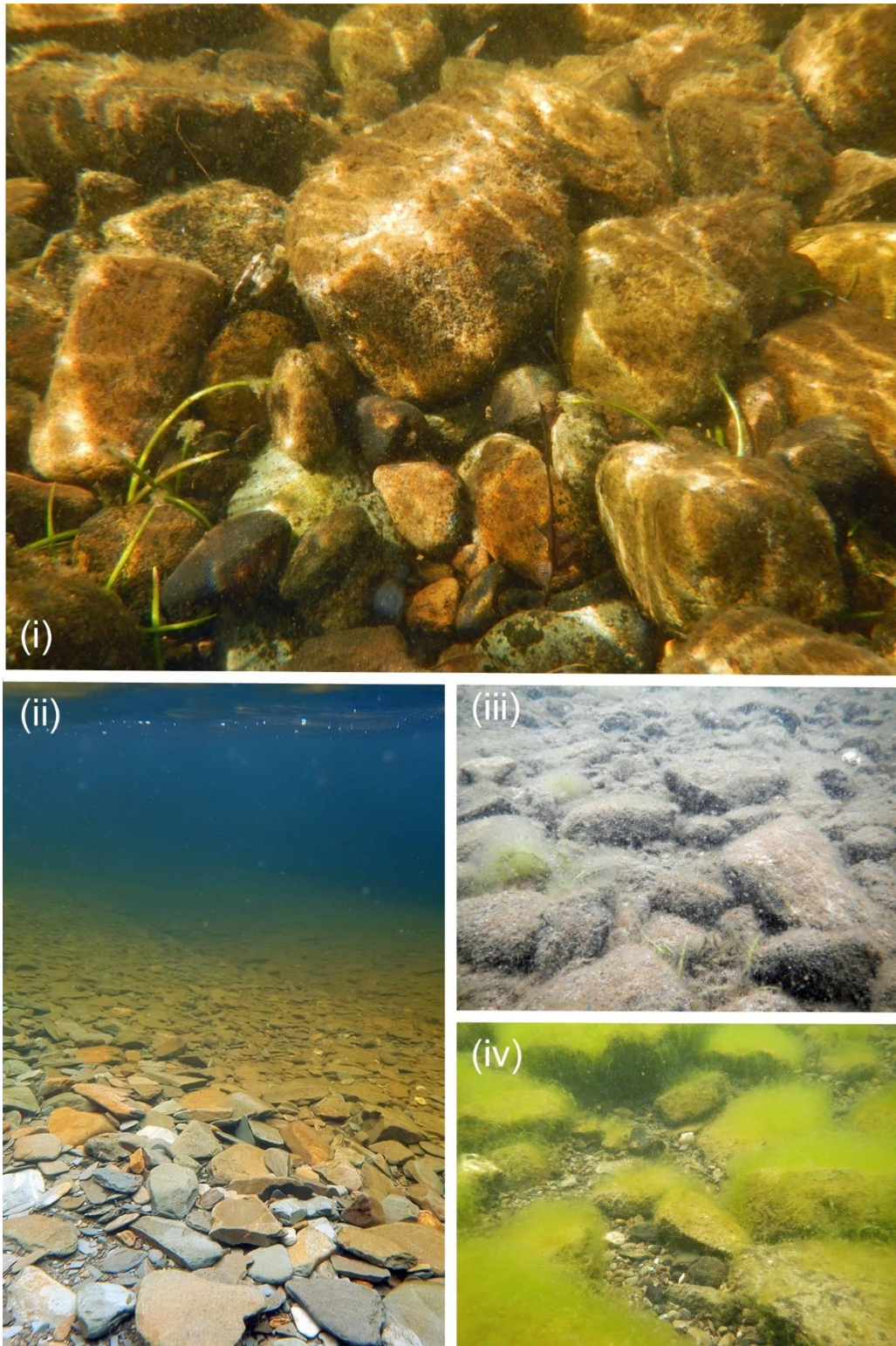


Figure 1.4 Low and moderate alkalinity littoral zones also varied in condition (i-iv) with some exposed shorelines with deep photic zones (ii) contrasting with littoral areas where sedimentation (iii) or opportunistic *Zygnematales* cover was high, but diatoms were sufficiently abundant in all of these habitats.

1.1.4. DIATOM CLASSIFICATION IN IRELAND

The most recent taxonomic assessment of the Irish diatom flora was provided by Niels Foged (Foged, 1977). Foged summarised the extent of diatom investigations completed up to that point and stated that no contemporary synopsis of the Irish freshwater diatom flora existed in that century. He concluded that the sampled area was no richer in species but indicated that several genera and species were absent relative to those found in similar temperate regions in Europe and America. Notably, he stated that *"many of the species have a much greater range of variation as regards the size and shape of valve, than I have observed previously in other areas of similar size"*. Such variation now provides the basis for new discoveries when modern-day diatom species concepts are applied (Mann, 1999; Medlin, 2018). Diatom-based ecological research and environmental monitoring for the Water Framework Directive (WFD) has since improved the situation and relevant publications are listed in Chapter 2 of this thesis. A provisional freshwater algal checklist has also recently been published (Guiry, 2019). But many reported species comprise of artificial aggregates of closely related taxa. This is in part a consequence of the lack of an indigenous freshwater algal taxonomic tradition in Ireland (Guiry et al., 2007). It does not suggest that Irish specimens have not been the focus of contemporary taxonomic investigations, they have, but these studies are few and referred to in my thesis discussion.

A focus of this thesis therefore is to explore taxonomic diversity in two widespread indicator taxa to investigate whether these are merely cosmopolitan elements within the established Holarctic flora. The implication of taxonomic resolution will also be

investigated in terms of the ecological niches identified with weighted averaging of the more finely resolved morphotaxa.

The objective of Chapter 3 is to improve our understanding of the taxonomy, ecology and distribution of *Brachysira* in lotic and lentic habitats in Ireland. The genus *Brachysira* was re-established by Round and Mann and is especially common in oligotrophic lakes and bogs, with a reported endemism in some regions (Metzeltin, 2007; Round and Mann, 1981; Round et al., 1990). It is similarly widely distributed in oligotrophic systems across a broad gradient of alkalinity in Ireland (Chen et al., 2008; Juggins et al., 2016; Leira et al., 2007; Leira et al., 2006). Its greater relative abundance exceeds that of the widely prevalent diatom *Achnanthes* Kützinger in peatland systems in particular. There is a tendency to operationally identify many analogous populations as *B. vitrea* in some routine studies but *B. brebissonii* R.Ross, *B. procera* Lange-Bertalot & Gerd Moser, *B. garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot and other less frequent but cosmopolitan species are also widely reported in Ireland.

The objective of Chapter 4 is to complete an equivalent investigation for the former *Cymbella microcephala*-complex. Its classification was previously examined when the new genus *Encyonopsis* Krammer was erected by Krammer (1997b). However, limited SEM was provided in some cases and to reappraise morphological diversity within this group I re-examined the original type material of *E. alpina* Krammer & Lange-Bertalot, *E. angusta* Krammer & Lange-Bertalot and *E. tavirana* Krammer with light (LM) and scanning electron (SEM) microscopy. The *Encyonopsis microcephala*-complex occurs across a range of oligo-mesotrophic freshwaters but in contrast to *Brachysira*, it is very abundant in calcifying habitats. Several taxa such as *E. krammeri* E.Reichardt and *E.*

alpina have been reported in Irish lakes but the majority of records are under the name *E. microcephala* (Grunow) Krammer. Given variability in the trophic sensitivity of its diatom metric scores it was hypothesised that a greater discrimination of morphospecies would be beneficial for environmental assessments. Concurrent with my examination of distinctive *Encyonopsis* populations in low alkalinity mesotrophic Irish lakes, *E. neerlandica* Van de Vijver, Verweij, van der Wal & A.Mertens was described for the first time.

Finally, in Chapter 5 I provide a synopsis of the three previous sections and discuss the findings in the broader context of the international literature in specific research areas. I consider the implications of diatom-based monitoring in an Irish lake context and consider where lake phytobenthos research might be developed in future. The implication of my new findings in terms of diatom taxonomy is also outlined.

1.2. RESEARCH OBJECTIVES

1. Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish Lakes

Littoral diatom assemblages are influenced by many environmental gradients that combine to structure epilithic communities and the significance of these factors will be assessed. Diatom communities at reference condition have distinct patterns so the response to environmental stressors can be evaluated and the effectiveness of environmental metrics can be determined once this has been established. The first aim is to identify the primary factors structuring epilithic diatom communities in Irish lakes. The second aim is to establish how communities of different lake types respond to environmental stressors. The third aim is to examine the effect of seasonality and its implication for monitoring programs.

Hypothesis 1: littoral diatom assemblages can be used for the ecological classification of lakes in Ireland and respond primarily to pH and nutrient gradients - but the role of other environmental factors may be significant.

Hypothesis 2: high and low alkalinity lake types have different reference communities, but their expected values are equivalent when evaluated using diatom metrics.

Hypothesis 3: Seasonality influences littoral diatom diversity with greater effects expected at moderate levels of nutrient enrichment.

2. The taxonomy, distribution and ecology of *Brachysira* and *Encyonopsis* in Irish waters.

Diatoms in the genus *Brachysira* and *Encyonopsis* frequently dominate benthic diatom assemblages in freshwaters. Documented taxa represent an aggregate of distinctive species or infraspecific forms, but this is frequently overlooked in ecological studies because of challenges with their identification. The first aim is to investigate morphological and ultrastructural variation within each of the aggregates using high-resolution electron microscopy with a view to establishing an improved classification of benthic diatoms in Irish waters. The second aim is to investigate the geographical distribution and ecological niches for taxa under the new classification.

Hypothesis 1: Diatoms in the genus *Brachysira* and *Encyonopsis* are taxonomic aggregates that can be resolved into a greater number of morphologically distinct species or morphotypes.

Hypothesis 2: established taxa arising out of a revision of *Brachysira* and *Encyonopsis* species aggregates have distinctive geographical distributions in Ireland with ecological niches that should be considered for environmental assessments.

1.3. THESIS CONTRIBUTION

Lake physicochemical data incorporated in this thesis was sourced primarily from AQUARIUS, the national database for the Water Framework Directive (WFD) chemical monitoring program in Ireland. Of the 91 lakes sampled to fulfil national WFD diatom monitoring objectives, approximately one-third were sampled by me and I processed or supervised processing of 220 lake and 212 river diatom samples that were generated subsequently. I completed all diatom counts, light microscopy (LM) and scanning electron microscopy (SEM) outlined in this thesis.

The thesis has partially or wholly contributed to several publications, or has been submitted for peer review, with my specific contribution specified in each case.

1. Kelly, M.G., Gómez-Rodríguez, C., Kahlert, M., Almeida, S.F.P., Bennett, C., Bottin, M., Delmas, F., Descy, J.-P., Dörflinger, G., Kennedy, B., Marvan, P., Opatrilova, L., Pardo, I., Pfister, P., Rosebery, J., Schneider, S., Vilbaste, S., 2012. Establishing expectations for pan-European diatom based ecological status assessments. *Ecological Indicators* 20, 177-186.

I participated in a workshop that planned this publication and provided data for it. I contributed to aspects of the preliminary data analysis and in a minor way to editing of the final text prior to submission.

2. Free, G., Tierney, D., Little, R., Kelly, F.L., Kennedy, B., Plant, C., Trodd, W., Wynne, C., Caroni, R., Byrne, C., 2016. Lake ecological assessment metrics in Ireland: relationships with phosphorus and typology parameters and the implications for setting nutrient standards, *Biology and Environment: Proceedings of the Royal Irish Academy*. JSTOR, pp. 191-204.

I provided diatom data and contributed to editing of the text prior to submission.

3. Kennedy, B., Allott, N., 2017. A review of the genus *Brachysira* in Ireland with the description of *Brachysira praegeri* and *Brachysira conamarae*, new raphid diatoms (Bacillariophyceae) from high status waterbodies. *Phytotaxa* 326, 1-27.
4. Kennedy, B., Buckley, Y., Allott, N., 2019. Taxonomy, ecology and analysis of type material of some small *Encyonopsis* with description of new species in Ireland. *Phytotaxa* 395, 89-128.

I conceived, designed, performed the analysis and wrote these papers with a contribution to final editing from my supervisors.

5. Kennedy, B., Buckley, Y., 2020. Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish lakes. *Ecological Indicators*, submitted.

I conceived, designed, performed the analysis and wrote this paper with a contribution from my supervisor during data analysis, writing and editing stages. Additional diatom metric scores sourced from EPA databases were incorporated in two graphs. This paper has been submitted to *Ecological Indicators* and is currently in review.

CHAPTER 2

Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish lakes.

This chapter has been submitted to ECOLOGICAL INDICATORS:

Kennedy, B., Buckley, Y., 2020. Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish lakes. Ecological Indicators, submitted.

2.1. ABSTRACT

Periphyton is increasingly used to assess the biological condition of lakes. The predominance of relatively shallow waterbodies in Ireland implies that benthic diatoms should have a prominent role as bioindicators; but epilithic diatom ecology in these Irish systems is poorly described. We characterised spring and summer diatom populations in diverse periphyton communities in 91 Irish lake waterbodies. Six assemblage clusters of diatom species were identified, and variation in their assemblages was predominately associated with environmental gradients of acidity-alkalinity, total phosphorus, and true colour. Diatom composition differed amongst high status lakes in three *a priori* defined lake alkalinity groups commonly used as a diatom typology for lake classification. However, when waterbodies were evaluated with a lake trophic diatom index (LTDI) their distribution of 'expected-values' was equivalent. The LTDI was highly correlated with total phosphorus but also had similar responses in the three lake alkalinity types. These findings indicated that lake typology had a limited role for lake diatom classification in Ireland. Matched spring and summer

sampling across wide environmental gradients allowed the role of seasonality to be investigated. Relatively few benthic diatoms had a significant seasonal indicator value suggesting that unbiased monitoring of the epilithon is possible during any month of the growing season in comparable temperate waterbodies. Factors to explain the greater magnitude of differences in seasonal LTDI scores apparent in some waterbodies were not identified. Given that lake diatom classification has now been validated, we suggest that the approach could be developed and used for more targeted assessment objectives.

2.2. INTRODUCTION

Periphyton is critical to biogeochemical cycling and sustains higher trophic levels via a contribution to food webs, particularly in shallow waterbodies (Vadeboncoeur and Steinman, 2002). The biological integrity of the productive littoral zone is increasingly threatened by a range of environmental stressors and its condition must be assessed to protect ecosystem functioning and lake biodiversity (Beklioglu *et al.*, 2016; Jeppesen *et al.*, 2015). Benthic algae are well established indicators for monitoring pollution effects in rivers (Stevenson, 2014). Equivalent negative impacts manifest in lakes (Auer *et al.*, 2010; Lambert *et al.*, 2008; Lowe, 1996) and the Water Framework Directive (EU, 2000) has incentivised lake periphyton research to ensure statutory objectives to monitor phytobenthos are fulfilled (Lyche Solheim *et al.*, 2019). While the effectiveness of both diatom and non-diatom algae has been evaluated (DeNicola *et al.*, 2004; Kelly *et al.*, 2008; King *et al.*, 2000; Schaumburg *et al.*, 2004), diatom-based approaches outnumber non-diatom metrics for ecological classification (Poikane *et al.*, 2016). The reasons for this are part pragmatic, in that the sampling and specialist taxonomy of only a single indicator group within a more complex community is

required, and partly because the earlier success of diatom monitoring in rivers suggested that the approach could also be easily adapted for lentic systems.

Lake diatom assemblages are reported to have a fast response time and sensitivity to a range of environmental stressors including nutrient enrichment, acidification, salinisation, metal contamination and hydromorphological alteration (Cantonati and Lowe, 2014; Evtimova and Donohue, 2016). Progress has been incremental since the earliest case studies (Köster and Hübener, 2001; Stockner and Armstrong, 1971) and diatom metrics are now used routinely to classify lake ecological status (Bennion *et al.*, 2014; Hendrickx and Denys, 2005; Pouličková *et al.*, 2004; Schaumburg *et al.*, 2004; Stenger-Kovács *et al.*, 2007). Their effectiveness for trophic assessment has been fully validated (Kelly *et al.*, 2012; Kelly *et al.*, 2008). However, characterisation of assemblages in waterbodies of contrasting type and biological condition is ongoing (Blanco *et al.*, 2014; Kelly *et al.*, 2019; Stenger-Kovács *et al.*, 2014) to help resolve key research gaps to improve the effectiveness of monitoring programs (Bennion *et al.*, 2010; DeNicola and Kelly, 2014; Poikane *et al.*, 2016). In order to evaluate ecological status the reference community 'expected' in the absence of anthropogenic impacts must be described (Pardo *et al.*, 2012; Stoddard *et al.*, 2006). Pristine condition is widely acknowledged to be the exception now given the extended legacy of anthropogenic impact, aerial deposition, and climate change over the last century in particular, affecting even the remotest European regions (Bennion *et al.*, 2004; Curtis *et al.*, 2005; Hübener *et al.*, 2015; Rimet *et al.*, 2019). Given these challenges, 'least disturbed' and 'best available' waterbodies instead often function as a practical benchmark for comparison (Bailey *et al.*, 2004; Moss, 2008; Pardo *et al.*, 2012; Stoddard *et al.*, 2006; Whittier *et al.*, 2007). This study incorporates high alkalinity oligotrophic

and marl lakes whose reference assemblages are more poorly described because of the confounding influence of intensive agriculture that often coincides with free-draining fertile areas in lowland limestone catchments (Bennion *et al.*, 2014; Lyche Solheim *et al.*, 2019; Pentecost, 2009). Similarly, a relatively lower level of deposited acidity in the extensive peatland lakes of the Atlantic periphery provides an opportunity to examine diatom assemblage response to environmental stressors in naturally acidic systems (Aherne and Curtis, 2003; Burton and Aherne, 2012; Flower *et al.*, 1994). Furthermore, a high diversity of lake types in close proximity reduces potential confounding identified in larger scale studies (Smucker and Vis, 2011; Soininen, 2007; Virtanen and Soininen, 2012).

Sediment-based diatom research and paleoecology have dominated diatom investigations to date in Ireland (Anderson *et al.*, 1993; Anderson and Rippey, 1994; Battarbee, 1978; Battarbee, 1986; Chen *et al.*, 2008; Donohue *et al.*, 2010; Flower *et al.*, 1994; Leira *et al.*, 2006; Murnaghan *et al.*, 2015; O'Dwyer and Taylor, 2010; Taylor *et al.*, 2006). Epilithic diatom ecology has received more limited attention with a regional focus (Barbiero, 2000; Blanco *et al.*, 2014; DeNicola *et al.*, 2004; Evtimova and Donohue, 2016; Round, 1959; Snell and Irvine, 2013; Sweeney and Linnane, 2010). A preliminary assessment of benthic algae found a good relationship with trophic parameters in Irish lakes but reported a poor correlation with diatom indices available at that time (DeNicola *et al.*, 2004). A Lake Trophic Diatom Index (LTDI) developed for UK waterbodies subsequently permitted the monitoring objectives of the WFD to be implemented in Ireland, but its application remains to be critically reviewed (Kelly *et al.*, 2007). In a European intercalibration comparison, Irish lake diatom classifications were reported to result in more stringent WFD classifications than the average of the

participating member states in some lake types (Kelly *et al.*, 2014). Conversely, when national metrics were evaluated, epilithic diatoms produced less stringent biological classifications, particularly in nutrient enriched waterbodies (Free *et al.*, 2016). The aim of this study is to identify the predominate epilithic diatom assemblages found in Irish lakes. It will establish the most important environmental variables influencing diatom composition to underpin their use in lake assessment.

Temporal variation has been identified as a research priority so that seasonal processes expected in dynamic lake systems can be clearly separated from the effects of anthropogenic stressors (McCabe and Cyr, 2006; Rimet *et al.*, 2019; Stevenson *et al.*, 1996; Virta *et al.*, 2020). Seasonality is synonymous with phytoplankton succession (Köster and Pienitz, 2006; Reynolds, 1980) and is also a major control on river biofilms and sediment diatoms in lakes (Admiraal *et al.*, 1984; Kelly *et al.*, 2009; Round, 1972; Snell *et al.*, 2019). The interaction of a range of abiotic factors such as disturbance, temperature, light and nutrient regime, with secondary biotic interactions and top-down control are known to strongly regulate patterns of algal succession in lakes (Kahlert *et al.*, 2002; Leira and Cantonati, 2008; Rimet *et al.*, 2019). However, the combined implication of these complex processes for monitoring programs targeting the epilithon is less well established (Bielczyńska, 2015; Cantonati and Lowe, 2014; Hall and Smol, 2010; King *et al.*, 2006). Temporal variation in periphyton communities has been examined in many single and multiple lake case-studies (Barbiero, 2000; Castenholz, 1960; Cattaneo and Kalff, 1978; Cocquyt, 1999; Jasprica and Hafner, 2005; Jüttner *et al.*, 2010; Kahlert *et al.*, 2002; King *et al.*, 2002; Müller, 1994). Significant structural change is reported in some situations (King *et al.*, 2002; McGowan *et al.*, 2018; Rimet *et al.*, 2015), but seasonality is found to be unimportant in others

(Fidlerová and Hlúbíková, 2016; Novais *et al.*, 2012). A greater understanding of how diatom assemblages are structured seasonally across environmental gradients is required so findings can be generalised and potential bias for lake monitoring programs identified (Kelly *et al.*, 2012; King *et al.*, 2006).

Here we investigate diatom assemblages in two seasons and examine the use of diatoms to indicate the health of the overall phytobenthos by:

- i) grouping lakes according to the compositional variation of their diatom assemblages;
- ii) identifying the major environmental gradients that structure assemblages in spring and summer seasons;
- iii) validating the efficacy of epilithic diatoms for a Water Framework Directive classification of Irish lakes.

2.3. MATERIALS AND METHODS

2.3.1. STUDY LAKES AND DIATOM SAMPLING

Epilithic diatoms sampled in 91 lakes (210 samples) were used to investigate the significance of environmental factors that determine the composition of diatom assemblages in lake phytobenthos in Ireland (Figure 2.1). Of the sampled lakes, 100 stations in 86 waterbodies were sampled in both spring and summer seasons. Waterbodies were selected to maximise the range in trophic condition for three broad *a priori* lake alkalinity groups reported to have a typological significance in European lakes, enabling the results of this study to be interpreted in the context of the international findings (Bennion *et al.*, 2014; Kelly *et al.*, 2014). Threshold alkalinities of <10, 10-50 and >50 mg/L CaCO₃ categorised lakes as a Low (n=38), Moderate (n=11) or High (n=42) alkalinity lake type, respectively. Long term monitoring data in 18 candidate-reference waterbodies (LA n=10; MA n=2; HA n=6) was considered as a basis to inform the expected value of the Lake Trophic Diatom Index. Diatoms were sampled in spring (March-April) and summer (July-September) with a minimum 10-week interval between successive sampling dates. Diatoms were collected according to European Committee for Standardization guidelines by brushing the top surface of at least five cobble-sized rocks, collected at approximately 0.5 metres water depth along the lake shoreline (CEN, 2003). A littoral station located in the main waterbody area of each lake was sampled and enclosed bays, areas adjacent to river inflows or sites of major recreational activity were avoided where possible. Samples were preserved with Lugol's Iodine for laboratory processing.

Diatoms were digested to remove organic material using concentrated sulphuric acid, potassium permanganate and oxalic acid, after a preliminary removal of carbonates using concentrated hydrochloric acid (CEN, 2003). Following rinsing in deionised water, a dilute suspension of the oxidised material was air-dried onto glass coverslips for 24 hours. Permanent glass slides were prepared by mounting the coverslip on the slide using Naphrax® (refractive index=1.74; Brunel Microscopes Ltd., UK). The relative abundance of diatoms was calculated by counting single or multiple non-overlapping transects through the centre of each coverslip, until typically 400-500 random non-planktonic diatom valves were enumerated at x1000 with a Nikon E80i microscope equipped with phase contrast (Plan Fluor x100 oil immersion objective n.a.=1.3).

2.3.2. ENVIRONMENTAL DATA

Lake physicochemical data was obtained from AQUARIUS, a national database for the surface water monitoring program in Ireland (EPA, 2006). A large number of participatory laboratories contribute to this database and methodologies and citations for the individual parameter tests are not provided here. For larger lakes with multiple open water and diatom sampling locations, the monitoring point in closest proximity to the diatom station was selected, or from surrogate shoreline locations when open water data was unavailable. The five-months preceding diatom collection was typically selected to generate a seasonal average value for each parameter. Where less than three data points were available then annual means were reported in these less frequently monitored waterbodies. Physicochemical parameters common to all lakes included alkalinity (alk), conductivity (cond), pH, true colour (TC), silica (Si), total oxidised nitrogen (TON), total ammonia (NH₄), nitrite (NH₃), total phosphorus (TP) and

chlorophyll *a* (chl_a). Spatial and physical variables were extracted from EPA databases and a Geographical Information System. The spatial variables included station easting (X), northing (Y) and altitude (alt), and physical variables incorporated catchment area (CA), lake area (LA), maximum lake depth (maxD) and station exposure. Fetch was also incorporated as a novel proxy for shoreline exposure and estimated as the maximum open water distance to the diatom station (fetch_{max}), or as maximum distance to shoreline in the direction of south-westerly prevailing winds in Ireland (fetch_{sw}). Average wind speed for the diatom sampling location was estimated with 20m and 30m rasters obtained from the Sustainable Energy Authority of Ireland Wind Mapping System (SEAI) and the wind map for Northern Ireland (DfE). Percentage catchment landcover was characterised using nine summary groups based on categories of landuse classified in CORINE Land Cover (European Environment Agency) in (Table 2.2) and a table of lake parameters and environmental characteristics is provided in Appendix A.

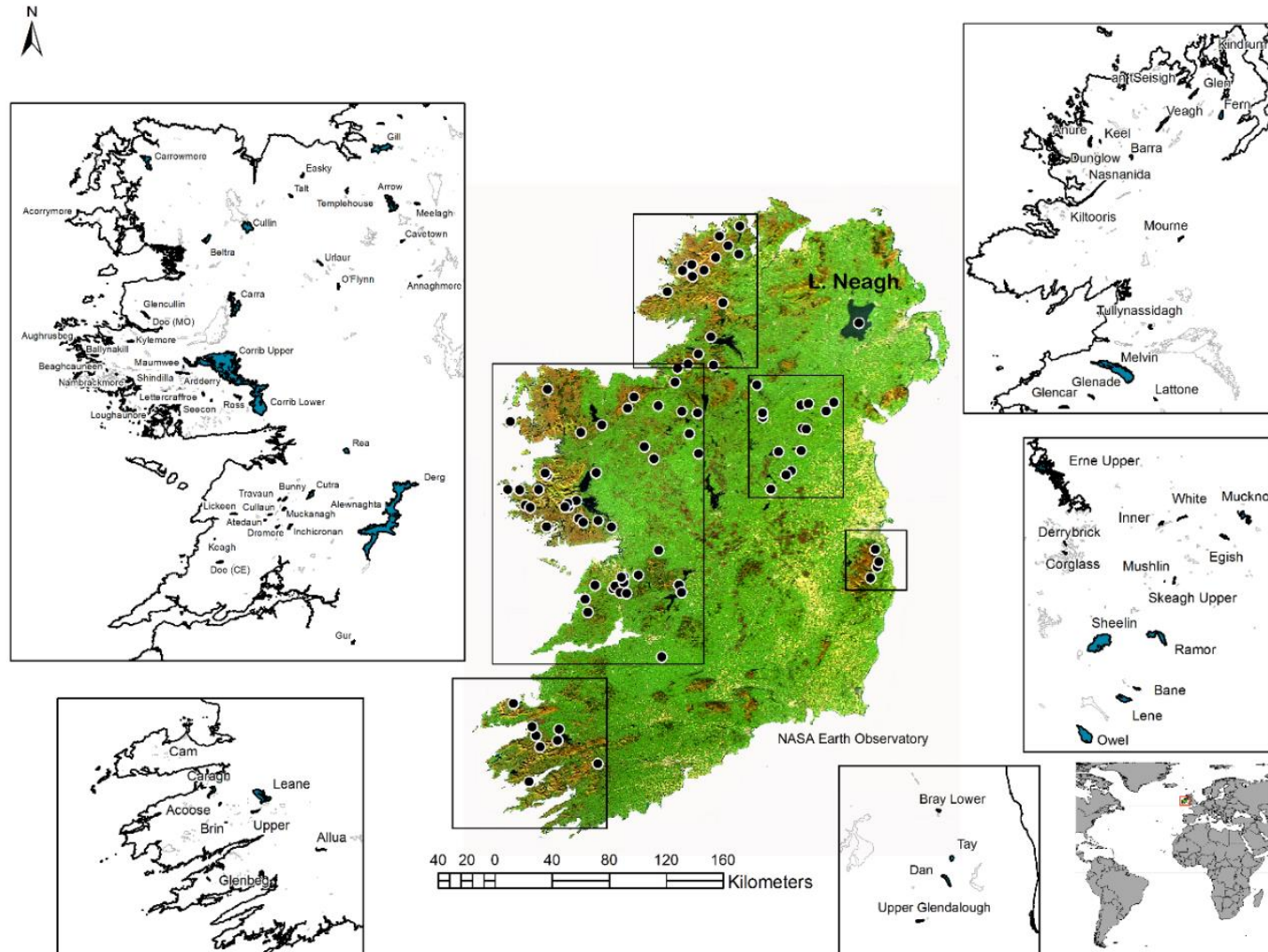


Figure 2.1 Geographic location of the 91 core study lakes in Ireland.

2.3.3. DIATOM IDENTIFICATION

Dominant taxa ($\geq 2\%$ relative abundance) were identified to species level where possible using standard floras (Krammer and Lange-Bertalot, 1986, 1988, 1991, 2004; Lange-Bertalot *et al.*, 2017; Lange-Bertalot and Metzeltin, 1996), and Kennedy and Allott (2017) for *Brachysira* (Kützing) and Kennedy *et al.* (2019) for *Encyonopsis* (Krammer). Unidentifiable girdle views were enumerated separately and allocated *pro-rata* to species or reported at genus level where this was not feasible. Common diatoms with an abundance $> 5\%$ in at least 10 lakes with their mean abundance and indicator value are listed in Appendix B.

2.3.4. STATISTICAL ANALYSIS

Statistical analysis was completed in R statistical software (R Development Core Team, 2018; version 3.4.4) with multivariate analysis done primarily in *vegan* (Oksanen *et al.*, 2018) and R packages specified below.

Principal Component Analysis (PCA) of the transformed water chemical parameters was used to provide a preliminary characterisation of the lake dataset (all variables with the exception of pH were $\log_{10}(x+1)$ transformed). Physical and landuse variables were projected passively *a posteriori* to this PCA ordination biplot to identify correlative associations between these supplementary variables and water chemistry with the *vegan* function *envfit*. Percentage landcover data was arcsine-square-root transformed to help normalise distributions and reduce skewness. To assess the significance of differences in lake physicochemistry between lake types and season a robust ANOVA was performed in the package WRS2 (Mair and Wilcox, 2020) with Bonferroni corrected *p*-values reported where statistically significant.

To group lakes according to their diatom composition a robust form of non-hierarchical k-means analysis called partitioning around medoids (PAM) was implemented in the *cluster* package (Maechler, 2019). Diatoms with relative abundance $\geq 2\%$ were square-root transformed and analysed based on a Bray-Curtis dissimilarity matrix. Statistics from a silhouette plots analysis and PCA of clustering results helped determine the final number of groups to retain and interpret. A pairwise analysis of similarity (ANOSIM) was used to check for the significance of the overall difference in the seasonal results (Clarke, 1993). Abundant diatoms and indicator taxa identified with the package *Indicspecies* (De Caceres and Jansen, 2016) were used in conjunction with boxplots of species richness and diversity to characterise assemblages and relate them to the lake environmental conditions in each cluster. Diatom species richness (S) and diversity (Shannon H') were calculated after samples were adjusted to standard counts of 350 valves using rarefaction.

The principal environmental factors structuring diatom assemblages were identified with Constrained Correspondence Analysis (Ter Braak, 1987) separately for each season, after Detrended Correspondence Analysis (DCA) confirmed unimodal responses on the first axis (Ter Braak and Smilauer, 2002) in spring and summer, respectively (length $4.02 \text{ SD}_{\text{spring}}$ and $5.04 \text{ SD}_{\text{summer}}$). CCA models were built by manually adding variables one at a time based on the Akaike Information Criterion (AIC) with Monte Carlo permutation tests used to confirm significance of individual terms and the final model at a 5% level (i.e. $p < 0.05$). Alkalinity had a high variance inflation factor so was omitted from the CCA analysis with the subsequent alkalinity, conductivity, pH gradient referred to as an alkalinity-pH gradient. The results of cluster analysis were plotted with convex hulls in the CCA biplot to help illustrate patterns in

lake diatom response. Partial CCA was used to determine the relative importance of two main categories of explanatory variables in structuring the diatom assemblages (physicochemical versus physical-spatial) and to evaluate their conditional effects.

To validate the suitability of epilithic diatoms for WFD classification a metric called the Lake Trophic Diatom Index (LTDI) developed to assess the condition of phytobenthos in UK lakes, was calculated (WFD-UKTAG, 2008). The LTDI is a weighted average of diatom nutrient sensitivity scores with scoring taxa assigned a score of 1 for the most sensitive, to 5 for the most tolerant group, respectively (Zelinka and Marvan, 1961). The ratio of the 'observed-value' to the 'expected-value' enables the LTDI to be expressed as an Ecological Quality Ratio (EQR) after a rescaling adjustment as follows ($EQR = 100 - \text{observed} / 100 - \text{expected}$). A subdivision of the 0-1 EQR scale categorises the five status classes specified in the normative definitions of the WFD (Annex V) i.e. high, good, moderate, poor, and bad status. An expected value of the LTDI for Irish lakes was explored by assessing the distribution of metric scores from a selection of candidate reference waterbodies after screening for compatibility with WFD reference criteria using a combination of paleolimnology, lake chemistry, landuse data and field evaluation of periphyton communities (Leira *et al.*, 2006; O'Dwyer and Taylor, 2010). Regression and analysis of covariance (ANCOVA) was used to test the significance of the relationship of EQR for each lake type and physicochemical variables. Optimal models in regression analysis were confirmed after the most parsimonious model was established to be more than 2 AIC units lower than the alternative significant model, with an ANOVA of nested models and F-ratio used to determine the significance of polynomial terms.

2.4. RESULTS

2.4.1. PHYSICOCHEMICAL AND ENVIRONMENTAL GRADIENTS

Annual and seasonal lake physicochemistry is summarised for the lake alkalinity types in Table 2.1 and Figure 2.2, with the range of values for the supplementary physical, spatial and landuse variables in Table 2.2. Results of robust ANOVA indicated that differences amongst lake alkalinity types were significant for all parameters with the exception of total ammonia (robust ANOVA; $p < 0.006$). Significant seasonal differences were only detected for silica and total oxidised nitrogen with a significant interaction effect identified only for the latter parameter with higher average concentration in high alkalinity lakes in spring (Figure 2.2). The cumulative percentage variance explained by the first three PCA axes on the 9 physicochemical parameters with the broken stick model was 76% (Jackson, 1993) and two major gradients were identified (Figure 2.3). PC axis-1 reflected the strong influence of the highly correlated pH, alkalinity and conductivity and their negative association with true colour. Of the 15 supplementary variables (Table 2.2), axis-1 was most strongly associated with the landcover categories pasture and bog. PC axis-2 was characterised as a nutrient gradient owing to the influence of total phosphorus, ammonia and chlorophyll *a*. PC axis-3 was also a trophic gradient with a greater influence of the correlated parameters total oxidised nitrogen and silica that were also significantly correlated with pasture (Appendix C, Figure C.2). While total phosphorus was significantly correlated with landcover it was only weakly associated with the acidity-alkalinity gradient in this data. Relationships between physical factors and lake water chemistry were also low (Appendix D, Figure D.1). Correlations amongst some supplementary variables

primarily indicated that smaller sized lakes tended to be situated in smaller catchments at higher altitude.

Oligotrophic to mesotrophic lakes dominated the dataset (Vollenweider and Kerekes, 1982) especially at lower alkalinities, but a trophic gradient was present to some extent for all three lake alkalinity groups in the *a priori* typology (Appendix C, Figure C.1). The 42 high alkalinity waterbodies included candidate reference lakes with low nutrients and chlorophyll *a* in the lower right quadrant and graduated to hypertrophic lakes in the upper right of the ordination plot. The 38 low alkalinity oligo-mesotrophic lakes were less dispersed along the trophic gradient by comparison but were more highly coloured and had a greater pH range (Appendix C, Figure C.1; Table 2.1). Candidate reference lakes for this type were dispersed throughout the lower left quadrant and had relatively low colour and low total phosphorus. More highly coloured lakes with low pH or elevated total phosphorus were distributed in the upper left quadrant. Fewer moderate alkalinity lakes were sampled, and these occupied a median position at the centre of the ordination with chemical characteristics intermediate between other types. Their colour was comparable to the low alkalinity group, but average total phosphorus levels were higher.

Seasonal difference in physicochemistry between individual lakes was often lower than differences amongst lakes with the result that many waterbodies group close together in the PCA biplot, but variability is greater in others, e.g. L. O'Flynn and L. Nasnanida (Appendix C, Figure C.1). Several waterbodies near alkalinity thresholds between *a priori* types were categorised as low alkalinity in spring but subsequently grouped with the moderate alkalinity lakes owing to higher summer alkalinity levels.

Parameter	All types annual	LA spring	LA summer	MA spring	MA summer	HA spring	HA summer
Alkalinity (mg/L CaCO ₃)	3-215 (65)	3-10 (5)	3-8 (5)	16-49 (30)	11-45 (25)	54-215 (119)	51-209 (111)
Total Ammonia (mg/L NH ₄ -N)	0.005-0.102 (0.019)	0.01-0.035 (0.018)	0.01-0.035 (0.018)	0.01-0.035 (0.024)	0.01-0.02 (0.015)	0.005- 0.102 (0.025)	0.01-0.074 (0.017)
Chlorophyll <i>a</i> (µg/L)	0.5-71.3 (6.6)	0.5-7.8 (2.38)	0.9-12.9 (3.58)	1.0-17 (6.2)	3.08-20.8 (8.3)	0.6-55.7 (4.8)	1-58.6 (7.9)
Conductivity (µS ^{-cm})	32-479 (187)	32-153 (85)	33-121 (71)	98-262 (161)	75-256 (129)	156-479 (277)	159-424 (263)
pH	5.1-8.9 (7.4)	5.4-7.2 (6.4)	5.4-7.8 (6.7)	6.9-7.6 (7.4)	7.0-7.9 (7.4)	7.5-8.4 (8.0)	7.8-8.9 (8.2)
Silica (mg/L Si)	0.11-6.63 (1.37)	0.43-1.79 (0.88)	0.10-1.90 (0.57)	1.10-3.10 (1.9)	0.40-1.80 (0.8)	0.56-6.63 (2.31)	0.27-3.59 (1.34)
Total Oxidised Nitrogen (mg/L N)	0.03-1.33 (0.26)	0.04-0.50 (0.18)	0.09-0.24 (0.16)	0.03-0.50 (0.28)	0.03-0.22 (0.16)	0.04-1.33 (0.43)	0.06-0.8 (0.21)

Table 2.1 The range and mean (parenthesis) physicochemistry of the 91 studied lakes with seasonal data for low (LA), moderate (MA) and high alkalinity (HA) lake types where available.

Parameter	All types annual	LA spring	LA summer	MA spring	MA summer	HA spring	HA summer
Total Phosphorus (mg/L P)	0.004-0.136 (0.019)	0.004-0.030 (0.009)	0.004-0.021 (0.008)	0.005-0.043 (0.021)	0.007-0.051 (0.019)	0.005- 0.131 (0.02)	0.005- 0.117 (0.019)
True Colour (Hazen)	3-211 (51)	10.9-165.5 (61.9)	11.8-164.6 (61.3)	29-102 (65)	19.6-177.2 (70.0)	4.5-97 (38.7)	2.5-72.6 (29)

Table 2.1 (Continued) The range and mean (parenthesis) physicochemistry of the 91 studied lakes with seasonal data for low (LA), moderate (MA) and high alkalinity (HA) lake types where available.

Supplementary	Symbol	LA	MA	HA	Candidate-Reference
Lake area (km ²)	area	0.06-9.2 (1.1)	0.04-19.5 (2.6)	0.06-381.8 (19.5)	0.1-1.7 (0.7)
Max. lake depth (m)	maxD	2.9-49.9 (19.9)	1.9-75.0 (17.6)	3.4-45.0 (17.8)	6.0-49.9 (17.5)
Easting	X	-	-	-	-
Northing	Y	-	-	-	-
Catchment area (km ²)	CA	0.2-162.6 (29.3)	0.23-557.8 (86.2)	1.1-10,643.0 (631.7)	0.2-120 (25.2)
Lake altitude (m)	altitude	4.6-375 (94.7)	6.5-189.9 (55.7)	1.8-60.8 (48.2)	9.0-137.8 (38.7)
Maximum fetch (km)	fetch_max	0.2-4.9 (1.2)	0.2-4.9 (1.7)	0.4-23.8 (2.7)	0.2-2.5 (1.0)
South-westerly fetch (km)	fetch_SW	0-2.9 (0.8)	0-2.9 (0.7)	0-23.8 (1.9)	0-2.5 (0.6)
Average wind speed (m ^{-sec})	wind	3.4-10.2 (5.4)	3.5-6.8 (5.2)	3.8-9.0 (5.0)	4.1-6.8 (5.0)
% Corine Landcover	AGRICULTURE_OTHER	0-16.9 (1.8)	0-99.7 (16.6)	0-36.8 (10.7)	0-8.9 (1.7)
% Corine Landcover	ARABLE	0-2.4 (0.06)	0-0.05 (0.04)	0-13 (1.2)	0-2.2 (0.2)
% Corine Landcover	BOG	18-100.0 (69.1)	0-63.0 (24.0)	0-58.6 (13.3)	0-100 (52.4)
% Corine Landcover	CONIFEROUS	0-77.7 (6.9)	0-22.0 (5.1)	0-21.1 (1.8)	0-11.7 (1.0)

Table 2.2 Supplementary physical, spatial and catchment landuse variables with variable codes and the range and mean (parenthesis) provided in low (LA), moderate (MA) and high (HA) alkalinity lakes. The mean and range for reference and best-available lakes (Candidate-Reference) is also provided.

Supplementary	Symbol	LA	MA	HA	Candidate-Reference
% Corine Landcover	FOREST	0-44.0 (5.3)	0-43.4 (17.4)	0-26.9 (6.3)	0-14 (4.6)
% Corine Landcover	NATURAL	0-56.7 (8.1)	0-7.3 (1.3)	0-90 (6.6)	0-90 (19.3)
% Corine Landcover	PASTURE	0-21.0 (1.8)	0-72.2 (26.2)	0-94.9 (45.8)	0-53.5 (14.4)
% Corine Landcover	URBAN	0-0.1 (0.004)	0-4.7 (0.6)	0-5.4 (0.7)	0-1.8 (0.1)
% Corine Landcover	WATER	0-31.3 (7.0)	0-30.7 (8.9)	1-39.5 (12.8)	0-14.9 (7.3)

Table 2.2 (Continued) Supplementary physical, spatial and catchment landuse variables with variable codes and the range and mean (parenthesis) provided in low (LA), moderate (MA) and high (HA) alkalinity lakes. The mean and range for reference and best-available lakes (Candidate-Reference) is also provided.

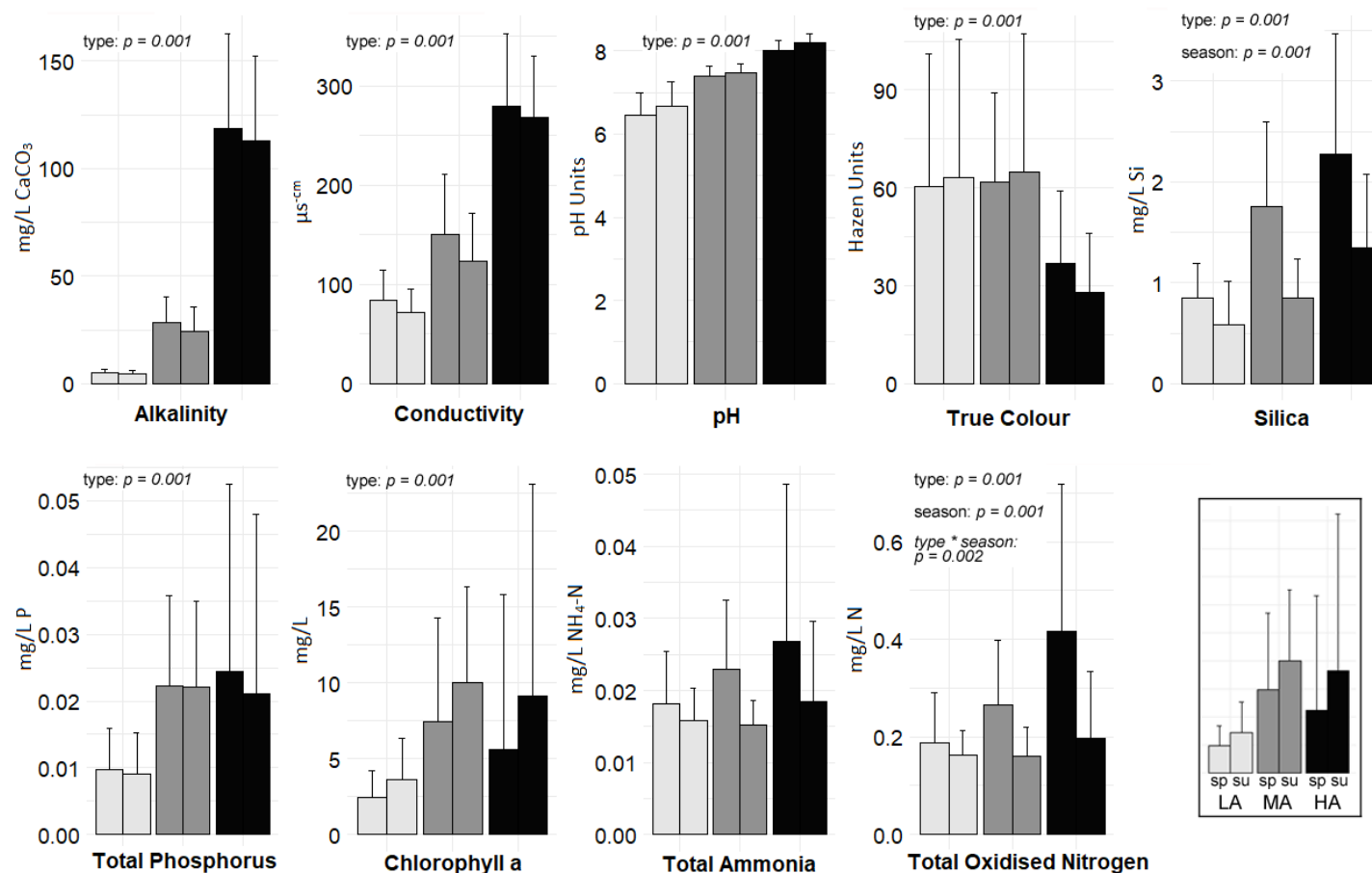


Figure 2.2 Mean and 95% confidence interval of lake physicochemical variables for the three a priori lake types in spring (sp) and summer (su) seasons, with results of Bonferroni corrected tests of significance between lake type, season, and their interaction indicated where significant.

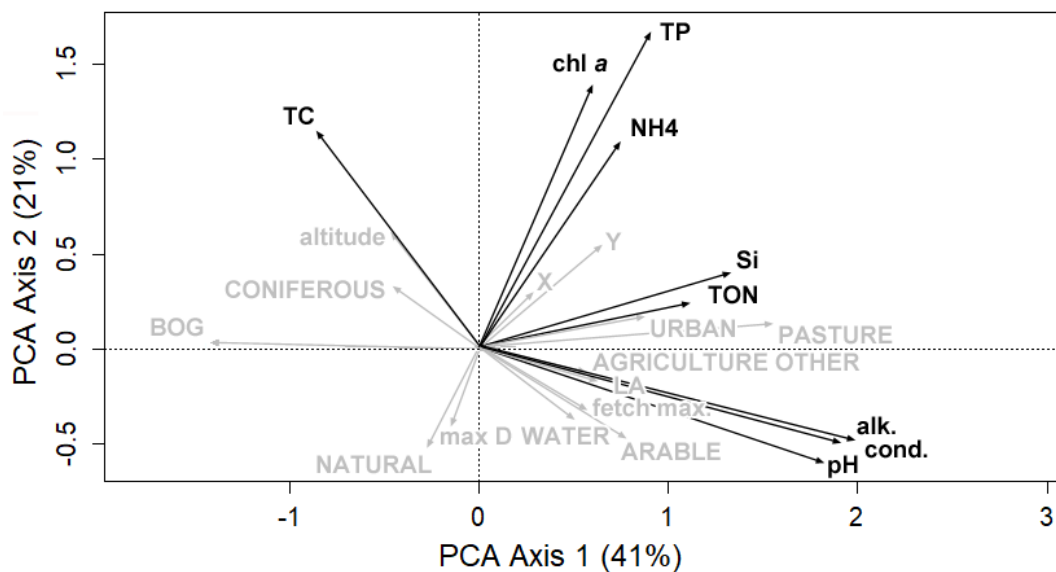


Figure 2.3 Principal Component Analysis (PCA) ordination of lake physicochemistry in the 91 study lakes, displaying PCA axes 1-2 with physicochemical variables displayed as black arrows and significant supplementary variables represented passively in the ordination as grey arrows. Codes and abbreviations used for all variables are provided in Table 2.1-2.2 and a biplot of PCA axes 1-3 is provided in Appendix C, Figure C.2.

2.4.2. EPILITHIC DIATOM ASSEMBLAGES

Littoral periphyton communities were compositionally highly diverse. They comprised of simple thin biofilms, or more complex communities with extensive macroalgae and cyanobacterial crusts. Diatoms were occasionally also macroscopically conspicuous, particularly in spring. In larger western lakes (e.g. Lough's Corrib, Mask and Conn) *Didyomosphenia geminata* (Lyngbye) Mart.Schmidt was prolific on stable substrate at exposed shorelines. Submerged mucilaginous colonies of *Cymbella* C.Agardh and *Encyonema lacustre* (C.Agardh) Pantocsek were attached to crusts of *Schizothrix* Kützing ex Gomont in some calcareous waterbodies. The filamentous *Diatoma ehrenbergii* Kützing was abundant in spring at groundwater inflows and some nutrient enriched shorelines. *Eunotia pectinalis* (Kützing) Rabenhorst developed extensive mats in a low alkalinity nutrient impacted lake but was less prolific in other oligotrophic

waterbodies. The centric diatoms *Stephanodiscus* Ehrenberg, *Cyclotella* (Kützing) Brébisson and *Aulacoseria* Thwaites were the commonest planktonic forms and dominated strews in summer in eutrophic lakes.

Of 450 non-planktonic diatom taxa identified in the 210 samples from 91 lakes, 247 taxa in 50 genera had a relative abundance of $\geq 2\%$ in at least one sample. With the exception of distinctive diatoms and *Brachysira* Kützing and *Encyonopsis* Krammer where Irish populations have been critically examined, taxonomic and multivariate analysis proceeded principally on species complexes. *Achnantheidium minutissimum* (Kützing) Czarnecki was ubiquitous and abundant in all three lake types. It was species rich (e.g. *A. affine*, *A. lineare*, *A. exile*, *A. altergracillima*, *A. caledonica*, *A. eutrophilum*, *A. straubianum*) with *A. hoffmannii* Van de Vijver *et al.*, *A. neomicrocephalum* Lange-Bertalot & Staab and *A. zhakovschikovii* Potapova reported for the first time in Ireland. However, many populations were not easily differentiated, and detailed taxonomic investigations of this dominant group in the epilithon of freshwaters in Ireland is required. *Encyonopsis* and *Brachysira* were the next most abundant genera and were restricted mostly to lakes of higher and lower alkalinity respectively, with some exceptions e.g. *B. neglectissima* Lange-Bertalot and *E. neerlandica* Van de Vijver *et al.* occurred in high and lower alkalinity lakes, respectively. Some generalist genera occurred in almost all lakes (e.g. *Encyonema*, *Fragilaria*, *Gomphonema*) while others were associated with specific lake types (e.g. LA: *Frustulia*, *Eunotia*, *Stauroforma* or HA: *Amphora*, *Mastogloia*). A high diversity of unidentified *Nitzschia* and *Navicula* were recorded but these were rarely abundant relative to more predominate diatom complexes (of e.g. *Fragilaria capucina*, *G. pumilum*, *G. parvulum* and *C. affinis*).

The majority of diatoms were distributed independently of season (Appendix B). While microscopic counts of *D. ehrenbergii* and *D. tenuis* suggested a preference for spring, only *D. moniliformis* had a statistically significant indicator value, as did *N. lanceolata*, *G. tergestinum*, *G. occultum*, *G. olivaceoides* and *G. olivaceum*. Nitrogen fixing *E. adnata* and *E. sorex* were identified as significant summer indicators with *M. lacustris* and *N. capitatoradiata*. Significant differences in diatom species number and diversity were found between some lake's clusters identified below, but there were no significant seasonal differences in diversity within the individual groups (Appendix G, Figure G.1).

2.4.3. DIATOM COMPOSITIONAL GROUPS

Cluster analysis identified six to seven groups of diatoms as optimal with a nominal difference in the classification of a small number of lakes amongst two clusters with either of these solutions (Appendix E). The following six clusters or groups were characterised with reference to their dominant and significant indicator taxa, species diversity and lake environmental characteristics (see Appendix F and Appendix G for detailed characterisation).

Cluster-1: Circumneutral oligo-mesotraphentic

Assemblages were dominated by *A. minutissimum* and mesotraphentic diatoms in circumneutral lakes with low to moderate nutrient levels in catchments with mixed landuse.

Cluster-2: Low alkalinity oligotraphentic

Assemblages were dominated by *Brachysira*, acidophilus diatoms and *A. minutissimum* exemplifying oligotrophic, weakly-acidic peatland lakes on siliceous geology, incorporating low alkalinity reference waterbodies.

Cluster-3: Acidophilous

This small distinctly more acidophilus assemblage was characterised by an absence of *Achnantheidium* and occurred in highly coloured peatland lakes at higher elevation.

Cluster-4: Alkalibiontic oligo-mesotraphentic

Assemblages were dominated by *Achnantheidium* and *Encyonopsis* in well buffered lakes with low to intermediate nutrient levels with mixed peat and pasture landcover.

Cluster-5: Alkalibiontic oligotraphentic

Assemblages were dominated by *Encyonopsis* and alkalibiontic indicators of oligotrophic to weakly mesotrophic calcium bicarbonate rich lakes and incorporated several high alkalinity reference waterbodies.

Cluster-6: Alkalibiontic eutraphentic

Achnantheidium and eutraphentic diatoms with high species richness and Shannon diversity occurred in lakes with elevated total phosphorus and catchments with variable landcover categories.

2.4.4. DIATOM-ENVIRONMENT RELATIONSHIPS

A CCA on 100 samples in 86 lakes where diatoms were assessed at the same monitoring location in both seasons identified 8 and 9 explanatory variables as

significant in spring and summer, respectively ($p < 0.05$). CCA-tripplots (Figure 2.4) broadly reflected the major pattern in lake physicochemistry identified in PCA (Figure 2.3). Diatom assemblages were strongly associated with pH-conductivity and wind speed to a lesser extent on CCA axis-1. Candidate reference lakes with low total phosphorus and low colour on the western seaboard contrasted strongly with the acidophilous (Cluster-3) and eutraphentic (Cluster-6) groups in the ordinations. The acidophilous assemblage had the most distinctive position and was negatively correlated with pH and conductivity and strongly associated with increasing colour and wind speed. The overlap in oligotraphentic to eutraphentic lakes positioned to the right of the ordination were indicative more of a gradient in trophic condition along CCA axis-2 in these alkalibiontic waterbodies. The circumneutral oligo-mesotraphentic cluster comprised of many moderate alkalinity lakes dispersed around the centre of the ordination and these overlapped several other groups. While the characterised diatom clusters had an equivalent pattern in both spring and summer CCAs, individual lakes comprising the clusters varied, with approximately one third of lakes changing group in different seasons. However, these variable lakes were predominately associated with the circumneutral oligo-mesotraphentic assemblage and clusters located to the right of the CCA ordinations. Both reference and nutrient impacted lakes changed cluster identity between the seasons but there was no obvious directional change associated with spring or summer in the latter group. Cluster identity in the acidophilous lake assemblages and many reference lakes in the low alkalinity oligotraphentic group was constant in both seasons. Overall difference in diatom assemblages between the seasons was not significant (ANOSIM Global $R = 0.014$, $P = 0.042$). Partial-CCA indicated that the unique effect of lake physicochemistry

on diatom variation was greater than that of the combined physical and spatial variables and it was marginally higher in the summer dataset (Table 2.3.).

Season	Explanatory variables	% variance explained	<i>p</i> -value
Spring	All significant CCA variables	19.2	0.001
	Significant Physicochemical variables	11.8	0.001
	Significant Physical-spatial variables	6.2	0.001
	Shared variance	1.2	-
Summer	All significant CCA variables	21.2	0.001
	Significant Physicochemical variables	15.0	0.001
	Significant Physical-spatial variables	4.3	0.001
	Shared variance	1.9	-

Table 2.3 Results of partial canonical correspondence analysis (pCCA) comparing the pure and shared effect of two groups of environmental variables (physicochemical versus physical-spatial defined in the methods) that were found to be significant in the spring and summer ordinations illustrated in figure 2.4.

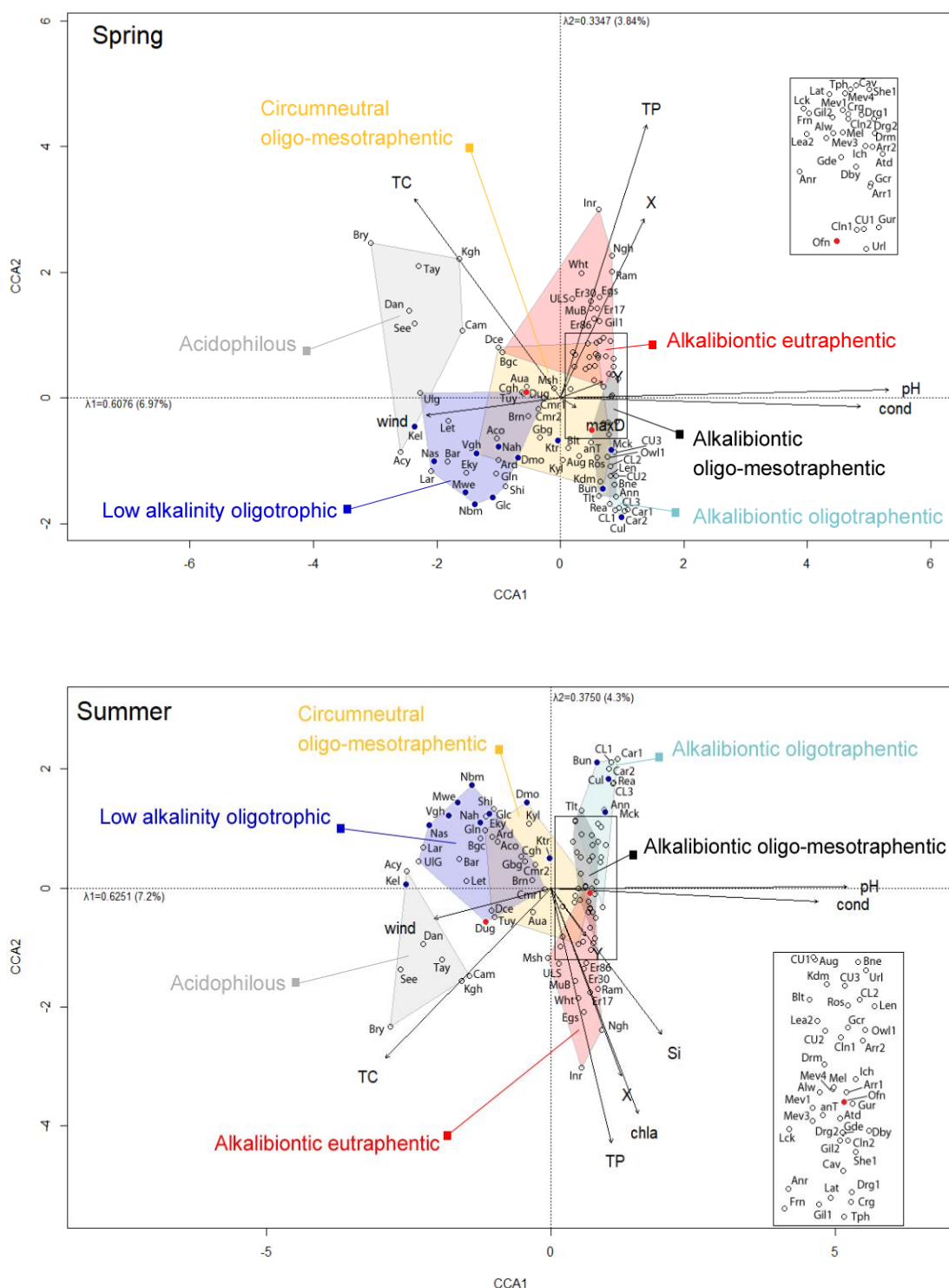


Figure 2.4 Canonical Correlation Analysis (CCA) triplots for spring and summer seasons showing the distribution of lakes along the first two axes based on the relationships between diatom taxa and significant environmental variables. Groups resulting from cluster analysis are fitted to convex hulls and lakes coloured with blue or red circles identify candidate reference lakes discussed in the text.

2.4.5. WFD CLASSIFICATION

Reference Condition

To establish an 'expected value' of the Lake Trophic Diatom Index in Irish lakes for the denominator of EQR calculations, metric scores from 18 candidate reference waterbodies sampled at varying frequencies over a 10-year interval since 2007 (117 samples) were compiled. These waterbodies were considered in conjunction with paleoecological assessments reported in 2006 and 2010 (Figure 2.5). Five low alkalinity lakes met strict reference screening criteria having low levels of anthropogenic landcover and reference condition validated with paleolimnology (L. Maumwee, Veagh, Nahasleam, Keel & Upper). A further five lakes lack paleoecological data, but their catchments are minimally disturbed and not intensively managed (L. Nambrackmore, Nasnanida, Travaun, Glencullin & Doo). Two lakes (L. Bunny & Kiltorris) have high cover of pasture but nevertheless reference condition was confirmed in these waterbodies. Other lakes lack paleoecological data (L. Skeardeen) or assemblages were found to deviate from reference conditions (L. Cullaun & Muckanagh). These latter lakes characterised as 'best-available' (*sensu* Stoddard *et al.* 2008) are retained to assess variation in LTDI scores amongst high quality marl lakes. Other lakes (L. O'Flynn, Dunglow & McNeen) were confirmed to be in reference condition but there were issues with the quality of paleoecological data in some cases (Leira *et al.*, 2006). A reliable chronology could not be established with certainty for L. O'Flynn and proliferation of planktonic and benthic cyanobacteria (*Microcystis* Lemmermann and *Microcoleus* Desmazières ex Gomont) with variable phosphorus levels suggested a decline in ecological condition. While reference status was confirmed in L. Dunglow its peatland catchment is extensively modified for turbary

and variable phosphorus levels and increased variance in LTDI scores indicated a possibility of a response to increased catchment pressure for this waterbody. While somewhat a circular argument, diatom assemblages in the latter two lakes did not group as closely as other reference waterbodies in the CCA ordinations (Figure 2.4). Reference status in L. McNean was based on a short sediment core with a limited chronology, while WFD classifications indicate that this lake does not meet good status objectives currently (O'Boyle *et al.*, 2019). When these latter lakes are discounted, the median LTDI increased progressively from LTDI = ~7.5 until a levelling off at LTDI = ~23. The upper limit in this range is comparable to that reported from a larger paleoecological assessment of low alkalinity lakes in the UK (LTDI = 20) and the value adopted in Ireland during a formal EU intercalibration exercise (Kelly *et al.*, 2014). There was no indication of any grouping of LTDI scores by lake alkalinity type (Figure 2.5).

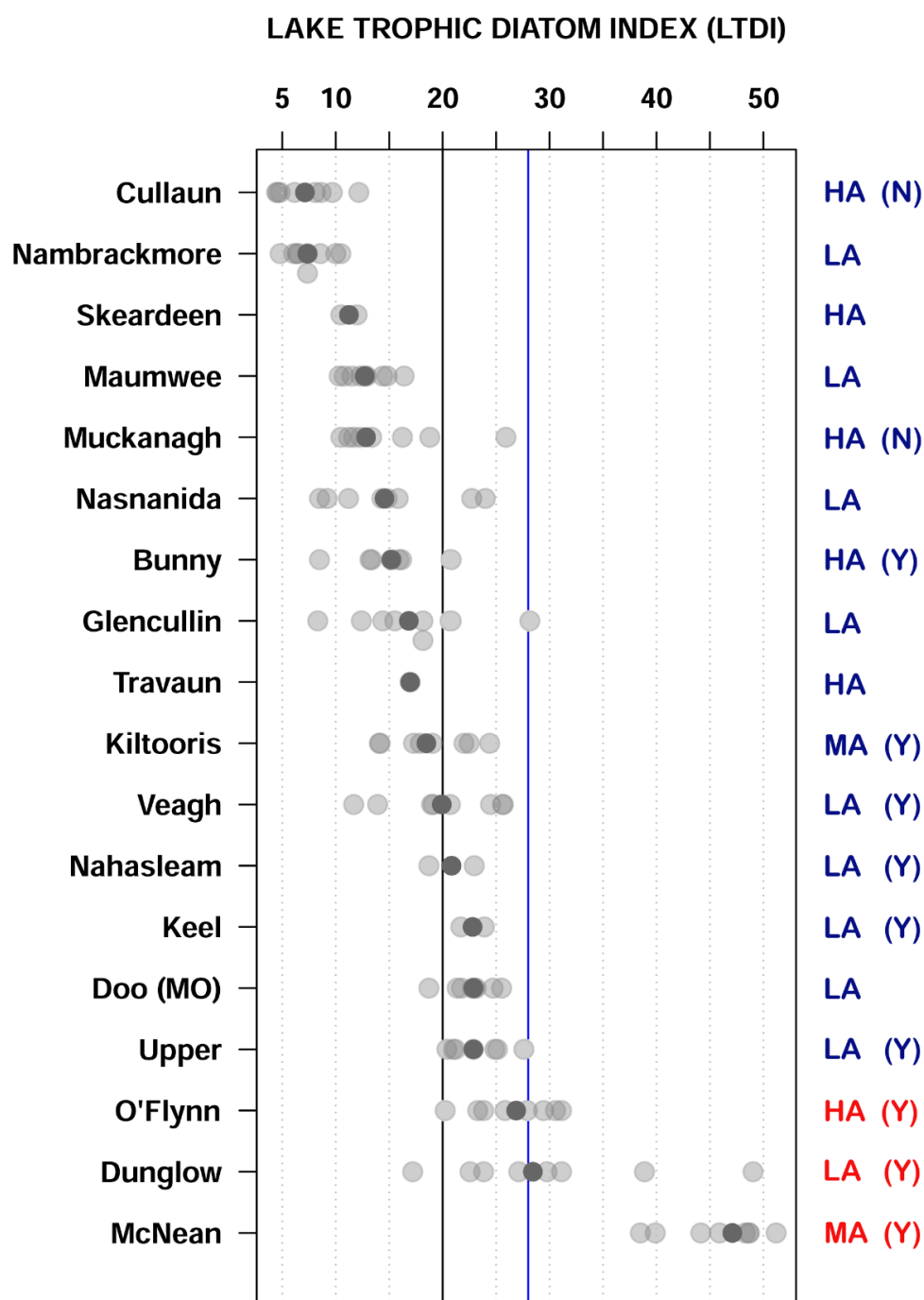


Figure 2.5 Scatterplot of the Lake Trophic Diatom Index (LTDI) for the compiled candidate reference lakes. Grey points represent LTDI scores and the series is ordered by increasing median LTDI value identified with darker circles. Lakes are identified as low (LA), moderate (MA) and high (HA) alkalinity. Where results of paleolimnology are available then reference condition when validated is indicated with a (Y) or alternatively with (N). A vertical line at LTDI = 20 indicates the expected value of the LTDI used for WFD classification. The blue vertical line at LTDI = 28 indicates the position of the high-good status class boundary. Three lakes where reference condition is in doubt are indicated in red.

2.4.6. DIATOM CLASSIFICATION & ECOLOGICAL QUALITY RATIO (EQR)

The relationship between diatom Ecological Quality Ratios (EQR; with expected value of LTDI = 20 for all lake types) and the physicochemical parameters was evaluated in a multiple stepwise regression analysis, separately for both seasons. Based on the *a priori* assumption that diatom EQRs based on the Lake Trophic Diatom Index should be most significantly related to total phosphorus, the relative proportion of explained variance in the optimal models was compared with a model based only on total phosphorus. Given the smaller sample size and shorter trophic gradients in lower alkalinity groups, models were also assessed after removal of three waterbodies with a large influence on performance (Figure 2.6). It was suspected that open water chemistry did not accurately reflect the nutrient condition at these shoreline stations. Average lake EQR was used for larger lakes with multiple sampling stations. A model that included total phosphorus and ammonium in spring had only marginally higher explanatory power ($R^2_{\text{spring}} = 0.60$, $p < 0.01$) compared to the single parameter phosphorus model ($R^2_{\text{spring}} = 0.59$, $p < 0.0001$) when evaluated with the AIC. The optimal model in summer incorporated conductivity, alkalinity, and total phosphorus but it also had only marginally higher explanatory power ($R^2_{\text{summer}} = 0.61$, $p < 0.0001$) relative to the total phosphorus model ($R^2_{\text{summer}} = 0.57$, $p < 0.0001$).

A significant proportion of the variability in EQR was explained by total phosphorus in high alkalinity lakes and was broadly comparable in both seasons (Table 2.4; Figure 2.6). The strength of relationships decreased and was more seasonally variable in moderate and low alkalinity lake groups but remained highly significant. Lakes with a comparable range of total phosphorus ($\leq 0.05 \text{ mg}^{-\text{L}}\text{-P}$) were used to test for differences in the regression lines amongst the three lake alkalinity types (Appendix

H). The assumption of homogeneity of regression slopes in the EQR-phosphorus relationships was confirmed in ANCOVA, with nonsignificant interaction terms for total phosphorus and lake type in both spring, $F(2, 75) = 1.0329$, $p = 0.36$ and summer $F(2, 78) = 0.4585$, $p = 0.63$. There was a significant effect of lake type on EQR after controlling for total phosphorus only in the summer data, $F(2, 80) = 4.122$, $p = 0.02$. Tukey post hoc tests revealed that the covariate adjusted mean of the moderate alkalinity lakes was significantly greater than both low alkalinity (difference = 0.13, $t = 2.57$, $p < 0.031$) and high alkalinity lakes (difference = 0.13, $t = 2.8$, $p < 0.0181$) in summer, while the low and high alkalinity types were not significantly different (difference = 0.0, $t = 0.006$, $p < 1.0$). Histograms of diatom Ecological Quality Ratio (EQR) for lake diatom samples monitored over a ten-year interval in the three lake alkalinity types is provided in Appendix I, Figure I.1.

Seasonal variability in the LTDI decreased markedly in lakes with index scores clustered below $LTDI = 18-20$ and there was an indication that average LTDI was slightly higher in spring samples at higher values of the metric (Figure 2.7). Exploratory analysis of potential covariates (e.g. lake exposure and seasonal water chemistry parameters) and lake groups (e.g. lake types and assemblage clusters) failed to detect factors to explain patterns of residual variability in the LTDI scores. The most seasonally variable scores were centred around the middle of the LTDI gradient and often associated with major shifts in the relative abundance of *A. minutissimum*, *A. pyrenaicum*, *E. microcephala*, *Diatoma*, *Amphora* and *Navicula* between seasons, but there was no clear directional change associated with spring or summer.

Lake Type	Season	R ² -adjusted	<i>p</i> -value	Model
All	SP	0.53	<0.0001	TP - quadratic
All	SU	0.58	<0.0001	TP - quadratic
HA	SP	0.65	<0.0001	TP - quadratic
HA	SU	0.70	<0.0001	TP - quadratic
MA	SP	0.20 (0.62)	0.13 (0.0124)	TP - linear
MA	SU	0.25 (0.50)	0.23 (0.0030)	TP - linear
LA	SP	0.15 (0.28)	0.008 (0.0005)	TP - linear
LA	SU	0.40	<0.0001	TP - linear

Table 2.4 Results of regression analysis quantifying the strength and significance of the relationships between total phosphorus (TP) and Lake Trophic Diatom Index EQRs in all lakes (ALL) and the low (LA), moderate (MA) and high (HA) alkalinity lake groups in spring (SP) and summer (SU). Values in parenthesis are statistics for LA and MA lakes with potential outlier lakes removed prior to model fitting.

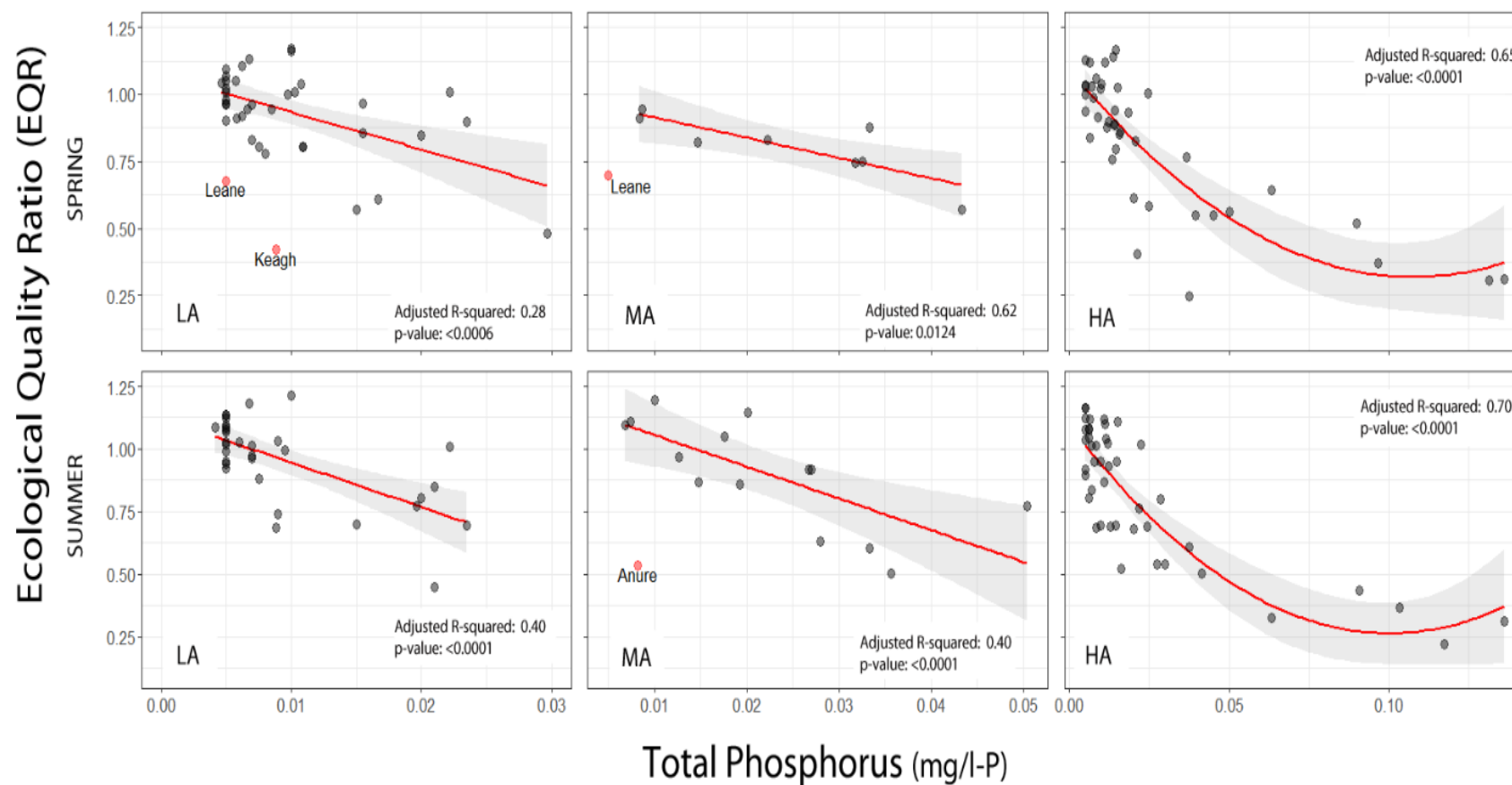


Figure 2.6 Relationships between mean lake total phosphorus concentrations and Ecological Quality Ratio (EQR) with 95% confidence limits for low (LA), moderate (MA) and high (HA) alkalinity lake types in spring and summer. Outlier lakes are identified.

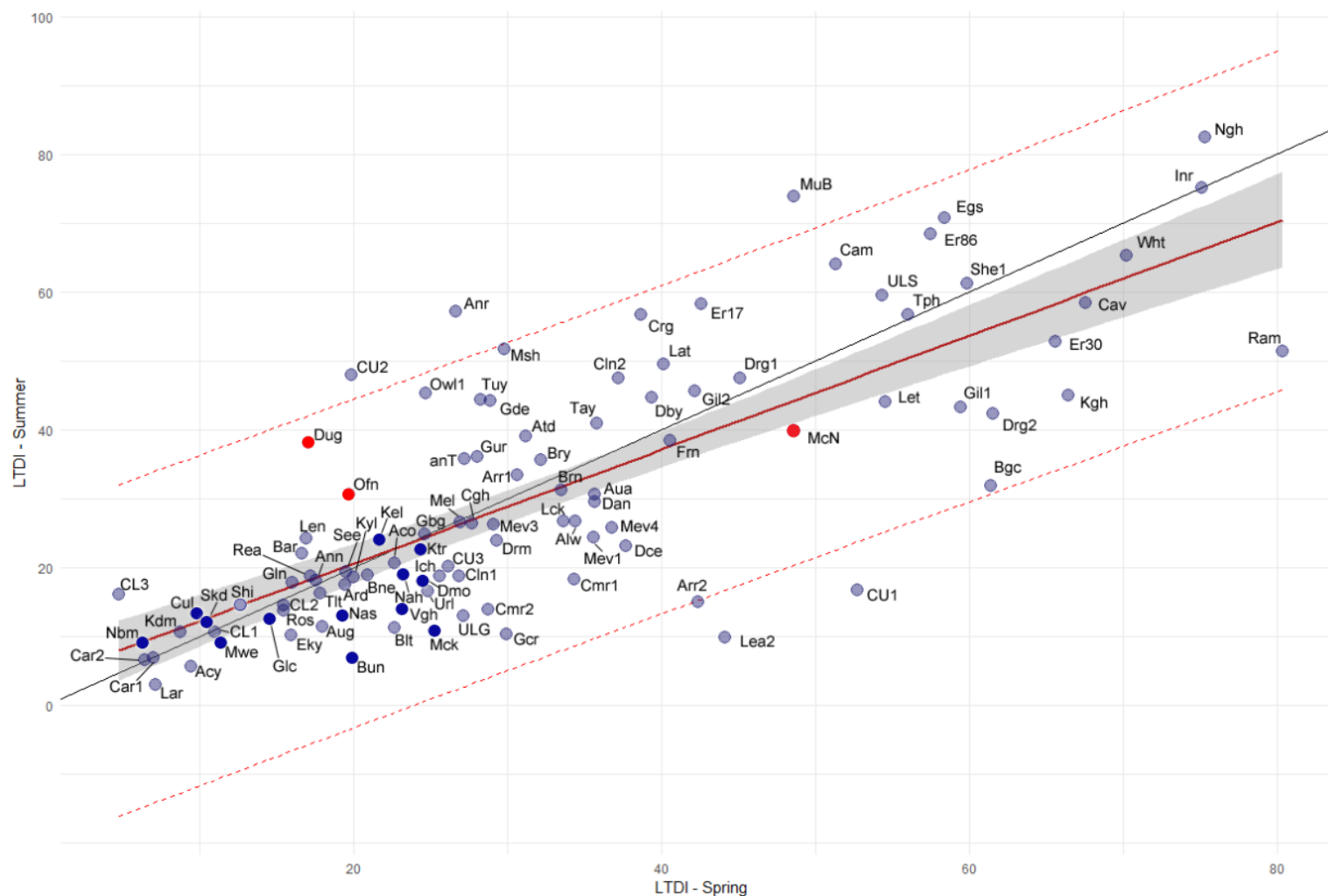


Figure 2.7 Regression of spring and summer LTDI metric values with fitted confidence (grey) and prediction (red) intervals, and a one to one line (black) for comparison. Candidate reference lakes are indicated in dark blue and red.

2.5. DISCUSSION

2.5.1. OVERVIEW

In recent assessments more than half of all monitored lakes in Ireland failed to meet Water Framework Directive good status objectives and nutrient enrichment was identified as the major stressor impacting lake ecological condition (DAERA 2020; O'Boyle *et al.*, 2019). This study evaluated lake physicochemistry with PCA for a range of these reference and impacted waterbodies and investigated their predominate epilithic diatom assemblages with cluster analysis and CCA. It established that the same major water chemistry gradients strongly associated with regional landcover patterns were also reflected in the distribution of six characteristic diatom assemblages. Our results revealed a highly significant relationship between total phosphorus and the Lake Trophic Diatom Index (LTDI) in lakes of contrasting alkalinity and established its efficacy for ecological classification. The clearest seasonal pattern that emerged was a consistently lower variability in metric scores in lakes with higher ecological quality ratios and unpredictable increases in metric variability with increasing nutrients thereafter.

2.5.2. LAKE ENVIRONMENTAL VARIATION

There were study limitations owing to the availability of fewer moderate alkalinity and nutrient impacted low alkalinity lakes in the dataset. Hydrogeochemistry in the Irish uplands indicates improving trends associated with reduced levels of atmospheric deposition, but biological validation of environmental quality is sporadic and the full gradient of acid-impacted lakes was also not incorporated in our study. (Aherne and Curtis, 2003; Battarbee *et al.*, 2014; Burton and Aherne, 2011; Leira *et al.*, 2006; O'Dwyer

and Taylor 2010). Nevertheless, we explored epilithic diatom diversity in waterbodies that represents the broad range of lake condition found in Ireland.

It is often challenging to fully separate the effects of covarying gradients of alkalinity and nutrients on aquatic communities (Bennion *et al.*, 2014; Fairchild and Sherman, 1993). However, while alkalinity was positively associated with landcover, total oxidised nitrogen and silica, it had a low correlation with total phosphorus in our study. Marl and non-marl calcifying lakes have a high sorption capacity for natural levels of bioavailable phosphorus that increase with alkalinity (Vighi and Chiaudani, 1985). Phosphorus levels in these oligotrophic waterbodies is also a consequence of the relatively low loading of reactive phosphate expected in high status rivers and groundwaters in limestone catchments in Ireland ($<10 \mu\text{g}^{-\text{L}}$ and $<15 \mu\text{g}^{-\text{L}} \text{PO}_4\text{-P}$, respectively; O'Boyle *et al.* 2019). Catchment cover of pasture and peat had the strongest association with the primary water chemistry gradient, while other categories of landcover and physical variables were less influential. This dominant influence accounts for the predominance of oligotrophic lakes in the western periphery, with nutrient enriched lakes increasing with increasing longitude as reported in both regional and national scale studies previously (Chen *et al.*, 2008; DeNicola *et al.*, 2004; Macintosh *et al.*, 2019; O'Boyle *et al.*, 2019). The close similarity of both the PCA and CCA results demonstrated that landcover, water chemistry and diatoms were also all very strongly coupled.

2.5.3. DIATOM COMPOSITION

Diatom populations varied greatly in terms of their abundance and composition across the network of lakes. In field observations, filamentous and colonial diatoms

were also conspicuous on occasion. *Didymosphenia* is widely distributed in Irish rivers but interestingly, it was more prolific in the eulittoral of three large western lakes, although frustules were rare in the diatom strews from these habitats. Anecdotal accounts confirm it from Lough's Corrib and Mask for at least three decades, where environmental conditions are equivalent to that reported for *Didymosphenia* blooms elsewhere (Cantonati and Lowe, 2014; Whitton *et al.*, 2009). Although average annual total phosphorus was often $\leq 10 \mu\text{g}^{-\text{L}}\text{-P}$, periods of greater eutrophication have resulted in declines in water quality in all of these lakes at various stages in the past (McCarthy *et al.*, 2000; McGarrigle & Champ, 1999; Murnaghan *et al.*, 2012). Increasing nutrient trends, infestations of *Dreissena*, climate change and its effect on organic phosphorus loading are a complex series of factors to relate to the pre-existing and future bloom events for these waterbodies.

The ordination analyses showed a clear distribution of candidate reference lakes that were all phosphorus limited and separated along a gradient of alkalinity-pH and true colour. Marl lakes were dominated by the alkalibiontic genera *Encyonopsis* and *Mastogloia*, analogous to calcareous alpine and karstic waterbodies in other regions (Bahls 2004; Feret *et al.*, 2017; Gaiser *et al.*, 2011; Lee *et al.*, 2014). Circumneutral to weakly-acidic waterbodies had a concomitant succession of *Achnanthyidium*, *Encyonopsis* and *Brachysira* that culminated with a greater relative abundance of the acidophilous *Tabellaria flocculosa* and *Eunotia* in response to increasing lake acidity and true colour. Marl lakes are naturally colour limited as a consequence of their calcareous lithologies and groundwater contribution in catchments with low organic soil cover. True colour is a proxy for dissolved organic carbon (DOC) and is of particular ecological importance for assemblages in low alkalinity waterbodies that have a

widespread distribution in Ireland (Dalton 2000; Fallu *et al.* 2002; Fork *et al.*, 2020). However, as a typological factor independent of anthropogenic influences it is problematic. The expected range of DOC is waterbody dependent and varies in response to natural catchment processes and climate (Free *et al.*, 2000; Webster *et al.*, 2008). But increased DOC is also generated with disturbance in peatlands and these have a prolonged history of degradation over millennia (Bradshaw and McGee, 1988; Leira *et al.*, 2006; Ryder *et al.*, 2014). The acidophilous assemblage in cluster-3 was more highly coloured and had a low pH range (pH 5-6) with the lowest species richness as reported from dystrophic and acidified waterbodies in other regions (Jüttner *et al.*, 2020; Kwadrans 2007; Schönfelder *et al.* 2002). Relative to the other low alkalinity reference lakes, Lough Keel was the most highly coloured lake. It had a lower average pH (pH = 5.5) and a distinctly more acidophilus assemblage of *Tabellaria flocculosa*, *Eunotia mucophila* and *E. incisa*. The possibility that L. Keel may have responded to an early phase of peatland disturbance was indicated in Leira *et al.* (2006) but nevertheless its reference status was verified. Its lake outlet is slightly modified for drinking water abstraction and macrophyte communities were found not to meet WFD objectives more recently, which may call its reference status into question. Nevertheless, pre-acidification assemblages equivalent to L. Keel have been reported from reference peatland lakes elsewhere (Battarbee *et al.*, 2011). Assuming it meets reference condition, its diatom assemblage may represent the extreme of naturally acidic or dystrophic lakes in Ireland.

A biological response to increased acid deposition has been established in higher altitude acid-sensitive lakes on the east coast where several the acid sensitive lakes are situated (Battarbee *et al.* 2014; Flower *et al.* 1994). The highly coloured L. Bray

Lower is the highest altitude waterbody in the dataset and was formerly signified to be anthropogenically acidified (Linnane and Murray, 2000). Its distinctive assemblage of *Psammothidium helveticum* and *Pinnularia subcapitata* is indicative of anthropogenic acidification, although *P. helveticum* dominated over *Pinnularia* and has a pH optimum that ranges from weakly-acidic to circumneutral in other studies (Juggins *et al.*, 2016; Lange-Bertalot *et al.*, 2017). Other acidobiontic indicators of higher acidity such as *E. subarcuatoides*, *Tabellaria quadrisepata* and *Eunotia exigua* were absent or less prevalent than reported previously, indicating a generally low acidification pressure in comparison to more significantly affected regions in Europe (Battarbee *et al.* 2014; Chen *et al.*, 2008; Leira *et al.*, 2006; Van Dam 1996; Van Dam *et al.* 1994). However, the ecological status of several very highly coloured lakes characterised as acid oligo-mesohumic to acid polyhumic is more questionable, given their mixed assemblages of *Psammothidium helveticum*, *Sellaphora* sp., *Frustulia erifugia*, *T. flocculosa*, *E. pectinalis* and *E. incsa* (Kwandrans and Eloranta, 2005; Kwandrans 2007). Although these lakes differed floristically from the low alkalinity lakes in the reference network, they were nevertheless classified as high and good status with the Lake Trophic Diatom Index. Lough Seecon for example had a high status LTDI value but achieves only a moderate WFD ecological classification owing to elevated total phosphorus and moderately impacted macrophyte community. Conspicuous littoral accumulations of *E. pectinalis* were a notable feature in this lake. *E. pectinalis* is also reported from weakly eutrophic habitats so the indicator sensitivity value for this diatom may need to be revised (Lange-Bertalot *et al.*, 2017). Lough Acorrymore also had a high status assemblage of *Brachysira garrensis* as often occurs in weakly-acidic peatland lakes in Ireland (Chen *et al.*, 2006; Kennedy and Allott, 2018).

But the filamentous green alga *Stigeoclonium* was very prolific. When it was microscopically examined extensive hair formation implicated in the acquisition of organic-phosphorus was apparent, possibly in response to degrading peat deposits and sheep grazing near the sampled shoreline (Gibson and Whitton, 1987). Such proliferations provide evidence for functional responses to enrichment that are not reflected in diatom metrics currently. They also provide potential opportunities for improvements in phytobenthos assessments in low alkalinity waterbodies. Conversely, Loughs Cam and Keagh and some low alkalinity lakes in other clusters had a greater abundance of the eutraphentic *Sellaphora nigri* than is typically encountered in low alkalinity lake contexts (Bennion *et al.*, 2014; Kelly *et al.*, 2014). These represented a clearer response to nutrient enrichment. Most of the nutrient impacted lakes were high alkalinity, but moderate alkalinity lakes and the low alkalinity Lough Beaghcauneen were identified as alkalibiontic-eutraphentic. Lough Beaghcauneen had high chlorophyll levels and a moderate status macrophyte community. Its spring diatom assemblage comprised of a reduced abundance of *Achnantheidium* with a greater representation of tolerant diatoms such as *Mayamaea permitis*, *Encyonema sileacium*, *E. minutum*, *Navicula* and *Nitzschia* in response to the increased nutrient pressures in that waterbody. All alkalibiontic-eutraphentic lakes generally had lower abundances of *Achnantheidium* and this group had the highest taxonomic diversity. Nutrient tolerant taxa featured prominently and included the genera *Rhoicosphenia*, *Nitzschia*, *Navicula*, *Encyonema*, *Gomphonema* and *Cocconeis*. These findings are consistent with that expected in nutrient impacted lakes elsewhere (Kelly *et al.*, 2014).

2.5.4. DIATOM ENVIRONMENT RESPONSE

The primary gradients influencing diatom composition were pH-alkalinity, total phosphorus and true colour. These results are generally consistent with those reported in western European lakes and are equivalent to surface sediment and periphyton investigations in Irish lakes previously (Chen *et al.*, 2008; DeNicola *et al.*, 2004; Gottschalk and Kahlert 2012; Kelly *et al.*, 2014; Snell and Irvine, 2013). The results of variance partitioning indicated that physical factors were less important in explaining diatom composition. While several studies have found lake depth to be significant (Feret *et al.*, 2017; Riato and Leira, 2020; Schönfelder *et al.*, 2002) our analyses agrees with others that indicated that morphology had a limited role (Bennion *et al.*, 2014; Blanco *et al.*, 2014, Chen *et al.*, 2008). Given the expectation that station exposure and physical disturbance might have a major influence, we incorporated lake fetch and average wind speed as explanatory variables in the multivariate analysis (Cantonati *et al.*, 2009; Cantonati and Lowe, 2014; Rimet *et al.*, 2019). For example, Rimet *et al.* (2015) reported a succession in benthic diatom guilds in Lake Geneva for a lake station frequently exposed to winds and waves, with an increased abundance of pioneer taxa adapted to the turbulent conditions. However, other within-lake studies have found less change in response to physical disturbance (McCabe and Cyr, 2006). Neither of our proxies fully captures the extent of local scale conditions prevailing in advance of diatom collection. Nevertheless, average wind speed was identified as having a significant correlation with diatom composition. It is possible that this finding more likely represented an association with some other latent variable given the similarity of the wind effect in both spring and summer ordinations. Wind speed was weakly correlated with other explanatory variables

including altitude, a variable that has been established to be important but in studies at greater elevation than occurs in Ireland (Feret *et al.*, 2017; Gottschalk and Kahlert, 2012). Linkages with unmeasured but potentially important climatic variables such as temperature will need to be clarified in future assessments.

2.5.5. LAKE REFERENCE CONDITIONS

A prerequisite to WFD classification is the establishment of waterbody types using abiotic criteria to group the distinctive reference communities expected in different natural environments. This process is intended to enable like-with-like comparisons between equivalent biological communities and improve the precision of biological classifications (Pardo *et al.*, 2012). While sediment and epiphytic diatoms studies incorporating Irish lakes have indicated lake depth to be a weakly significant factor, these investigations incorporated planktonic diatoms and were less relevant in our epilithic study (Blanco *et al.*, 2014; Chen *et al.*, 2008; Rodríguez-Alcalá *et al.*, 2020). As expected, diatom assemblages were characterised more by gradients in response to changes in water chemistry than with discrete groups, but nevertheless a simplified typology based on alkalinity provided an acceptable option to group waterbodies with similar characteristics (Hatton-Ellis, 2008; Kelly *et al.*, 2014; Søndergaard *et al.*, 2020). Given the extent of heterogeneity in the diatom flora of the generalised low alkalinity lake type, the requirement for a humic lake sub-group within the national typology remains to be evaluated in more detail. This may help to differentiate lakes with naturally high organic carbon, from those responding to anthropogenically increased DOC in short term events or with longer-term climate projections in the future (Doyle *et al.*, 2019; Monteith *et al.*, 2007).

We found no differences in the distribution of LTDI scores amongst lake types in the candidate reference network. This lack of differentiation is surprising in the context of the relationship of naturally increasing phosphorus and alkalinity, and relative to studies that have documented greater differences in trophic condition between types (Bennion *et al.*, 2014; Cardoso *et al.*, 2007; Schaumburg *et al.*, 2004; Vighi and Chiaudani, 1985). In low alkalinity lakes, the results from contemporary diatom analysis and paleoecological investigations are broadly comparable across regions (Bennion and Simpson, 2011; Free *et al.*, 2016; Leira *et al.*, 2006; Bennion *et al.*, 2014). Diatom-inferred total phosphorus (DI-TP) in Irish marl lakes is comparable to the range observed in low alkalinity waterbodies and supports the equivalency of LTDI scores between these lake types in Ireland (Leira *et al.*, 2006). As high alkalinity waterbodies were often represented by marl lakes and calcifying limestone lakes, the prospect that LTDI scores could be naturally higher at intermediate alkalinities where phosphorus co-precipitation is lower was also considered. There was only a single viable moderate alkalinity reference lake in our dataset. Its LTDI range and diatom-inferred TP ($<10 \mu\text{g}^{-\text{L}}$ DI-TP) was also equivalent to moderate and high alkalinity lakes, indicating a similarity of reference concepts across the alkalinity gradient as was established in the lake water chemistry.

The significant majority of paleo-investigated lakes of all alkalinity classes were often within or below a $10 \mu\text{g}^{-\text{L}}$ DI-TP limit, but the full range of DI-TP encompasses oligotrophic to oligo-mesotrophic conditions (Leira *et al.*, 2006; Taylor *et al.*, 2006). This was also established for lakes with paleo-core bottom dates that precede the 1850 period that is recommended as a pragmatic solution for defining lake reference conditions (Battarbee *et al.*, 2011). As found in Donohue *et al.* (2010) there is a

possibility that some of these waterbodies had already responded to anthropogenic pressures in advance of the 1850 timeline. But further paleolimnology and diatom sampling in an expanded moderate alkalinity network will be required to address this question more comprehensively.

A review of EQR scores provided a further opportunity to check for bias in the choice of expected value. If the chosen LTDI was too stringent then it would manifest as low EQRs in that lake type. We found that the distribution of EQRs in moderate alkalinity lakes indicated that the selected value was appropriate. In fact, moderate alkalinity lakes arguably had the best fit with a lower proportion of classifications exceeding the upper limit of the 0-1 EQR scale. The tail of EQR exceedances in low and high alkalinity lakes was more considerable by comparison. In normalised terms it was equivalent to an additional status class than provided for in the WFD. But this is a consequence of the extent of natural variability in LTDI scores across the network of near-pristine lakes. It was also within the range of variability typically observed in other biological quality elements using a type specific reference approach. While this study indicated that there was no requirement to adopt multiple reference concepts in different lake types, it is possible that a greater differentiation of diatom taxonomic complexes might provide a level of resolution sufficient to separate the different lake types (Kennedy *et al.*, 2019; Potapova and Hamilton, 2007; Pouličková *et al.*, 2017).

2.5.6. DIATOM METRIC CLASSIFICATION

Numerous studies evaluating the performance of regional diatom indices that subsequently were applied in other geographic areas have identified limitations of this approach (Potapova and Charles, 2007; González-Paz *et al.*, 2020). While some

well-established metrics have a broad applicability, transferability even between contiguous countries has shown to be problematic (Charles *et al.*, 2020; Pajunen *et al.*, 2020). This has potentially greater significance for lakes given biogeographical factors such as increased dispersal limitation that regulate periphyton composition in lentic waters. It was a possible factor explaining the weak relationship reported for littoral diatom metrics in Irish lakes previously (DeNicola *et al.*, 2004). Despite the high prevalence of taxonomic species complexes in our data, our results correspond to Fidlerová and Hlúbíková (2016) in validating the significant relationship between the LTDI developed for UK lake classifications and total phosphorus. In addition, we established that the LTDI responds to total phosphorus independently of covarying parameters such as alkalinity, pH and nitrogen that provided minimal explanatory power in our multiple regressions.

Several lakes had lower than expected LTDI scores and were treated as outliers given their strong influence on regression performance. It was more problematic for low alkalinity lakes where diatom nutrient correlations are often weaker (Bennion *et al.*, 2014; Denicola *et al.*, 2004; Deny, 2007; Kelly *et al.* 2014). Their omission was justified based on the limited gradient in our study and when seasonal surveys are averaged outliers are often less consequential. A comparable cluster in high alkalinity lakes however indicated that higher variability is not limited just to low alkalinity contexts. We do not consider this variability in metric scores solely as inherent “noise” in the assessment approach, or can it be just linked to physical disturbance in littoral habitats (Riato and Leira, 2020). Schneider *et al.*, (2020) also found diatom metrics can be poorly related to water nutrient concentrations because of nutrient uptake in algal biomass. This metric variation also needs to be considered in terms of resilience in the

periphyton after potential for localised deteriorations or missed events in open water chemical monitoring have been ruled out. Nevertheless, performance of the LTDI at low alkalinity was borderline and we provided examples where classifications were less stringent in some impacted waterbodies. Several justifications for their lower response to nutrient pressures have been proposed (DeNicola *et al.*, 2004; Fairchild and Sherman, 1993; Kelly *et al.* 2014). These included confounding by acidification, interactions with light, nitrogen and dissolved organic carbon, competition from soft algae or phytoplankton, or an interactions of the factors. The lakes with lowest LTDI scores in our study had a range of chlorophyll and true colour values but tended to be less acidic (ave. pH = 6.2). Diatom responses to nutrient enrichment have been reported in lakes with comparable water chemistry previously, suggesting that it is an increased acidification pressure that is also the most critical factor in controlling lake responses (DeNicola *et al.*, 2006; Schneider *et al.*, 2013).

From the extent of the variation observed in our study it is clear that a minimum lake number and gradient is required before the success of diatom metrics can be evaluated. The increased number of oligotrophic high alkalinity and nutrient impacted dystrophic lakes probably contributed to our findings, with a better opportunity to separate the effects of alkalinity and nutrients. Generally, our results indicated that disturbance or variability in littoral habitats did not represent a major obstacle to assessing eutrophication pressure. In fact, modest temporal and spatial replication provided explanatory power comparable to the levels of uncertainty observed for other assessment tools (Free *et al.*, 2016; Donohue *et al.*, 2009). This may be regionally or lake specific however, as diatom response is dependent on the extent of seasonal

nutrient fluxes and water level fluctuations that may be less extreme in Irish lakes than encountered in other regions.

2.5.7. FUTURE DEVELOPMENTS

After a decade of monitoring for the WFD, the question of the value of benthic diatoms for lake assessment has come full circle. A redundancy in information has been identified where multiple indicator groups are used to determine nutrient effects on biological communities (Kelly *et al.*, 2016; Schneider *et al.*, 2012; Schneider *et al.*, 2019). This same question is relevant for Irish lakes as the frequency of classifications determined by benthic diatoms under the one-out-all-out principle of the WFD is low. Factors associated with the lower stringency relate to both the sensitivity of the metric and the relative positioning of status class boundaries along the EQR scale. Free *et al.* (2016) compared the relationships of national monitoring tools and established macrophytes to have a much higher sensitivity to nutrient stress. They indicated that this was in part a consequence of the role of functional metrics in macrophyte classifications, whereas the diatom metric is based on compositional changes in assemblages only. When considered in terms of lake misclassification rates and phosphorus levels, the effective good-moderate threshold was elevated for diatoms and phytoplankton relative to macrophytes ($31 \mu\text{g}^{-\text{L}}\text{-P}$ versus $18 \mu\text{g}^{-\text{L}}\text{-P}$ for macrophytes). Unsatisfactory declines in lake quality occur at phosphorus levels below the higher threshold value (Free *et al.*, 2006). The phytoplankton metric does incorporate biomass in its calculations suggesting that it is the positioning of status classes that is the major factor in the lack of concordance between the methods. However, the good-moderate boundary has the greatest ecological underpinning of all the diatom status classes that are arbitrary divisions of the EQR scale and therefore

empirically based (Bennion *et al.*, 2014; Poikane *et al.*, 2016). Proposals more recently might provide alternatives for future boundaries based on different ecological justifications (Cantonati *et al.*, 2020; Wang *et al.*, 2019). But it is the lack of a functional component in the phytobenthos metric as required in the WFD that remains problematic. A diatom-macroalgal multimetric is in our view also the most effective way to build on existing approaches (Charles *et al.*, 2020). Differences in ecological information provided by diatoms and non-diatom algae have been investigated and incorporating functional attributes like algal abundance is not without its challenges (Denicola and Kelly, 2014; Kelly *et al.*, 2018). Nevertheless, we observed epilithic diatom biomass and cover of Zygnemataceae to proliferate before compositional change was reflected in some of the diatom classifications. While the opposite pattern also occurred as reported in other studies, for some periphyton communities green algae increase in response to nutrient stress and should be incorporated in littoral monitoring (Doddy *et al.*, 2019; Kelly *et al.*, 2018). Filamentous green algae are already assessed with macrophytes in Irish lakes and provide a valuable proxy for ecological disturbance and nutrient enrichment. While deemed a crude metric, it is also a negative indicator only, and opportunistic algal increases do not always occur in response to nutrient enrichment (Schneider *et al.*, 2019). The prevalence and integration of a greater range of organic-phosphorus specialists, heterocystous cyanobacteria and toxic cyanobacterial mats would more broadly encompass periphyton responses to environmental disturbance, while helping to improve communication of the significance of these assessments (Charles *et al.*, 2020).

Nevertheless, compliance with statutory surveillance monitoring obligations is a legitimate motivation for the continued use of diatoms in lake assessment. Even

without further development, long term monitoring trends have the potential to provide important insights for environmental management. Further roles for phytobenthos monitoring have been identified but while water level fluctuation has some relevance in an Irish lake context, the scale of the pressure is less extreme (Kelly *et al.*, 2016). Acidification and atmospheric deposition have a regional relevance restricted primarily to smaller waterbodies in the uplands, but future climate scenarios will have an even more pervasive effect in terms of nutrient and dissolved organic carbon fluxes in sensitive waterbodies. Given that lake diatom assessment has been validated, monitoring agencies should also have a clear vision for their intended application, while ensuring monitoring programmes are statistically optimised to meet these ambitions.

2.5.8. CONCLUSIONS

Epilithic diatom assemblages in a range of dystrophic to calcifying lakes were characterised and shown to have clear responses to alkalinity-pH, total phosphorus, and true colour gradients, confirming their effectiveness as indicators of lake physicochemical condition. Our study also demonstrated the transferability of the Lake Trophic Diatom Index (LTDI) for use in Irish lakes. Diatom populations in reference lakes differed floristically but we found no indication for differences in the distribution of their LTDI reference values. While there were variations in LTDI-total phosphorus relationships amongst three lake alkalinity types, the overall result also confirmed that lake type had a marginal influence on ecological classifications. Given the absence of large-scale studies investigating the potential implication of seasonal sampling it remains a core question for applied monitoring programs. We established that seasonal variation was more pronounced in lakes with higher metric scores, but

it was not possible to predict waterbodies with higher variation in advance of monitoring them. Nonetheless, modest spatial replication with repeated sampling in two seasons provided levels of uncertainty comparable to other lake metrics even when targeting littoral habitats.

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APPENDIX A

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SW_22_208	Acoose	Aco.sp	LA	spring	3.6	0.013	4.2	82.5	6.5	0.6	0.18	0.016	28.5	0.97	22.7
SW_22_208	Acoose	Aco.su	LA	summer	6.0	0.010	2.9	72.5	7.2	0.5	0.15	0.010	38.8	0.99	20.5
WE_33_1892	Acorrymore	Acy.sp	LA	spring	4.0	0.015	1.4	126.9	5.1	0.9	0.20	0.007	40.3	1.13	9.4
WE_33_1892	Acorrymore	Acy.su	LA	summer	4.0	0.015	1.4	126.9	5.1	0.9	0.20	0.007	40.3	1.18	5.6
SH_25_189	Alewnaghta	Alw.sp	MA	spring	48.7	0.035	1.7	156.2	7.6	3.1	0.30	0.015	97.7	0.82	34.4
SH_25_189	Alewnaghta	Alw.su	MA	summer	43.8	0.018	19.6	132.4	7.4	1.4	0.20	0.027	177.2	0.92	26.7
SH_26_669	Annaghmore	Ann.sp	HA	spring	166.7	0.060	3.5	344.0	8.2	2.8	0.27	0.007	20.7	1.03	17.5
SH_26_669	Annaghmore	Ann.su	HA	summer	114.2	0.029	4.0	261.9	8.2	1.9	0.15	0.012	14.8	1.02	18.2

Appendix A.1 Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_38_83	Anure	Anr.sp	LA	spring	9.8	0.015	2.8	118.9	7.1	1.4	0.16	0.006	70.5	0.92	26.7
NW_38_83	Anure	Anr.su	MA	summer	11.0	0.015	4.4	114.9	7.2	0.6	0.20	0.008	58	0.53	57.2
NW_38_61	an tSeisigh	anT.sp	MA	spring	43.3	0.035	13.8	224.3	7.3	1.6	0.20	0.008	29	0.91	27.2
NW_38_61	an tSeisigh	anT.su	HA	summer	51.6	0.015	2.3	222.7	7.8	1.0	0.20	0.006	19.4	0.8	35.7
WE_31_76	Ardderry	Ard.sp	LA	spring	5.5	0.015	1.0	69.0	6.6	0.7	0.20	0.010	123	1.01	19.4
WE_31_76	Ardderry	Ard.su	LA	summer	4.0	0.015	3.4	82.3	6.8	0.3	0.20	0.009	102.4	1.03	17.4
WE_35_159	Arrow	Arr.sp	HA	spring	113.7	0.010	3.3	247.8	8.2	2.7	0.14	0.015	23.3	0.79	36.5
WE_35_159	Arrow	Arr.su	HA	summer	108.5	0.014	3.1	244.8	8.3	1.8	0.10	0.008	19.4	0.95	24.2
SH_27_108	Atedaun	Atd.sp	HA	spring	164.7	0.015	1.0	366.9	8.1	1.4	0.50	0.016	21.7	0.86	31.2

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SH_27_108	Atedaun	Atd.su	HA	summer	166.6	0.015	6.3	338.1	8.1	0.9	0.30	0.022	29.6	0.76	39.1
SW_19_4	Allua	Aua.sp	LA	spring	4.0	0.010	1.0	106.7	6.8	1.3	0.46	0.008	12.5	0.8	35.7
SW_19_4	Allua	Aua.su	MA	summer	13.3	0.010	11.5	75.3	7.4	0.6	0.10	0.015	30.8	0.87	30.6
WE_32_436	Aughrusbeg	Aug.sp	HA	spring	54.0	0.022	8.1	335.3	7.9	0.2	0.20	0.015	27.6	1.03	18
WE_32_436	Aughrusbeg	Aug.su	HA	summer	54.0	0.022	8.1	335.3	7.9	0.2	0.20	0.015	27.6	1.11	11.3
NW_38_84	Barra	Bar.sp	LA	spring	4.5	0.029	1.0	68.3	5.9	0.6	0.18	0.005	54.8	1.04	16.7
NW_38_84	Barra	Bar.su	LA	summer	4.0	0.015	1.8	41.2	6.4	0.5	0.20	0.007	75.4	0.97	22.1
WE_32_402	Beaghcauneen	Bgc.sp	LA	spring	4.0	0.033	3.0	82.6	6.1	0.4	0.20	0.030	59.3	0.48	61.4
WE_32_402	Beaghcauneen	Bgc.su	LA	summer	4.0	0.015	12.9	96.2	6.8	0.3	0.20	0.021	27.3	0.85	31.9

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_32_479	Ballynakill	Bkl.su	MA	summer	21.5	0.015	5.6	170.0	7.0	0.9	0.20	0.010	23	1.2	4.2
WE_32_452	Beltra	Blt.sp	LA	spring	8.3	0.010	1.3	153.5	6.8	1.8	0.10	0.005	50.3	0.97	22.7
WE_32_452	Beltra	Blt.su	MA	summer	18.2	0.010	6.0	106.9	7.4	1.0	0.10	0.007	65.6	1.11	11.2
EA_07_270	Bane	Bne.sp	HA	spring	145.3	0.027	2.8	318.1	8.1	1.2	0.20	0.008	5.2	0.99	20.9
EA_07_270	Bane	Bne.su	HA	summer	135.0	0.014	4.6	300.9	8.4	0.4	0.13	0.007	4.2	1.01	19
SW_21_402	Brin	Brn.sp	LA	spring	8.0	0.028	3.3	100.8	6.9	0.6	0.18	0.007	11.5	0.83	33.5
SW_21_402	Brin	Brn.su	MA	summer	14.2	0.016	3.1	86.4	7.3	0.5	0.13	0.019	19.6	0.86	31.2
EA_10_28	Bray Lower	Bry.sp	LA	spring	5.0	0.025	1.2	36.4	6.0	2.0	0.16	0.020	95.5	0.85	32.1
EA_10_28	Bray Lower	Bry.su	LA	summer	5.0	0.025	1.2	36.4	6.0	2.0	0.16	0.020	95.5	0.8	35.6

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_27_114	Bunny	Bun.sp	HA	spring	146.0	0.014	1.0	341.0	8.2	0.6	0.47	0.005	8.5	1	19.9
WE_27_114	Bunny	Bun.su	HA	summer	128.0	0.013	1.2	300.8	8.3	0.4	0.10	0.005	7.8	1.16	6.9
SH_23_74	Cam	Cam.sp	LA	spring	3.0	0.013	2.1	109.3	5.8	0.4	0.18	0.017	44	0.61	51.3
SH_23_74	Cam	Cam.su	LA	summer	2.5	0.020	4.9	87.6	5.9	1.0	0.20	0.021	99.4	0.45	64
WE_30_347	Carra	Car.sp	HA	spring	159.3	0.035	3.9	357.9	8.3	1.9	0.65	0.015	22	1.17	6.7
WE_30_347	Carra	Car.su	HA	summer	113.6	0.019	2.8	288.4	8.3	0.7	0.16	0.005	7.6	1.17	6.7
SH_26_705	Cavetown	Cav.sp	HA	spring	129.3	0.010	1.0	284.9	8.1	3.1	0.25	0.021	75	0.41	67.6
SH_26_705	Cavetown	Cav.su	HA	summer	163.6	0.010	4.5	296.5	8.3	1.4	0.12	0.016	57.2	0.52	58.4
SW_22_207	Caragh	Cgh.sp	LA	spring	4.0	0.017	1.8	95.2	6.5	0.7	0.43	0.005	26.3	0.9	27.7

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SW_22_207	Caragh	Cgh.su	LA	summer	7.8	0.016	3.1	98.7	6.9	0.5	0.23	0.005	23.6	0.92	26.4
WE_30_666a	Corrib Lower	CL.sp	HA	spring	143.9	0.020	1.2	294.0	8.1	2.1	0.43	0.006	24.7	1.12	10.4
WE_30_666a	Corrib Lower	CL.su	HA	summer	101.2	0.016	2.4	229.7	8.3	1.0	0.23	0.005	15.2	1.08	13.8
WE_34_406a	Cullin	Cln.sp	HA	spring	96.8	0.010	5.2	256.7	8.0	2.1	0.23	0.016	48.8	0.85	32
WE_34_406a	Cullin	Cln.su	HA	summer	81.6	0.010	1.0	228.6	8.9	0.3	0.10	0.007	30	0.84	33
WE_33_1914	Carrowmore	Cmr.sp	LA	spring	5.3	0.010	4.8	130.4	6.6	1.1	0.13	0.016	83.6	0.86	31.5
WE_33_1914	Carrowmore	Cmr.su	MA	summer	15.6	0.011	4.5	123.4	7.4	0.6	0.10	0.018	89.5	1.05	16.1
NW_36_655	Corglass	Crg.sp	HA	spring	130.0	0.015	3.7	275.9	7.9	3.9	0.20	0.037	46.3	0.77	38.7
NW_36_655	Corglass	Crg.su	HA	summer	137.6	0.022	11.1	282.6	8.2	2.2	0.20	0.030	25.2	0.54	56.7

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_30_666b	Corrib Upper	CU.sp	HA	spring	91.6	0.017	1.7	212.6	8.2	1.6	0.29	0.007	19.3	0.84	32.9
WE_30_666b	Corrib Upper	CU.su	HA	summer	97.5	0.016	1.2	225.1	8.1	1.9	0.24	0.005	26.7	0.9	28.3
SH_27_115	Cullaun	Cul.sp	HA	spring	166.7	0.010	1.0	371.7	8.1	0.7	0.49	0.005	17.7	1.13	9.8
SH_27_115	Cullaun	Cul.su	HA	summer	153.4	0.015	3.1	350.6	8.3	0.3	0.16	0.006	16.2	1.08	13.3
WE_29_37	Cutra	Cut.su	MA	summer	34.4	0.015	4.7	102.9	7.5	0.8	0.20	0.020	175.6	1.14	8.5
EA_10_29	Dan	Dan.sp	LA	spring	2.5	0.028	1.1	37.5	5.4	0.9	0.13	0.011	142.3	0.8	35.6
EA_10_29	Dan	Dan.su	LA	summer	2.5	0.020	1.9	36.2	5.4	1.1	0.09	0.008	141.8	0.88	29.6
NW_36_400	Derrybrick	Dby.sp	HA	spring	137.0	0.015	1.8	283.7	8.0	3.6	0.20	0.013	20.3	0.76	39.4
NW_36_400	Derrybrick	Dby.su	HA	summer	139.8	0.018	8.5	280.0	8.2	3.1	0.20	0.024	12.2	0.69	44.7

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SH_28_82	Doo (CE)	Dce.sp	LA	spring	5.0	0.013	3.8	93.1	6.7	0.7	0.10	0.008	111.9	0.78	37.7
SH_28_82	Doo (CE)	Dce.su	LA	summer	5.0	0.014	7.3	90.1	7.1	0.2	0.09	0.007	104.1	0.96	23.1
WE_32_490	Doo (MO)	Dmo.sp	LA	spring	4.0	0.013	1.3	69.4	7.1	0.9	0.10	0.009	10.9	0.94	24.5
WE_32_490	Doo (MO)	Dmo.su	LA	summer	4.8	0.015	2.0	69.8	7.4	0.8	0.10	0.005	11.8	1.03	18
SH_25_191a	Derg	Drg.sp	HA	spring	203.3	0.006	0.7	389.8	8.3	1.8	1.03	0.025	70	0.58	53.3
SH_25_191a	Derg	Drg.su	HA	summer	201.1	0.011	2.9	404.1	8.3	1.4	0.79	0.013	57.5	0.69	44.8
SH_27_82	Dromore	Drm.sp	HA	spring	178.0	0.020	1.6	389.5	8.0	1.9	0.57	0.014	21.3	0.88	29.3
SH_27_82	Dromore	Drm.su	HA	summer	174.0	0.018	8.1	355.3	8.1	0.7	0.20	0.015	25.4	0.95	23.9
NW_38_692	Dunglow	Dug.sp	LA	spring	6.5	0.019	1.6	143.1	6.3	1.0	0.20	0.011	120.8	1.04	17

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_38_692	Dunglow	Dug.su	LA	summer	4.0	0.015	4.7	121.0	6.9	0.5	0.20	0.020	119.8	0.77	38.1
NW_36_671	Egish	Egs.sp	HA	spring	67.7	0.015	55.7	190.5	7.8	0.6	0.20	0.090	32	0.52	58.4
NW_36_671	Egish	Egs.su	HA	summer	83.0	0.026	48.6	216.7	8.3	1.7	0.20	0.103	32.6	0.37	70.7
WE_35_136	Easky	Eky.sp	LA	spring	4.0	0.023	2.5	62.4	6.7	1.2	0.10	0.005	60	1.05	16
WE_35_136	Easky	Eky.su	LA	summer	4.0	0.010	4.1	48.8	7.8	0.4	0.10	0.005	62.5	1.12	10.1
NW_36_672	Upper Lough Erne	Er.sp	HA	spring	72.8	0.032	2.0	257.8	7.7	1.5	0.91	0.050	53.8	0.56	55.2
NW_36_672	Upper Lough Erne	Er.su	HA	summer	93.7	0.023	7.7	288.4	8.0	1.5	0.12	0.041	46.1	0.5	59.8
NW_39_13	Fern	Frn.sp	MA	spring	24.0	0.028	4.0	133.0	7.3	1.9	0.32	0.032	74.3	0.74	40.5

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_39_13	Fern	Frn.su	MA	summer	31.6	0.018	13.1	126.1	7.3	1.8	0.20	0.050	85.2	0.77	38.4
SW_21_444	Glenbeg	Gbg.sp	LA	spring	4.0	0.014	7.8	94.1	7.2	0.6	0.10	0.007	12	0.94	24.6
SW_21_444	Glenbeg	Gbg.su	LA	summer	5.0	0.010	7.6	96.6	7.4	0.1	0.10	0.005	15.2	0.94	24.8
WE_35_139	Glencar	Gcr.sp	HA	spring	86.0	0.010	1.0	213.2	8.0	2.1	0.14	0.012	29.7	0.88	29.9
WE_35_139	Glencar	Gcr.su	HA	summer	93.0	0.012	2.6	224.5	8.0	1.4	0.22	0.005	19.4	1.12	10.3
WE_35_156	Glenade	Gde.sp	HA	spring	54.0	0.010	1.9	156.2	7.6	1.8	0.18	0.014	44	0.89	28.9
WE_35_156	Glenade	Gde.su	HA	summer	71.2	0.010	4.5	171.9	7.8	1.1	0.10	0.010	26.4	0.7	44.3
WE_35_158	Gill	Gil.sp	HA	spring	79.0	0.010	1.0	208.5	8.0	2.2	0.32	0.020	59	0.61	50.8
WE_35_158	Gill	Gil.su	HA	summer	76.3	0.010	4.3	202.3	8.1	1.3	0.20	0.014	53.4	0.7	44.4

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_32_487	Glencullin	Glc.sp	LA	spring	4.0	0.010	1.7	74.6	6.9	0.7	0.10	0.005	18.5	1.07	14.6
WE_32_487	Glencullin	Glc.su	LA	summer	4.8	0.010	3.3	46.7	7.3	0.3	0.10	0.005	27.8	1.09	12.5
NW_38_22	Glen	Gln.sp	LA	spring	4.5	0.021	1.0	88.6	6.6	1.3	0.16	0.006	72	1.05	16
NW_38_22	Glen	Gln.su	LA	summer	4.5	0.022	1.8	74.3	6.9	1.3	0.20	0.006	100.2	1.03	17.8
SH_24_99	Gur	Gur.sp	HA	spring	152.0	0.102	2.2	325.3	8.2	2.3	0.43	0.012	19	0.9	28.1
SH_24_99	Gur	Gur.su	HA	summer	99.0	0.015	8.0	225.4	8.4	1.0	0.20	0.028	15.4	0.8	36.1
SH_27_126	Inchicronan	Ich.sp	HA	spring	129.3	0.023	1.9	303.3	8.0	3.1	0.47	0.018	50.7	0.93	25.6
SH_27_126	Inchicronan	Ich.su	HA	summer	119.8	0.022	10.7	270.3	7.9	1.3	0.20	0.022	63.6	1.02	18.7
NW_36_526	Inner	Inr.sp	HA	spring	96.7	0.015	71.3	227.4	8.2	1.6	0.03	0.136	62.3	0.31	75.1

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_36_526	Inner	Inr.su	HA	summer	96.7	0.015	71.3	227.4	8.2	1.6	0.03	0.136	62.3	0.31	75.1
NW_38_670	Kindrum	Kdm.sp	HA	spring	65.0	0.015	22.0	280.1	7.9	3.1	0.20	0.014	38	1.14	8.7
NW_38_670	Kindrum	Kdm.su	HA	summer	70.0	0.028	6.8	282.7	7.8	1.4	0.20	0.011	34	1.12	10.6
NW_38_576	Keel	Kel.sp	LA	spring	5.3	0.020	2.7	61.0	5.5	1.6	0.20	0.005	111.3	0.98	21.7
NW_38_576	Keel	Kel.su	LA	summer	5.3	0.020	2.7	61.0	5.5	1.6	0.20	0.005	111.3	0.95	23.9
SH_28_64	Keagh	Kgh.sp	LA	spring	5.0	0.015	3.3	102.3	6.6	0.3	0.08	0.009	211.1	0.42	66.5
SH_28_64	Keagh	Kgh.su	LA	summer	5.0	0.015	3.3	102.3	6.6	0.3	0.08	0.009	211.1	0.69	45
NW_38_47	Kiltooris	Ktr.sp	MA	spring	23.3	0.032	2.9	262.0	6.9	2.1	0.50	0.009	59.7	0.95	24.4
NW_38_47	Kiltooris	Ktr.su	MA	summer	29.8	0.015	3.2	256.0	7.5	0.4	0.20	0.013	38.2	0.97	22.5

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_32_509b	Kylemore	Kyl.sp	LA	spring	8.5	0.015	1.6	56.5	7.1	0.6	0.20	0.010	24	1	20
WE_32_509b	Kylemore	Kyl.su	LA	summer	7.0	0.015	2.3	62.2	7.0	0.5	0.20	0.005	27	1.02	18.6
WE_31_177	Loughaunore	Lar.sp	LA	spring	4.0	0.015	8.5	139.0	6.8	0.5	0.20	0.010	54.3	1.16	7.1
WE_31_177	Loughaunore	Lar.su	LA	summer	4.0	0.015	8.5	139.0	6.8	0.5	0.20	0.010	54.3	1.21	3
NW_35_143	Lattone Lough	Lat.sp	MA	spring	35.8	0.020	7.3	170.8	7.6	1.1	0.03	0.033	71	0.75	40.1
NW_35_143	Lattone Lough	Lat.su	MA	summer	44.6	0.020	11.2	166.4	7.9	1.1	0.03	0.028	82.2	0.63	49.5
SH_28_85	Lickeen	Lck.sp	MA	spring	23.0	0.016	1.7	128.3	7.5	1.5	0.22	0.022	102	0.83	33.6
SH_28_85	Lickeen	Lck.su	MA	summer	22.6	0.015	10.2	123.9	7.5	0.8	0.20	0.027	94.4	0.92	26.7
SW_22_185	Leane	Lea.sp	MA	spring	11.8	0.010	0.8	110.4	7.1	0.9	0.26	0.005	26.8	0.69	45

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SW_22_185	Leane	Lea.su	MA	summer	23.9	0.015	4.8	127.7	7.6	0.7	0.22	0.007	27.6	1.1	12.3
EA_07_274	Lene	Len.sp	HA	spring	106.0	0.024	2.8	218.9	8.2	1.1	0.04	0.010	7	1.04	16.9
EA_07_274	Lene	Len.su	HA	summer	93.4	0.016	3.8	202.6	8.3	1.0	0.06	0.010	5.6	0.95	24.2
WE_30_344	Lettercraffroe	Let.sp	LA	spring	4.0	0.015	6.5	71.5	5.5	0.9	0.20	0.015	93	0.57	54.5
WE_30_344	Lettercraffroe	Let.su	LA	summer	4.0	0.015	6.9	67.5	6.3	0.4	0.24	0.015	64.2	0.7	44.1
SH_27_94	Muckanagh	Mck.sp	HA	spring	215.3	0.019	2.3	478.8	8.3	1.1	0.69	0.005	35.3	0.93	25.3
SH_27_94	Muckanagh	Mck.su	HA	summer	164.4	0.017	3.6	383.9	8.3	0.4	0.10	0.006	21.8	1.12	10.7
SH_26_711	Meelagh	Mel.sp	HA	spring	62.3	0.014	1.3	217.1	7.9	2.3	0.10	0.009	34	0.91	26.9
SH_26_711	Meelagh	Mel.su	HA	summer	64.0	0.010	2.4	233.0	8.1	0.9	0.10	0.005	16.7	0.92	26.5

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_35_160	Melvin	Mev.sp	HA	spring	59.6	0.016	3.0	170.9	7.9	1.7	0.13	0.021	81.7	0.83	33.8
NW_35_160	Melvin	Mev.su	HA	summer	52.8	0.010	5.1	159.8	7.8	0.7	0.10	0.012	62.3	0.93	25.4
NW_01_104	Mourne	Mrn.sp	LA	spring	5.8	0.021	1.2	53.2	6.0	0.7	0.04	0.005	88.5	1.02	18.4
NW_36_272	Mushlin	Msh.sp	MA	spring	18.3	0.015	17.6	69.0	7.7	0.7	0.20	0.033	39	0.88	29.8
NW_36_272	Mushlin	Msh.su	MA	summer	18.3	0.015	17.6	69.0	7.7	0.7	0.20	0.033	39	0.6	51.6
IE_NB_06_56	Muckno or Blayney	MuB.sp	HA	spring	53.7	0.052	12.6	174.9	7.7	4.6	0.97	0.063	45.7	0.64	48.6
IE_NB_06_56	Muckno or Blayney	MuB.su	HA	summer	64.0	0.074	46.2	207.1	8.2	1.2	0.36	0.063	34.2	0.33	73.9
WE_30_343	Maumwee	Mwe.sp	LA	spring	4.0	0.015	1.6	58.1	6.6	0.7	0.20	0.006	29.3	1.11	11.4

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_30_343	Maumwee	Mwe.su	LA	summer	4.0	0.015	2.3	61.9	6.6	0.2	0.20	0.005	27.4	1.14	9.1
WE_31_208	Nahasleam	Nah.sp	LA	spring	5.2	0.015	2.8	90.4	6.6	0.3	0.20	0.007	51.4	0.96	23
WE_31_208	Nahasleam	Nah.su	LA	summer	5.2	0.015	2.8	90.4	6.6	0.3	0.20	0.007	51.4	1.02	18.8
NW_38_67	Nasnanida	Nas.sp	LA	spring	4.0	0.035	5.0	120.6	5.8	0.7	0.20	0.005	59	1.01	19.3
NW_38_67	Nasnanida	Nas.su	LA	summer	4.0	0.027	1.2	96.1	6.0	0.4	0.20	0.005	65	1.09	13
WE_32_500	Nambrackmore	Nbm.sp	LA	spring	4.0	0.015	1.0	73.3	6.5	0.5	0.20	0.010	56	1.17	6.3
WE_32_500	Nambrackmore	Nbm.su	LA	summer	4.0	0.024	1.9	95.7	6.8	0.1	0.20	0.005	28.8	1.14	9
UKGBNI3NB0032	Neagh	Ngh.sp	HA	spring	103.6	0.067	30.9	305.5	8.4	1.5	0.55	0.131	40.4	0.31	75.3
UKGBNI3NB0032	Neagh	Ngh.su	HA	summer	107.5	0.040	58.6	305.7	8.5	1.5	0.07	0.117	24	0.22	82.5

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
SH_26_693	O'Flynn	Ofn.sp	HA	spring	122.7	0.046	5.7	274.4	8.1	2.3	0.60	0.025	77	1	19.7
SH_26_693	O'Flynn	Ofn.su	HA	summer	101.2	0.010	3.6	264.3	8.3	0.6	0.22	0.011	48	0.87	30.6
SH_26_703	Owel	Owl.sp	HA	spring	112.1	0.043	4.8	247.4	8.2	0.9	0.13	0.010	6.9	1.02	18.3
SH_26_703	Owel	Owl.su	HA	summer	104.2	0.016	5.1	233.6	8.4	0.7	0.09	0.008	5.8	0.68	45.3
EA_07_275	Ramor	Ram.sp	HA	spring	65.3	0.011	60.0	191.7	8.8	1.4	0.09	0.038	44.5	0.25	80.4
EA_07_275	Ramor	Ram.su	HA	summer	65.3	0.011	60.0	191.7	8.8	1.4	0.09	0.038	44.5	0.61	51.3
WE_29_194	Lough Rea	Rea.sp	HA	spring	145.7	0.045	2.4	297.0	8.2	2.1	0.20	0.005	4.5	1.04	17.2
WE_29_194	Lough Rea	Rea.su	HA	summer	108.4	0.015	2.8	242.8	8.4	1.5	0.20	0.008	2.5	1.02	18.8
WE_30_345	Ross	Ros.sp	HA	spring	130.0	0.028	1.9	292.2	7.5	3.6	0.53	0.008	47	1.06	15.4

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
WE_30_345	Ross	Ros.su	HA	summer	141.2	0.021	3.5	311.1	8.0	2.3	0.34	0.006	30.2	1.08	13.8
WE_31_98	Seecon	See.sp	LA	spring	4.6	0.019	6.6	91.7	5.4	1.6	0.17	0.022	197	1.01	19.5
WE_31_98	Seecon	See.su	LA	summer	4.6	0.019	6.6	91.7	5.4	1.6	0.17	0.022	197	1.01	19.4
SH_26_709	Sheelin	She.sp	HA	spring	161.5	0.029	2.0	346.6	8.0	6.5	1.33	0.045	34.8	0.55	56
SH_26_709	Sheelin	She.su	HA	summer	169.4	0.018	6.2	344.3	8.5	3.4	0.56	0.020	16.2	0.68	45.6
WE_31_171	Shindilla	Shi.sp	LA	spring	4.0	0.015	1.0	52.5	6.7	0.8	0.20	0.005	42	1.09	12.6
WE_31_171	Shindilla	Shi.su	LA	summer	4.8	0.015	2.3	66.0	6.8	0.4	0.20	0.005	31.6	1.07	14.6
WE_29_181	Skeardeen	Skd.sp	HA	spring	158.3	0.037	3.2	306.2	7.8	0.3	0.11	0.011	10.4	1.12	10.5
WE_29_181	Skeardeen	Skd.su	HA	summer	158.3	0.037	3.2	306.2	7.8	0.3	0.11	0.011	10.4	1.1	12

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
EA_10_25	Tay	Tay.sp	LA	spring	2.5	0.028	1.2	35.8	5.4	0.8	0.13	0.011	165.5	0.8	35.8
EA_10_25	Tay	Tay.su	LA	summer	2.5	0.014	1.8	35.1	5.4	1.2	0.13	0.009	164.6	0.74	40.9
WE_34_405	Talt	Tlt.sp	HA	spring	66.7	0.010	1.0	164.3	7.8	2.6	0.10	0.005	25.7	1.03	17.8
WE_34_405	Talt	Tlt.su	HA	summer	66.6	0.012	2.1	172.9	8.1	1.6	0.10	0.006	25.8	1.05	16.3
WE_35_157	Templehouse	Tph.sp	HA	spring	160.7	0.017	2.1	324.7	7.8	2.6	0.62	0.039	87.3	0.55	56.1
WE_35_157	Templehouse	Tph.su	HA	summer	202.4	0.017	6.8	416.1	8.2	2.6	0.18	0.027	72.6	0.54	56.8
N/A	Travaun	Trv.su	HA	summer	201.0	0.014	1.2	301.0	8.1	0.4	0.04	0.005	7	1.04	17
NW_36_651	Tullynasiddagh	Tuy.sp	LA	spring	9.0	0.020	3.1	73.1	6.6	0.5	0.10	0.024	72.8	0.9	28.3
NW_36_651	Tullynasiddagh	Tuy.su	LA	summer	9.0	0.020	3.1	73.1	6.6	0.5	0.10	0.024	72.8	0.69	44.4

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
EA_10_32	Upper Glendalough	ULG.sp	LA	spring	4.2	0.023	0.9	32.2	6.6	1.5	0.50	0.006	48.3	0.91	27.2
EA_10_32	Upper Glendalough	ULG.su	LA	summer	4.2	0.018	0.9	32.6	6.2	1.9	0.17	0.004	47.7	1.09	13
EA_07_267	Upper Lough Skeagh	ULS.sp	MA	spring	26.3	0.015	17.0	97.7	7.5	2.9	0.37	0.043	56	0.57	54.3
EA_07_267	Upper Lough Skeagh	ULS.su	MA	summer	30.4	0.020	20.8	99.9	7.7	0.8	0.20	0.036	36.2	0.51	59.6
SW_22_186	Upper	Upr.su	LA	summer	6.9	0.010	2.7	60.7	7.0	0.5	0.13	0.005	28.8	0.99	20.8
SH_26_689	Urluar	Url.sp	HA	spring	76.3	0.020	5.4	258.7	8.1	1.7	0.25	0.014	57.3	0.94	24.8
SH_26_689	Urluar	Url.su	HA	summer	104.8	0.012	5.9	264.8	8.2	2.0	0.12	0.011	34.2	1.04	16.6

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Season	Alkalinity (mg/L CaCO ₃)	Total Ammonia (mg/L NH ₄ -N)	Chlorophyll <i>a</i> (µg/L)	Conductivity (µS ^{-cm})	pH	Silica (mg/L Si)	Total Oxidised Nitrogen (mg/L N)	Total Phosphorus (mg/L P)	True Colour (Hazen)	Ecological Quality Ratio (EQR)	Lake Trophic Diatom Index (LTDI)
NW_38_80	Veagh Upper	Vgh.sp	LA	spring	4.3	0.010	1.0	83.7	6.2	0.6	0.13	0.005	45.1	0.96	23.1
NW_38_80	Veagh Upper	Vgh.su	LA	summer	5.0	0.010	1.7	70.6	6.2	0.5	0.10	0.005	36.5	1.08	14
NW_36_647	White	Wht.sp	HA	spring	60.0	0.085	12.5	177.0	7.6	3.9	0.83	0.097	46.7	0.37	70.2
NW_36_647	White	Wht.su	HA	summer	83.8	0.028	41.8	215.1	8.0	1.9	0.30	0.091	32.6	0.43	65.3

Appendix A.1 (Continued) Lake average seasonal physicochemical variables and diatom metric scores

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
SW_22_208	Acoose	Aco.sp	LA	0.67	18.8	75221	85199	11.2	156	1050	0	4.4
WE_33_1892	Acorrymore	Acy.sp	LA	0.14	37.3	57824	305775	1.8	188	485	301	10.2
SH_25_189	Alewnaghta	Alw.sp	MA	0.55	10.0	176522	191281	26.0	31	1071	1071	4.3
SW_19_4	Allua	Aua.sp	LA	1.36	28.4	118246	65268	108.4	88	935	695	3.8
NW_38_61	an tSeisigh	anT.sp	MA	0.24	20.7	204298	436409	0.8	29	583	583	5.8
SH_26_669	Annaghmore	Ann.sp	HA	0.53	15.9	190055	283811	3.2	47	748	748	5
NW_38_83	Anure	Anr.sp	LA	1.33	11.9	181793	416638	36.9	39	985	985	5.6
WE_31_76	Ardderry	Ard.sp	LA	0.81	9.4	98493	245918	15.4	38	796	796	4.9
WE_35_159	Arrow	Arr.sp	HA	12.47	39.5	178036	311001	66.2	53	2997	0	4.8
SH_27_108	Atedaun	Atd.sp	HA	0.38	8.5	129984	188440	305.8	16	794	794	4.4
WE_32_436	Aughrusbeg	Aug.sp	HA	0.50	14.3	55899	258536	2.1	5	934	934	9
WE_32_479	Ballynakill	Bkl.su	MA	0.62	10.7	64978	257939	7.5	7	1907	1155	6.4
EA_07_270	Bane	Bne.sp	HA	0.75	16.0	255075	271515	3.2	113	1097	1097	5.8
NW_38_84	Barra	Bar.sp	LA	0.63	11.3	192947	412356	19.6	89	1096	137	5.6
WE_32_402	Beaghcauneen	Bgc.sp	LA	0.19	15.3	68063	247050	16.4	15	544	236	6

Appendix A.2 Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
WE_32_452	Beltra	Blt.sp	LA	4.03	29.8	108108	299018	97.9	15	2923	2923	3.6
EA_10_28	Bray Lower	Bry.sp	LA	0.26	45.7	313618	216390	1.5	375	528	325	7.3
SW_21_402	Brin	Brn.sp	LA	0.25	12.8	78712	77412	5.2	113	873	859	4.7
WE_27_114	Bunny	Bun.sp	HA	1.03	12.9	137416	196280	12.1	17	1505	869	4.3
SH_23_74	Cam	Cam.sp	LA	0.08	15.0	59967	107816	4.7	209	223	223	5.5
SW_22_207	Caragh	Cgh.sp	LA	4.93	40.4	70276	88496	162.6	13	1910	0	3.4
WE_30_347	Carra	Car.sp	HA	15.64	20.5	117197	275295.29	109.1	15	3862	3862	4.5
WE_33_1914	Carrowmore	Cmr.sp	LA	9.15	2.9	83122	329850	87.9	5	4937	1690	5.6
SH_26_705	Cavetown	Cav.sp	HA	0.64	20.0	183991	297837	4.8	81	1590	1590	5.1
NW_36_655	Corglass	Crg.sp	HA	0.34	4.5	234475	309155	1.1	48	858	0	4.8
WE_30_666a	Corrib Lower	CL.sp	HA	50.63	12.0	127373	237965	3021.0	2	3357	3357	5.3
WE_30_666b	Corrib Upper	CU.sp	HA	115.68	45.0	114014	249527	1633.0	2	4449	0	5.3
SH_27_115	Cullaun	Cul.sp	HA	0.50	24.8	131940	190509	120.4	16	1137	1137	4.3
WE_34_406a	Cullin	Cln.sp	HA	10.24	3.4	123555	304639	819.8	8	4173	4173	4.4
WE_29_37	Cutra	Cut.su	MA	3.84	16.5	148441	198383	123.8	34	2872	2872	4.7

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
EA_10_29	Dan	Dan.sp	LA	1.03	38.0	315732.93	202517.18	62.8	204	2231	2231	4.6
SH_25_191a	Derg	Drg.sp	HA	116.51	40.0	184185	201062	10643.7	26	5009	4480	4.7
NW_36_400	Derrybrick	Dby.sp	HA	0.36	4.9	235060	310994	2.0	48	771	0	4.5
SH_28_82	Doo (CE)	Dce.sp	LA	1.30	14.8	111339	172285	22.8	86	780	648	6.3
WE_32_490	Doo (MO)	Dmo.sp	LA	1.55	40.0	83399	268718	27.4	13	1984	816	5.2
SH_27_82	Dromore	Drm.sp	HA	0.49	19.4	134495	185985	31.8	17	790	790	4.4
NW_38_692	Dunglow	Dug.sp	LA	0.65	7.8	178549	411372	37.7	17	966	966	5.6
WE_35_136	Easky	Eky.sp	LA	1.19	13.7	144751	323800	10.7	183	1813	1813	6.3
NW_36_671	Egish	Egs.sp	HA	1.12	12.8	278685	314055	9.3	161	751	751	5.2
NW_39_13	Fern	Frn.sp	MA	1.81	4.9	218149.08	422426.4	206.7	19	2381	443	4
WE_35_158	Gill	Gil.sp	HA	13.81	37.0	178271.52	335095.29	391.8	7	6669	6669	3.8
NW_38_22	Glen	Gln.sp	LA	1.68	14.0	209877	428283	123.0	27	981	742	4.1
WE_35_156	Glenade	Gde.sp	HA	0.74	11.3	183147	345696	14.9	58	1881	281	4.7
SW_21_444	Glenbeg	Gbg.sp	LA	0.66	31.1	70116	53788	7.5	73	1815	592	6.1
WE_35_139	Glencar	Gcr.sp	HA	1.15	19.5	175807	343540	41.0	23	2287	2287	4.6

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
WE_32_487	Glencullin	Glc.sp	LA	0.34	13.5	81529	269969	6.1	35	877	235	4.9
SH_24_99	Gur	Gur.sp	HA	0.79	6.0	164181	141243	3.7	75	1325	1325	5.6
SH_27_126	Inchicronan	Ich.sp	HA	1.17	18.8	139305	186047	28.6	26	721	721	4.9
NW_36_526	Inner	Inr.sp	HA	0.61	5.8	261727	317682	4.6	80	1250	1250	4.7
SH_28_64	Keagh	Kgh.sp	LA	0.07	5.7	110191	181276	0.4	183	417	417	7
NW_38_576	Keel	Kel.sp	LA	0.11	9.9	184628	416746	3.7	138	978	185	6.3
NW_38_47	Kiltooris	Ktr.sp	MA	0.43	6.0	167480	397619	5.5	9	1069	80	6.8
NW_38_670	Kindrum	Kdm.sp	HA	0.61	12.3	219357	443349	4.3	10	1705	1705	5.9
WE_32_509b	Kylemore	Kyl.sp	LA	1.34	29.8	76067	258198	20.8	16	2279	2279	5.2
NW_35_143	Lattone Lough	Lat.sp	MA	0.31	15.7	199877	345407	6.4	59	680	0	4.1
SW_22_185	Leane	Lea.sp	MA	19.52	75.0	93051	85851	557.8	20	4899	0	3.5
EA_07_274	Lene	Len.sp	HA	4.16	22.8	252367	268115	13.1	92	4324	3030	5.3
WE_30_344	Lettercraffroe	Let.sp	LA	0.82	17.7	105710	237659	4.3	153	781	545	5.4
SH_28_85	Lickeen	Lck.sp	MA	0.84	16.8	116356	190930	9.1	68	2517	118	5.2
WE_29_194	Lough Rea	Rea.sp	HA	3.01	21.0	162604	214806	12.0	85	2517	1659	5.4

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
WE_31_177	Loughaunore	Lar.sp	LA	0.06	13.1	83123	232030	0.5	85	375	171	7.7
WE_30_343	Maumwee	Mwe.sp	LA	0.28	8.7	97772	248221	3.9	48	740	0	5.4
SH_26_711	Meelagh	Mel.sp	HA	1.16	15.3	190146	311761	6.7	50	2213	177	3.8
NW_35_160	Melvin	Mev.sp	HA	22.06	41.6	188118	353084	246.0	23	5972	0	4.2
NW_01_104	Mourne	Mrn.sp	LA	0.67	10.1	207747	390193	8.5	170	1946	1946	4
SH_27_94	Muckanagh	Mck.sp	HA	0.96	14.4	136980	192403	34.3	18	1218	443	4.4
IE_NB_06_56	Muckno or Blayney	MuB.sp	HA	3.56	12.0	283163	319726	110.4	90	1905	0	5
NW_36_272	Mushlin	Msh.sp	MA	0.04	1.9	262566	301039	0.2	190	223	220	6
WE_31_208	Nahasleam	Nah.sp	LA	0.28	6.1	97285	244063	0.3	36	359	242	
WE_32_500	Nambrackmore	Nbm.sp	LA	0.10	9.8	71667	245522	0.2	25	258	210	5.8
NW_38_67	Nasnanida	Nas.sp	LA	0.15	8.0	185548	407919	2.0	68	648	648	4.9
UKGBNI3NB0032	Neagh	Ngh.sp	HA	381.79	30.0	235486	326342	4450.0	10	23821	23821	6.75
SH_26_693	O'Flynn	Ofn.sp	HA	1.37	14.3	158520	279114	16.8	76	1144	649	5.5
SH_26_703	Owel	Owl.sp	HA	10.22	22.8	242082	256438	44.6	96	6163	1021	5.5

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
EA_07_275	Ramor	Ram.sp	HA	7.13	5.5	259416	286871	236.9	84	2655	1941	5.2
WE_30_345	Ross	Ros.sp	HA	1.39	10.0	119265	236493	37.7	5	848	810	4.5
WE_31_98	Seecon	See.sp	LA	0.26	3.0	108710	235426	8.8	106	573	573	5
SH_26_709	Sheelin	She.sp	HA	18.16	13.7	244954	282453	253.8	65	3896	3896	5.1
WE_31_171	Shindilla	Shi.sp	LA	0.66	21.8	95493	245773	8.5	38	734	0	5.4
WE_29_181	Skeardeen	Skd.sp	HA	0.06		138924	199139		19			
WE_34_405	Talt	Tlt.sp	HA	0.97	39.80	140162	314548	4.9	135	1834	0	5
EA_10_25	Tay	Tay.sp	LA	0.50	34.86	316298	208405	20.1	246	1006	1006	6.6
WE_35_157	Templehouse	Tph.sp	HA	1.19	6.25	161908	318157	273.9	55	2031	2031	4.4
N/A	Travaun	Trv.su	HA	0.28	8.00	135453	196688	4.0	17	729	0	4.4
NW_36_651	Tullynasiddagh	Tuy.sp	LA	0.47	11.33	198254	365794	3.5	108	1136	415	5.3
SW_22_186	Upper	Upr.su	LA	1.67	35.95	92530	82546	113.0	20	543	543	4.2
EA_10_32	Upper Glendalough	ULG.sp	LA	0.35	29.58	310943	196202	18.3	133	1295	1295	4.6

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	Lake area (km ²)	Max. lake depth (m)	Easting	Northing	Catchment area (km ²)	Lake altitude (m)	Maximum fetch (km)	South-westerly	Average wind speed (m ^{-sec})
NW_36_672	Upper Lough Erne	Er.sp	HA	32.18	27.00	229345	333538	3352.3	38	2640	2640	6
EA_07_267	Upper Lough Skeagh	ULS.sp	MA	0.61	15.47	265325	300897	4.4	149	681	681	6
SH_26_689	Urluar	Url.sp	HA	1.15	10.96	150752	289399	24.7	80	874	486	5.4
NW_38_80	Veagh Upper	Vgh.sp	LA	1.53	49.85	202272	421312	28.6	45	2463	2463	4.1
NW_36_647	White	Wht.sp	HA	0.54	8.58	267814	319106	134.0	80	436	436	4.7

Appendix A.2 (Continued) Supplementary physical, spatial and morphological variables.

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
SW_22_208	Acoose	Aco.sp	LA	0.9	0.0	68.6	0.0	7.7	16.4	0.0	0.0	6.4
WE_33_1892	Acorrymore	Acy.sp	LA	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
SH_25_189	Alewnaghta	Alw.sp	MA	7.0	0.0	15.8	22.0	35.7	0.0	17.1	0.0	2.4
SW_19_4	Allua	Aua.sp	LA	9.3	0.0	44.4	14.2	6.3	3.1	21.0	0.0	1.9
NW_38_61	an tSeisigh	anT.sp	MA	0.0	0.0	35.3	0.0	18.7	0.0	10.5	4.7	30.7
SH_26_669	Annaghmore	Ann.sp	HA	0.0	0.0	0.0	0.0	0.0	7.6	66.9	0.0	25.5
NW_38_83	Anure	Anr.sp	LA	0.7	0.0	79.0	0.0	0.2	8.0	8.7	0.0	3.5
WE_31_76	Ardderry	Ard.sp	LA	0.0	0.0	70.3	14.9	2.7	0.0	0.0	0.0	12.1
WE_35_159	Arrow	Arr.sp	HA	14.3	1.3	8.6	2.5	12.2	4.7	36.1	0.0	20.3
SH_27_108	Atedaun	Atd.sp	HA	8.8	0.5	2.5	1.5	10.9	36.3	36.4	0.2	2.8
WE_32_436	Aughrusbeg	Aug.sp	HA	36.8	0.0	36.8	0.0	0.0	0.0	0.0	0.0	26.5
WE_32_479	Ballynakill	Bkl.su	MA	12.4	0.0	10.9	0.0	43.4	0.0	24.7	0.0	8.6
EA_07_270	Bane	Bne.sp	HA	2.0	0.0	0.0	0.0	0.0	0.0	69.9	0.0	28.1
NW_38_84	Barra	Bar.sp	LA	0.0	0.0	69.8	0.0	0.0	26.9	0.0	0.0	3.3
WE_32_402	Beaghcauneen	Bgc.sp	LA	0.0	0.0	68.7	0.0	0.0	0.0	0.0	0.0	31.3

Appendix A.3 CORINE catchment landcover in the study lakes

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
WE_32_452	Beltra	Blt.sp	LA	16.9	0.0	55.6	7.6	13.4	2.2	0.0	0.0	4.4
EA_10_28	Bray Lower	Bry.sp	LA	0.0	0.0	79.6	0.0	0.0	0.0	0.0	0.0	20.4
SW_21_402	Brin	Brn.sp	LA	9.1	0.0	23.3	0.0	0.0	56.7	5.4	0.0	5.5
WE_27_114	Bunny	Bun.sp	HA	0.0	2.2	8.8	0.0	10.0	36.8	34.7	0.0	7.4
SH_23_74	Cam	Cam.sp	LA	0.0	0.0	77.9	20.3	0.0	1.8	0.0	0.0	0.0
SW_22_207	Caragh	Cgh.sp	LA	10.0	0.0	59.0	1.8	8.3	13.0	3.6	0.0	4.3
WE_30_347	Carra	Car.sp	HA	20.8	1.2	8.3	1.1	7.1	0.0	44.7	0.0	16.9
WE_33_1914	Carrowmore	Cmr.sp	LA	4.9	0.0	57.9	9.9	10.5	0.0	6.0	0.1	10.7
SH_26_705	Cavetown	Cav.sp	HA	34.9	0.1	1.8	0.0	7.1	0.0	40.3	0.0	15.7
NW_36_655	Corglass	Crg.sp	HA	0.0	0.0	0.0	0.0	0.0	0.0	60.5	0.0	39.5
WE_30_666a	Corrib Lower	CL.sp	HA	16.2	3.2	19.8	1.2	5.4	1.7	42.1	0.8	9.5
WE_30_666b	Corrib Upper	CU.sp	HA	19.6	2.3	24.6	1.5	6.8	2.9	27.6	0.6	14.2
SH_27_115	Cullaun	Cul.sp	HA	6.3	0.2	2.8	0.0	8.5	41.4	34.9	0.0	5.9
WE_34_406a	Cullin	Cln.sp	HA	25.7	1.4	29.7	2.1	8.8	0.1	22.9	1.1	8.2
WE_29_37	Cutra	Cut.su	MA	8.9	0.0	18.2	17.9	37.8	0.0	13.6	0.0	3.6

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
EA_10_29	Dan	Dan.sp	LA	0.2	0.0	69.7	9.5	12.9	3.7	1.3	0.0	2.7
SH_25_191a	Derg	Drg.sp	HA	6.2	2.9	12.2	2.7	7.9	0.7	61.8	1.2	4.5
NW_36_400	Derrybrick	Dby.sp	HA	0.0	0.0	0.0	0.0	0.0	0.0	76.2	0.0	23.8
SH_28_82	Doo (CE)	Dce.sp	LA	3.2	2.4	21.3	6.0	44.0	0.0	15.4	0.0	7.6
WE_32_490	Doo (MO)	Dmo.sp	LA	0.0	0.0	49.6	2.7	2.8	37.8	0.0	0.0	7.1
SH_27_82	Dromore	Drm.sp	HA	11.3	2.4	2.4	3.5	26.9	0.0	49.2	0.9	3.4
NW_38_692	Dunglow	Dug.sp	LA	7.1	0.0	78.6	0.0	9.7	0.0	0.0	0.0	4.6
WE_35_136	Easky	Eky.sp	LA	0.0	0.0	85.9	0.8	1.3	0.0	0.0	0.0	12.0
NW_36_671	Egish	Egs.sp	HA	9.4	0.0	0.0	0.0	0.0	0.0	74.6	2.2	13.8
NW_39_13	Fern	Frn.sp	MA	18.3	0.0	35.6	1.0	15.9	7.3	18.5	0.4	2.9
WE_35_158	Gill	Gil.sp	HA	23.4	1.3	23.3	6.4	13.2	4.7	23.0	0.4	4.4
NW_38_22	Glen	Gln.sp	LA	3.5	0.0	66.7	2.6	5.7	14.9	2.2	0.0	4.4
WE_35_156	Glenade	Gde.sp	HA	9.2	0.0	58.6	1.9	0.0	18.8	6.4	0.0	5.2
SW_21_444	Glenbeg	Gbg.sp	LA	0.0	0.0	60.7	0.0	3.0	27.1	0.0	0.0	9.3
WE_35_139	Glencar	Gcr.sp	HA	28.8	0.0	37.9	0.7	6.2	12.6	10.9	0.0	2.9

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
WE_32_487	Glencullin	Glc.sp	LA	0.0	0.0	85.9	0.0	0.0	7.9	0.0	0.0	6.2
SH_24_99	Gur	Gur.sp	HA	6.8	0.0	0.0	0.0	0.5	0.0	69.8	0.0	22.8
SH_27_126	Inchicronan	Ich.sp	HA	20.1	1.2	16.5	2.1	23.2	0.0	32.4	0.0	4.6
NW_36_526	Inner	Inr.sp	HA	0.0	0.0	0.0	21.1	7.8	0.0	56.4	0.0	14.7
SH_28_64	Keagh	Kgh.sp	LA	0.0	0.0	99.2	0.1	0.0	0.0	0.7	0.0	0.0
NW_38_576	Keel	Kel.sp	LA	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
NW_38_47	Kiltooris	Ktr.sp	MA	0.0	0.0	29.5	0.0	3.6	2.3	51.2	0.0	13.4
NW_38_670	Kindrum	Kdm.sp	HA	20.3	0.0	44.7	0.0	0.0	0.0	20.6	0.0	14.4
WE_32_509b	Kylemore	Kyl.sp	LA	0.0	0.0	62.2	4.2	6.9	20.1	0.0	0.0	6.6
NW_35_143	Lattone Lough	Lat.sp	MA	8.8	0.0	63.0	1.7	0.0	0.0	21.5	0.0	5.0
SW_22_185	Leane	Lea.sp	MA	4.1	0.0	45.8	4.6	10.3	5.0	23.2	1.7	5.3
EA_07_274	Lene	Len.sp	HA	0.0	0.0	0.0	0.0	6.2	0.0	58.7	2.0	33.1
WE_30_344	Lettercraffroe	Let.sp	LA	0.0	0.0	32.8	30.1	16.3	0.0	0.0	0.0	20.9
SH_28_85	Lickeen	Lck.sp	MA	18.3	0.0	9.9	8.5	18.0	0.0	34.9	0.0	10.4
WE_29_194	Lough Rea	Rea.sp	HA	3.6	0.0	0.0	0.0	0.0	0.0	64.8	5.4	26.2

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
WE_31_177	Loughaunore	Lar.sp	LA	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
WE_30_343	Maumwee	Mwe.sp	LA	0.0	0.0	92.0	0.0	0.0	0.0	0.0	0.0	8.0
SH_26_711	Meelagh	Mel.sp	HA	11.0	13.0	11.6	7.5	23.3	0.0	16.3	0.0	17.2
NW_35_160	Melvin	Mev.sp	HA	13.8	0.0	17.2	2.5	7.8	3.9	8.7	0.3	9.2
NW_01_104	Mourne	Mrn.sp	LA	0.0	0.0	73.8	12.9	5.2	0.0	0.0	0.0	8.0
SH_27_94	Muckanagh	Mck.sp	HA	7.9	0.0	6.7	0.0	14.0	6.9	53.5	0.0	11.0
IE_NB_06_56	Muckno or Blayney	MuB.sp	HA	4.8	0.3	1.7	0.3	0.8	0.0	84.1	4.4	3.7
NW_36_272	Mushlin	Msh.sp	MA	99.7	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0
WE_31_208	Nahasleam	Nah.sp	LA	0.0	0.0	71.2	11.7	8.1	0.0	0.0	0.0	9.0
WE_32_500	Nambrackmore	Nbm.sp	LA	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
NW_38_67	Nasnanida	Nas.sp	LA	0.0	0.0	85.1	0.0	0.0	0.0	0.0	0.0	14.9
UKGBNI3NB0032	Neagh	Ngh.sp	HA	15.2	1.3	5.5	1.2	1.1	2.6	62.1	2.7	8.3
SH_26_693	O'Flynn	Ofn.sp	HA	8.9	0.0	32.5	0.5	5.7	0.0	41.7	1.8	9.0
SH_26_703	Owel	Owl.sp	HA	7.1	1.9	1.0	1.7	5.5	0.0	55.5	1.4	25.7
EA_07_275	Ramor	Ram.sp	HA	11.4	0.1	0.6	1.1	3.5	0.0	78.8	1.1	3.4

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
WE_30_345	Ross	Ros.sp	HA	19.1	2.6	39.0	3.2	13.7	0.0	18.4	0.0	4.1
WE_31_98	Seecon	See.sp	LA	0.0	0.0	18.2	77.7	0.0	0.0	0.0	0.0	4.1
SH_26_709	Sheelin	She.sp	HA	3.3	0.6	2.1	0.8	3.3	0.7	80.9	1.1	7.4
WE_31_171	Shindilla	Shi.sp	LA	0.0	0.0	84.5	4.8	1.7	0.0	0.0	0.0	9.1
WE_29_181	Skeardeen	Skd.sp	HA	0.0	0.0	00.0	0.0	0.0	0.0	0.0	0.0	0.0
WE_34_405	Talt	Tlt.sp	HA	0.0	0.0	52.1	1.5	0.6	0.0	25.0	0.0	20.7
EA_10_25	Tay	Tay.sp	LA	0.0	0.0	81.1	2.9	10.7	2.3	0.0	0.0	3.0
WE_35_157	Templehouse	Tph.sp	HA	10.8	4.7	13.0	2.0	7.8	1.2	59.1	0.5	0.9
N/A	Travaun	Trv.su	HA	0.0	0.0	0.0	0.0	0.0	90.0	0.0	0.0	10.0
NW_36_651	Tullynasiddagh	Tuy.sp	LA	0.0	0.0	74.9	6.6	0.0	0.0	4.3	0.0	14.2
SW_22_186	Upper	Upr.su	LA	2.3	0.0	71.4	0.4	7.6	15.5	0.5	0.0	2.3
EA_10_32	Upper Glendalough	ULG.sp	LA	0.0	0.0	57.6	19.2	6.3	14.6	0.0	0.0	2.3
NW_36_672	Upper Lough Erne	Er.sp	HA	7.0	0.0	7.4	4.1	1.6	2.5	72.6	0.5	4.1
EA_07_267	Upper Lough Skeagh	ULS.sp	MA	4.9	0.0	0.0	0.0	7.5	0.0	72.3	0.0	15.3
SH_26_689	Urluar	Url.sp	HA	3.3	3.6	29.2	0.0	5.6	0.0	49.8	0.5	8.1

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes.

Lake code	Lake	Lake ID	Lake Type	AGRICULTURE _OTHER	ARABLE	BOG	CONIFEROUS	FOREST	NATURAL	PASTURE	URBAN	WATER
NW_38_80	Veagh Upper	Vgh.sp	LA	0.0	0.0	50.5	0.0	8.1	36.1	0.0	0.0	5.3
NW_36_647	White	Wht.sp	HA	1.2	0.2	0.7	0.5	0.5	0.0	94.9	1.0	1.0

Appendix A.3 (Continued) CORINE catchment landcover in the study lakes.

APPENDIX B

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	195	88.4	27.3		
<i>Amphora pediculus</i> (Kützing) Grunow in A.W.F.Schmidt	115	38.4	2.2		
<i>Nitzschia</i> Hassall	108	6.8	0.5		
<i>Navicula cryptotenella</i> Lange-Bertalot	102	12.1	0.7		
<i>Encyonema silesiacum</i> (Bleisch) D.G.Mann in Round, R.M.Crawford & D.G.Mann	92	24.6	1.3		
<i>Nitzschia lacuum</i> Lange-Bertalot	86	10.4	0.5		
<i>Navicula cryptotenelloides</i> Lange-Bertalot	85	17.9	0.8		
<i>Navicula</i> sp. indet.	84	11.2	0.5		
<i>Sellaphora nigri</i> (De Notaris) C.E.Wetzel & L.Ector	80	40.4	1.6		
<i>Fragilaria gracilis</i> Østrup	80	32.0	1.0		
<i>Encyonopsis microcephala</i> form 1	79	70.0	4.7	4	
<i>Tabellaria flocculosa</i> (Roth) Kützing	78	82.6	2.3		
<i>Denticula tenuis</i> Kützing	73	7.8	0.5		

Appendix B A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Gomphonema lateripunctatum</i> E.Reichardt & Lange-Bertalot	72	11.0	1.0		
<i>Encyonopsis minuta</i> Krammer & E.Reichardt	71	18.8	1.2		
<i>Cymbella affinis</i> Kützing	70	43.7	1.5		
<i>Encyonema minutum</i> (Hilse) D.G.Mann in Round, R.M.Crawford & D.G.Mann	68	15.2	0.9		
<i>Fragilaria capucina</i> Desmazières	68	32.8	0.6		
<i>Karayevia clevei</i> (Grunow) Bukhtiyarova	65	10.9	0.3		
<i>Cocconeis lineata</i> Ehrenberg	62	6.9	0.4		
<i>Brachysira microcephala</i> form 1 (Grunow) Compère	61	33.8	1.8	2	
<i>Encyonopsis subminuta</i> Krammer & Reichardt	61	26.7	0.9		
<i>Fragilaria perminuta</i> (Grunow) Lange-Bertalot in Krammer & Lange-Bertalot	60	11.2	0.6		
<i>Nitzschia dissipata</i> (Kützing) Rabenhorst	60	11.5	0.5	6	
<i>Encyonopsis krammeri</i> E.Reichardt	59	44.7	3.0	5	
<i>Cymbella helvetica</i> Kützing	55	12.3	0.4	5	
<i>Achnanthes</i> Bory	54	5.2	0.2		

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Navicula subalpina</i> E.Reichardt	53	3.1	<1.0	5	
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	53	26.5	0.8	6	
<i>Pseudostaurosira brevistriata</i> (Grunow) D.M.Williams & Round	53	8.3	0.4		
<i>Encyonopsis neerlandica</i> Van de Vijver, Verweij, van der Wal & A.Mertens	52	55.1	1.7	2	
<i>Navicula reichardtiana</i> Lange-Bertalot	51	9.2	0.3	6	
<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	50	19.8	0.6		
<i>Navicula gregaria</i> Donkin	49	19.1	0.4	6	
<i>Navicula tripunctata</i> (O.F.Müller) Bory in Bory de Saint-Vincent	49	20.3	0.4	6	
<i>Fragilaria tenera</i> (W.Smith) Lange-Bertalot	49	7.1	0.4		
<i>Encyonema neogracile</i> Krammer	48	22.8	0.6		
<i>Amphora indistincta</i> Levkov	47	14.9	0.4		
<i>Gomphonema parvulum</i> (Kützing) Kützing	47	10.6	0.3		
<i>Gomphonema olivaceum</i> (Hornemann) Brébisson	46	14.9	0.4	6	spring
<i>Nitzschia paleacea</i> (Grunow) Grunow in van Heurck	45	20.3	0.4	6	
<i>Cocconeis neothumensis</i> Krammer	45	16.8	0.4		

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Eunotia incisa</i> W.Smith ex W.Gregory	44	13.9	0.6	3	
<i>Stauroforma exiguiformis</i> (Lange-Bertalot) R.J.Flower, V.J.Jones & Round	43	28.9	0.6	2	
<i>Amphora inariensis</i> Krammer	43	4.6	0.1		
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Lange-Bertalot	42	4.3	<1.0	6	
<i>Fragilaria vaucheriae</i> (Kützing) J.B.Petersen	41	22.7	0.4	6	
<i>Fragilaria</i> Lyngbye	41	23.1	0.3		
<i>Cocconeis euglypta</i> Ehrenberg	41	10.9	0.3	6	
<i>Nitzschia fonticola</i> (Grunow) Grunow in Van Heurck	40	17.0	0.5	6	
<i>Staurosirella pinnata</i> (Ehrenberg) D.M.Williams & Round	40	15.7	0.4		
<i>Cymbella hustedtii</i> Krasske	40	13.4	0.3		
<i>Diatoma tenuis</i> C.Agardh	40	7.4	0.2		
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützing) Grunow	40	6.0	0.2	6	
<i>Eunotia</i> Ehrenberg	40	4.7	0.2		
<i>Encyonopsis alpina</i> Krammer & Lange-Bertalot in Krammer	37	30.8	1.0	5	
<i>Brachysira microcephala</i> form 2	37	14.8	0.8	2	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Encyonopsis microcephala</i> form 2	37	6.7	0.2		
<i>Gomphonema pumilum</i> agg.	36	36.9	0.9		
<i>Nitzschia palea</i> (Kützing) W.Smith	35	3.1	<1.0	6	
<i>Epithemia sorex</i> Kützing	35	48.1	0.7		summer
<i>Ulnaria delicatissima</i> (W.Smith) Aboal & P.C.Silva	35	8.0	0.3		
<i>Gomphonema olivaceoides</i> Hustedt 1950	33	12.7	0.4		spring
<i>Mayamaea permitis</i> (Hustedt) K.Bruder & Medlin	33	9.6	0.3		
<i>Nitzschia gracilis</i> Hantzsch	32	8.6	0.2		
<i>Brachysira neglectissima</i> Lange-Bertalot in Werum & Lange-Bertalot	31	36.0	0.3	5	
<i>Geissleria</i> Lange-Bertalot & Metzeltin	31	4.7	0.1	6	
<i>Frustulia crassinervia</i> (Brébisson ex W.Smith) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin	29	7.8	0.3	2	
<i>Navicula</i> indet. small forms	29	20.0	0.3		
<i>Peronia fibula</i> (Brébisson ex Kützing) R.Ross	28	19.9	0.4	2	
<i>Brachysira microcephala</i> form 3	27	31.2	1.0	2	
<i>Encyonopsis hibernica</i> B.Kennedy, Y.Bukley & Allott	27	25.8	0.7		

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Rossithidium pusillum</i> (Grunow) Round & Bukhtiyarova	27	11.4	0.2		
<i>Achnantheidium pyrenaicum</i> (Hustedt) H.Kobayasi	26	56.5	1.2	4	
<i>Mastogloia lacustris</i> (Grunow) Grunow in Van Heurck	26	49.5	1.1	5	summer
<i>Gomphonema pumilum</i> (Grunow) E.Reichardt & Lange-Bertalot	26	33.8	0.8		
<i>Frustulia erifuga</i> Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin	26	14.6	0.5	3	
<i>Amphora</i> Ehrenberg ex Kützing	25	3.4	<1.0	6	
<i>Brachysira brebissonii</i> R.Ross in Hartley, R.Ross & D.M.Williams	25	12.3	0.2	2	
<i>Brachysira garrensis</i> (Lange-Bertalot & Krammer) Lange-Bertalot in H.Lange-Bertalot & Gerd Moser	24	54.4	1.0		
<i>Diatoma moniliformis</i> (Kützing) D.M.Williams	24	18.1	0.3		spring
<i>Staurosira elliptica</i> (Schumann) Williams & Round	24	6.0	0.2		
<i>Gomphonema exilissimum</i> (Grunow) Lange-Bertalot & E.Reichardt in Lange-Bertalot & Metzeltin	23	18.2	0.3	3	
<i>Sellaphora saugerresii</i> (Desmazières) C.E.Wetzel & D.G.Mann	23	9.0	0.2	3	
<i>Nitzschia sociabilis</i> Hustedt	23	9.6	0.1	6	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Cymbella</i> C.Agardh	23	5.0	0.1		
<i>Encyonopsis carraensis</i> B.Kennedy, Y.Buckley & Allott	22	39.0	0.9	5	
<i>Eunotia pectinalis</i> (Kützing) Rabenhorst	22	50.2	0.6	3	
<i>Psammothidium subatomoides</i> (Hustedt) Bukhtiyarova & Round	22	49.9	0.4	3	
<i>Platessa oblongella</i> (Østrup) C.E.Wetzel, Lange-Bertalot & Ector	22	6.7	0.1	3	
<i>Frustulia saxonica</i> Rabenhorst	21	7.2	0.2	2	
<i>Planothidium granum</i> (M.H.Hohn & Hellerman) Lange-Bertalot	20	3.4	<1.0	6	
<i>Brachysira intermedia</i> (Østrup) Lange-Bertalot in Lange-Bertalot & Gerd Moser	20	12.7	0.2	2	
<i>Encyonema cespitosum</i> Kützing	20	5.6	0.1	5	
<i>Encyonopsis</i> aff. <i>tavirana</i> Krammer	19	25.1	0.4		
<i>Eunotia implicata</i> Nörpel, Lange-Bertalot & Alles in Alles, Nörpel-Schempp & Lange-Bertalot	19	12.6	0.2		
<i>Diatoma ehrenbergii</i> Kützing	19	21.2	0.2		
<i>Psammothidium helveticum</i> (Hustedt) Bukhtiyarova & Round	18	44.3	0.6	3	
<i>Nitzschia diversa</i> Hustedt	16	2.3	<1.0	5	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Staurosirella martyi</i> (Héribaud-Joseph) E.A.Morales & K.M.Manoylov	16	2.3	<1.0	6	
<i>Staurosira</i> Ehrenberg	16	15.6	0.3		
<i>Epithemia adnata</i> (Kützing) Brébisson	16	4.7	0.1		summer
<i>Nitzschia perminuta</i> (Grunow) M.Peragallo	16	6.5	0.1	2	
<i>Fistulifera saprophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	16	9.8	0.1		
<i>Staurosirella oldenburgiana</i> (Hustedt) Morales	16	4.8	0.1		
<i>Eunotia mucophila</i> (Lange-Bertalot, Nörpel-Schempp & Alles) Lange-Bertalot in Metzeltin & Lange-Bertalot	15	36.1	0.4		
<i>Gomphonema tergestinum</i> (Grunow) Fricke	14	<1.0	<1.0		spring
<i>Gomphonema angustum/pumilum</i> agg.	14	19.3	0.2		
<i>Nitzschia frustulum</i> (Kützing) Grunow in Cleve & Grunow	14	6.7	0.2	6	
<i>Eunotia naegelii</i> Migula	14	5.8	0.1	2	
<i>Fragilaria rumpens</i> (Kützing) G.W.F.Carlson	14	6.9	0.1		
<i>Nitzschia archibaldii</i> Lange-Bertalot	14	5.7	0.1	6	
<i>Psammothidium ventrale</i> (Krasske) Bukhtiyarova & Round	13	2.1	<1.0	3	
<i>Navicula capitatoradiata</i> H.Germain ex Gasse	13	10.6	0.2	6	summer

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Fragilaria mesolepta</i> Rabenhorst	12	33.1	0.3		
<i>Sellaphora difficillima</i> (Hustedt) C.E.Wetzel, L.Ector & D.G.Mann in Wetzel <i>et al.</i>	12	13.2	0.2	3	
<i>Kobayasiella subtilissima</i> (Cleve) Lange-Bertalot	11	3.9	<1.0	2	
<i>Psammothidium altaicum</i> (Poretzky) Bukhtiyarova in Bukhtiyarova & Round	11	2.3	<1.0	3	
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt in Kanitz	11	21.3	0.2	2	
<i>Delicatophycus delicatulus</i> (Kützing) M.J.Wynne	11	11.9	0.2	5	
<i>Pseudostaurosira robusta</i> (Fusey) D.M.Williams & Round	11	4.8	0.1		
<i>Staurosirella</i> D.M.Williams & Round	11	5.6	0.1		
<i>Eunotia exigua</i> (Brébisson ex Kützing) Rabenhorst	11	4.9	0.1		
<i>Navicula leptostriata</i> Jørgensen	10	4.2	<1.0	2	
<i>Gomphonema acuminatum</i> Ehrenberg	10	3.5	<1.0	2	
<i>Encyonema reichardtii</i> (Krammer) D.G.Mann in Round, R.M.Crawford & D.G.Mann	10	3.3	<1.0	6	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Psammothidium lacus-vulcani</i> (Lange-Bertalot & Krammer) L.N.Bukhtiyarova in L.N.Bukhtiyarova & Round	10	2.4	<1.0	1	
<i>Pinnularia subcapitata</i> W.Gregory	10	14.6	0.1	3	
<i>Achnanthes didyma</i> Hustedt	10	14.1	0.1		
<i>Brachysira praegeri</i> B.Kennedy & Allott	9	17.4	<1.0	2	
<i>Nitzschia inconspicua</i> Grunow	9	7.8	<1.0	6	
<i>Karayevia carissima</i> (Lange-Bertalot) Bukhtiyarova	9	7.7	<1.0	2	
<i>Gomphonema occultum</i> Reichardt & Lange-Bertalot	9	4.3	<1.0	5	spring
<i>Navicula notha</i> J.H.Wallace	9	2.2	<1.0	2	
<i>Psammothidium abundans</i> (Manguin) Bukhtiyarova & Round	9	2.0	<1.0	2	
<i>Adlafia minuscula</i> var. <i>muralis</i> (Grunow) Lange-Bertalot	8	11.1	<1.0	2	
<i>Fragilariforma virescens</i> (Ralfs) Williams & Round	8	6.6	<1.0	3	
<i>Achnanthes kuelbsii</i> Lange-Bertalot in Lange-Bertalot & Krammer	8	2.5	<1.0	2	
<i>Nitzschia solgensis</i> Cleve-Euler	7	4.7	<1.0	6	
<i>Tabellaria quadrisepata</i> B.M.Knudson	7	4.6	<1.0	2	
<i>Pinnularia perirrorata</i> Krammer	6	4.0	<1.0	3	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Sellaphora atomoides</i> (Grunow) Wetzel & Van de Vijver	6	2.3	<1.0	3	
<i>Navicula lanceolata</i> (Agardh) Ehrenberg	5	<1.0	<1.0		spring
<i>Surirella roba</i> Leclercq	5	4.9	<1.0	3	
<i>Adlafia minuscula</i> (Grunow) Lange-Bertalot	5	3.8	<1.0	3	
<i>Epithemia goeppertiana</i> Hilse	5	2.8	<1.0	5	
<i>Eunotia trinacria</i> Krasske	5	2.1	<1.0	3	
<i>Rossithidium anastasiae</i> (Kaczmarska) Potapova	4	34.8	<1.0	3	
<i>Nitzschia</i> sp. form 4	4	5.7	<1.0	6	
<i>Pinnularia appendiculata</i> (C.Agardh) Schaarschmidt	4	5.5	<1.0	3	
<i>Eunotia</i> girdle indet.	4	5.3	<1.0	3	
<i>Epithemia smithii</i> Carruthers	4	3.7	<1.0	5	
<i>Chamaepinnularia soehrensii</i> (Krasske) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin	4	3.6	<1.0	3	
<i>Nupela fennica</i> (Hustedt) Lange-Bertalot in Krammer & Lange- Bertalot	4	3.1	<1.0	2	
<i>Kolbesia ploenensis</i> (Hustedt) Kingston	4	3.0	<1.0	2	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

Diatom taxa (> 5.0% and occurring in at least 10 samples with all significant indicator taxa identified)	No. samples	Max. abundance (%)	Average abundance (%)	Significant Indicator for Cluster	Significant Seasonal Indicator
<i>Eunotia denticulata</i> (Brébisson ex Kützing) Rabenhorst	3	2.2	<1.0	2	
<i>Nitzschia</i> aff. <i>subtilioides</i> Hustedt	2	12.4	<1.0	3	
<i>Eunotia microcephala</i> Krasske	2	2.4	<1.0	3	
<i>Chamaepinnularia mediocris</i> (Krasske) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin	2	2.3	<1.0	3	

Appendix B (Continued) A list of all common diatom taxa with abundance and indicator categories identified.

APPENDIX C

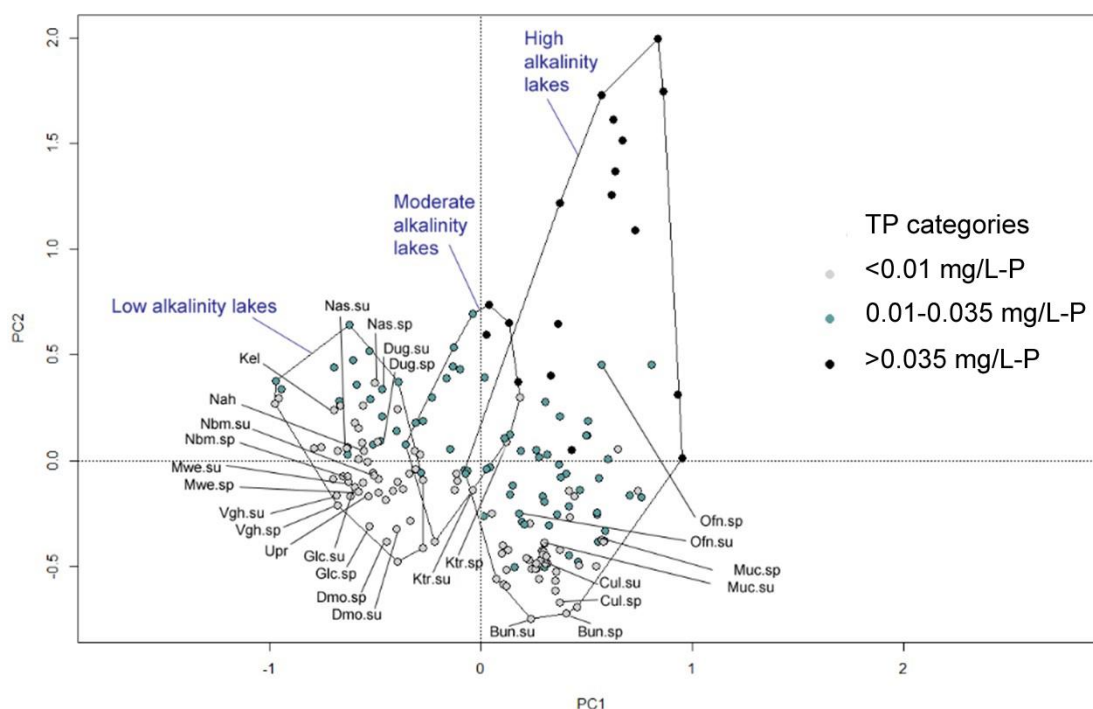


Figure C.1 PCA plots of axis-1 and axis-2 of 91 studied lakes with lakes grouped by Low (LA), Moderate (MA) and High (HA) alkalinity types in convex hulls along PC-1 and categorised by total phosphorus indicating a trophic gradient along PC-2. Labelled samples refer to candidate reference lakes or waterbodies discussed in the text.

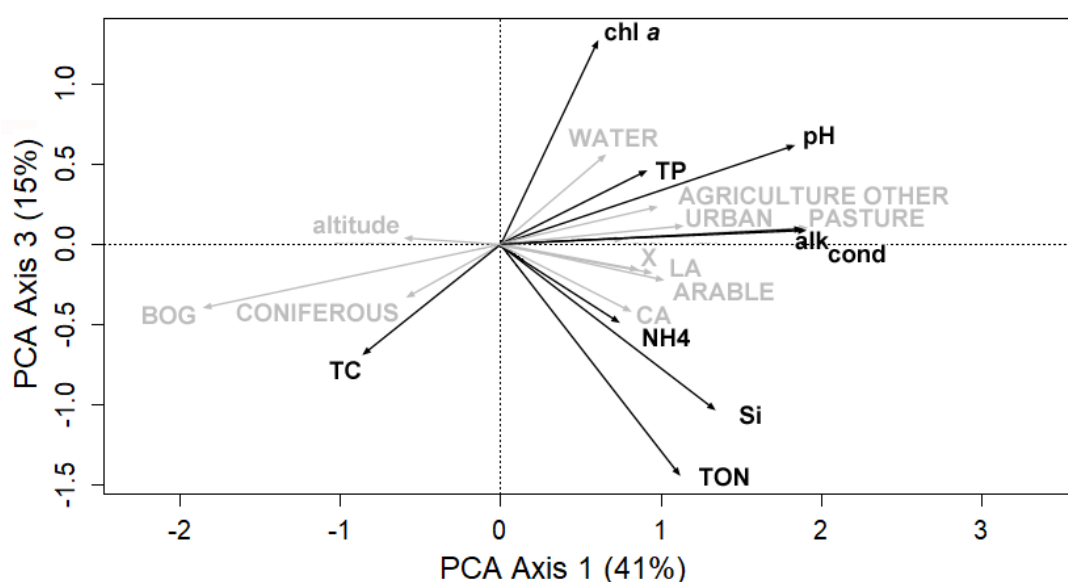


Figure C.2 PCA plots of axis-1 and axis-3 of 91 studied lakes with physiochemical parameters displayed as black arrows and significant supplementary variables represented passively in the ordination as grey arrows.

APPENDIX D

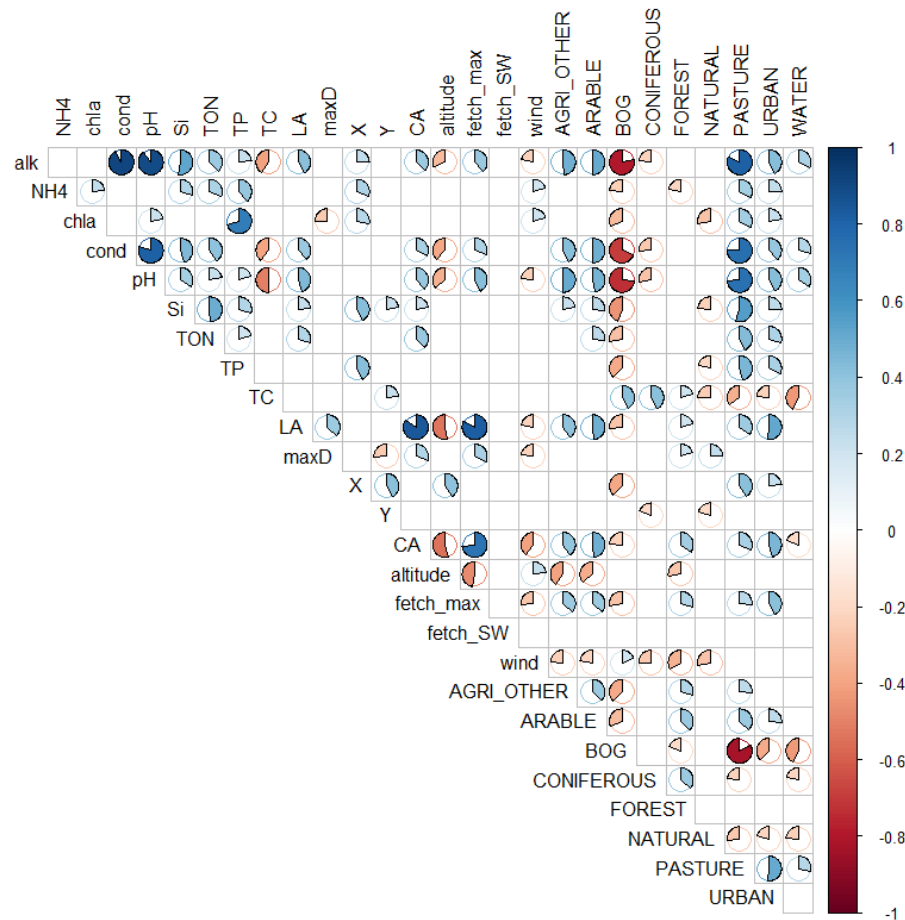


Figure D.1 Correlation matrix of explanatory variables with significant Pearson correlations ($p < 0.05$) coloured blue (positive) or red (negative) and circles filled conditional on the strength of the association between the variables. The meaning of environmental codes is explained in the introduction and in Appendix A.

Non-hierarchical Clustering (PAM) of Diatom Community (Bray-Curtis)

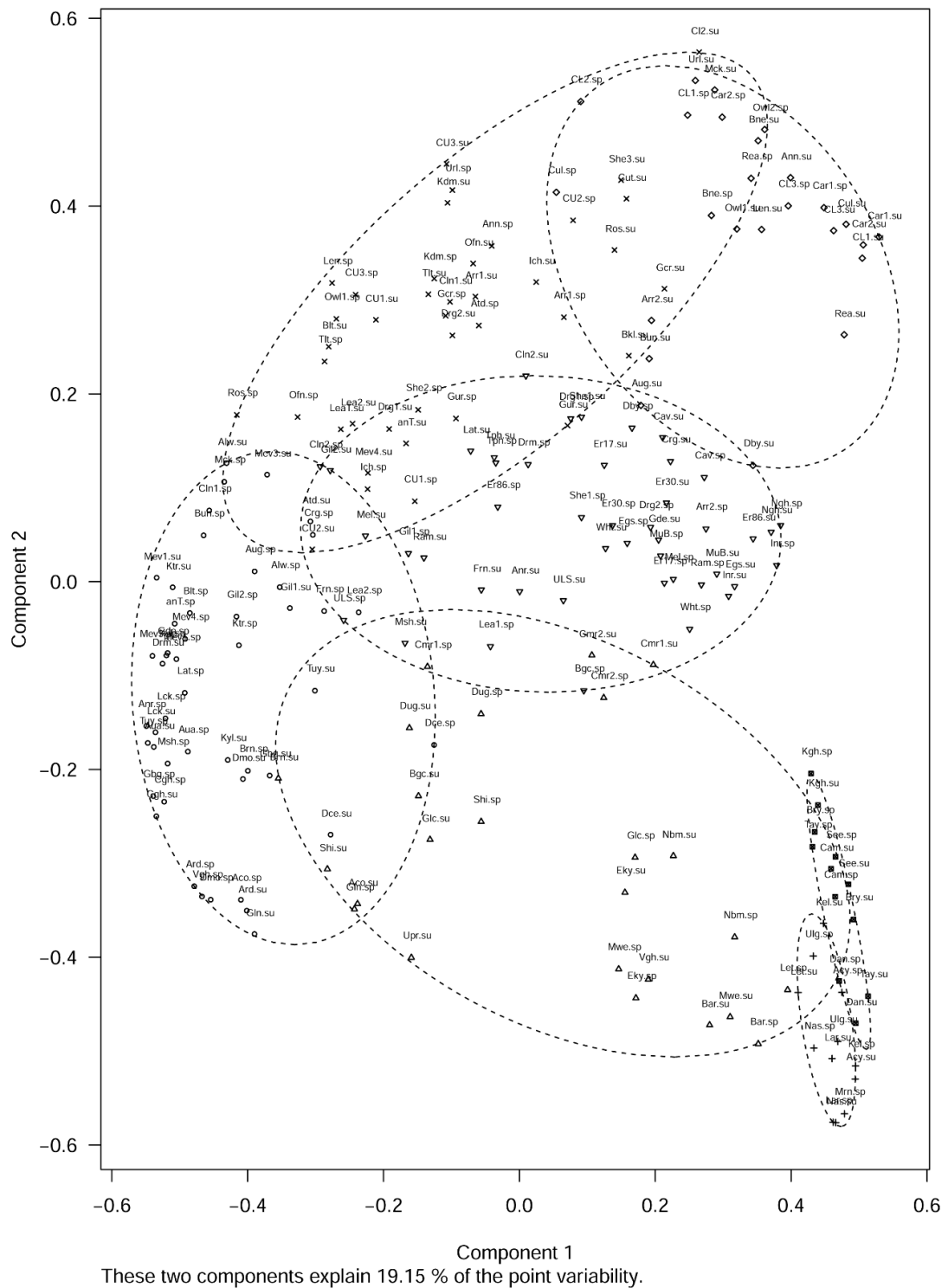


Figure E.2 Non-hierarchical Clustering (PAM) of Diatom Community (Bray-Curtis) 7-Cluster

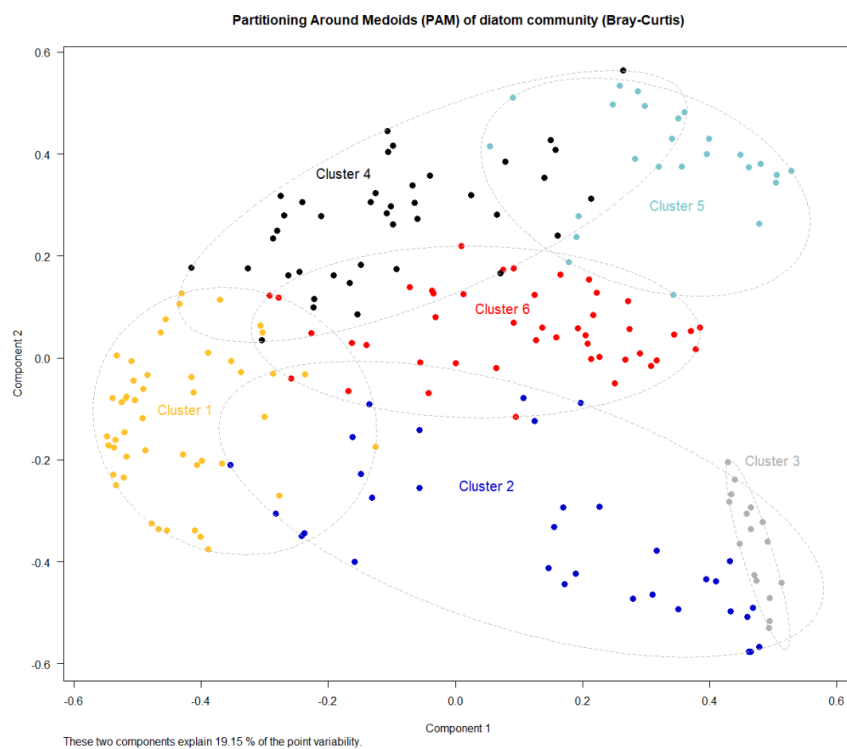


Figure E.3 Partitioning around Medoids (PAM) of diatom community (Bray-Curtis) 6-Cluster

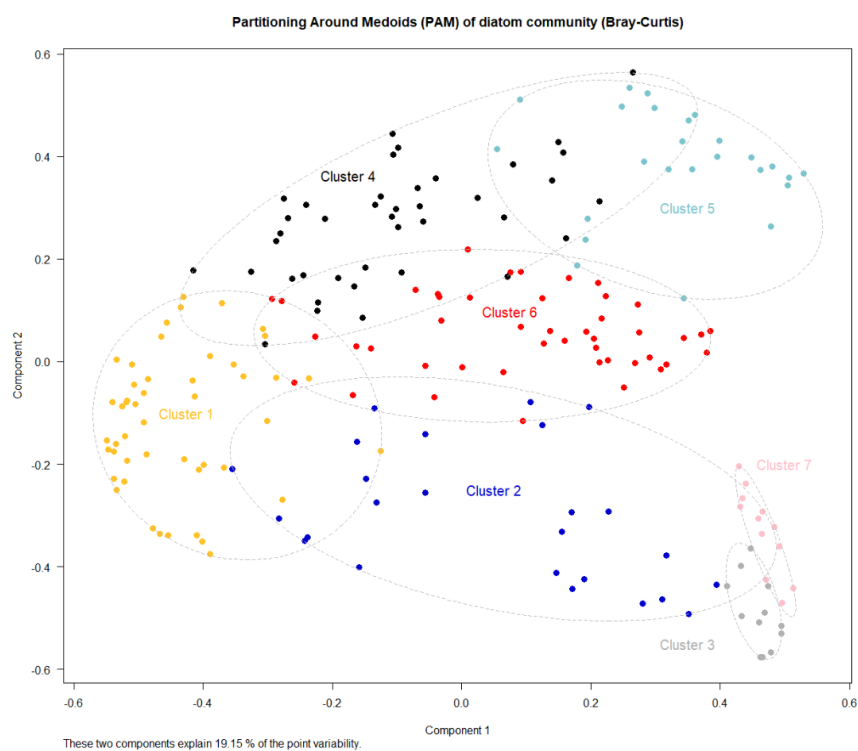


Figure E.4 Partitioning around Medoids (PAM) of diatom community (Bray-Curtis) 7-Cluster

APPENDIX F

DETAILED DESCRIPTION OF 6 CLUSTERS OBTAINED IN CLUSTER ANALYSIS.**Cluster-1: Circumneutral oligo-mesotraphentic**

The circumneutral *A. minutissimum* dominated the group and had a high average abundance (61%). The mesotraphentic *E. sileacium* (Bleisch) D.G.Mann and *F. gracilis* Østrup also occurred in a majority of lakes but were numerically subordinate by comparison (Van Dam *et al.*, 1994). *E. minutum* (Hilse) D.G.Mann, *B. microcephala* (Grunow) Compère, *E. neerlandica* Van de Vijver, Verweij, van der Wal & A.Mertens, *Tabellaria flocculosa* (Roth) Kützing and *Nitzschia lacuum* Lange-Bertalot were found in under half of lakes in the cluster but have a high abundance in several waterbodies. *Psammothidium lacus-vulcani* (Lange-Bertalot & Krammer) L.N.Bukhtiyarova was the only significant indicator taxon for the group but it was never abundant. The cluster has significantly lower average Shannon diversity which may be an artefact in part owing to merging of *Achnantheidium*. Diatom assemblages and chemical parameters were characteristic of circumneutral lakes with low to moderate nutrient levels in catchments of mixed landuse.

Cluster-2: Low alkalinity oligotraphentic

The group was also characterised by the occurrence of *A. minutissimum* but *Brachysira* was more prevalent and a significant indicator for the cluster. Many lakes have an increased density of *E. neerlandica* and *F. gracilis*. A greater representation of acidophilus taxa including *T. flocculosa*, *Peronia fibula* (Brébisson ex Kützing) R.Ross, and *Stauroforma exiguiformis* (Lange-Bertalot) R.J.Flower, V.J.Jones & Round exemplify these oligotrophic, weakly-acidic peatland lakes on siliceous geology. The group includes many low alkalinity reference lakes with low LTDI values, but several waterbodies of higher trophic status overlap waterbodies in cluster 1.

Cluster-3: Acidophilous

This small group of lakes had a distinctive acidophilous assemblage of *T. flocculosa*, *Eunotia* Ehrenberg and *Psammothidium* L.Buhtkiyarova & Round and characterised by an absence of *Achnanthyidium*. *B. garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot, *Pinnularia subcapitata* W.Gregory and *E. neogracile* Krammer were also prevalent. Average species richness was lowest of all groups, especially in spring. These highly coloured peatland lakes occurred at higher elevation and maximum lake depth with a diatom flora indicative of higher lake acidity. Nutrient levels were variable with elevated LTDI scores in response to increased representation of motile eutraphentic *Sellaphora nigri* (De Notaris) C.E.Wetzel & L.Ector in lakes with higher total phosphorus.

Cluster-4: Alkalibiontic oligo-mesotraphentic

A. minutissimum and small *Encyonopsis* Krammer were equally represented and dominated the cluster. *A. pediculus* (Kützing) Grunow, *C. affinis* Kützing agg., *C. helvetica* Kützing, *G. lateripunctatum* E.Reichardt & Lange-Bertalot, *G. minutum* (C. Agardh) C. Agardh, *Navicula cryptotenella* Lange-Bertalot and *A. pyrenacium* (Hustedt) H.Kobayasi were also well represented in several waterbodies. The latter taxon and *Encyonopsis microcephala* sp. 1 were significant indicator species for this group. Lakes were characterised as well buffered with low to intermediate nutrient levels. Catchment landcover comprises of mixed peat and pasture and there was significant overlap of this group with clusters 5 and 6.

Cluster-5: Alkalibiontic oligotraphentic

Achnanthyidium while present in most lakes was significantly reduced relative to small *Encyonopsis*, including *E. krammeri* E.Reichardt, *E. carraensis* B.Kennedy, Y.Buckley & Allott and *E. alpina* Krammer & Lange-Bertalot which dominated and were significant indicators for the group. The alkalibiontic *Mastogloia lacustris* (Grunow) Grunow, *B. neglectissima* Lange-Bertalot, *N. subalpina* E.Reichardt, *N. diversa* Hustedt and the genus *Delicatophycus* M.J.Wynne were also significant indicators in these oligotrophic to weakly mesotrophic calcium bicarbonate rich lakes (Lange-Bertalot *et al.*, 2017). *C. affinis*, *C. hustedtii* Krasske, *A. pediculus*, *A. indistincta* Levkov and the nitrogen-fixing *Epithemia* Kützing were also significant indicators and present in several waterbodies.

While the epilithon of high status marl lakes was dominated by cyanobacteria a distinctive diatom layer on the external surface of these complex crusts was always present (Kennedy *et al.*, 2012). While some well buffered marl lakes had a relatively high percentage cover of pasture, they nevertheless had low nutrients, low colour and low LTDI scores.

Cluster-6: Alkalibiontic eutraphentic

A. minutissimum was again the most abundant taxon but was accompanied by *A. pediculus* and a high diversity of genera associated with increased nutrient tolerance including *Cocconeis* Ehrenberg, *Encyonema* Kützing, *Gomphonema* Ehrenberg, *Fragilaria* Lyngbye, *Navicula*, *Nitzschia* Hassall and *Rhoicosphenia* Grunow. Although catchment landcover was variable, percentage pasture was higher on average than other clusters. Alkalinity and pH are comparable to cluster 4 but lower than cluster 5. These lakes are characterised by a eutraphentic flora and increased motile indicator taxa, species richness and Shannon diversity in response to elevated total phosphorus.

APPENDIX G

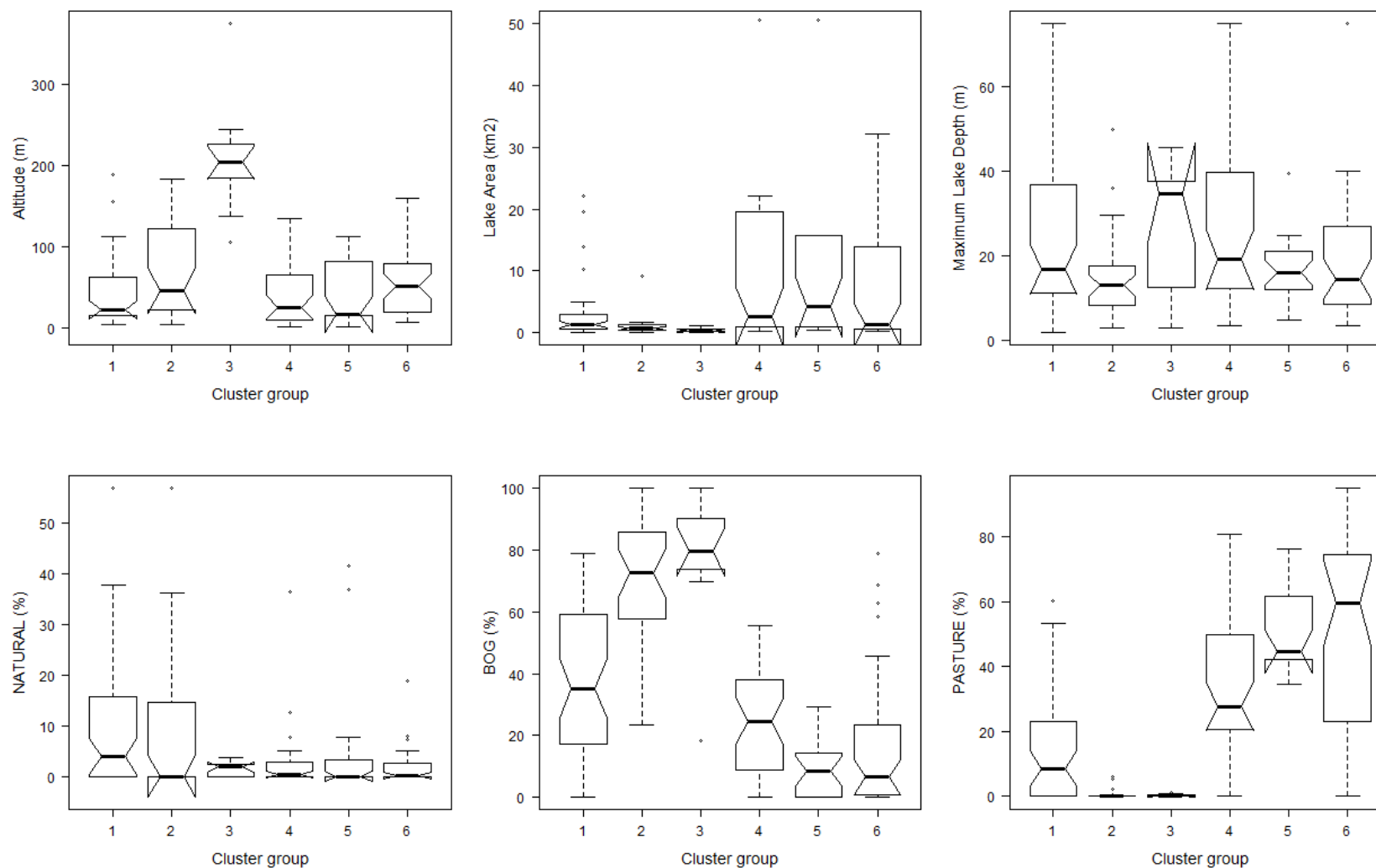


Figure G.1 The distribution of physicochemical and landuse variables amongst the six clusters. The inner line is the median, the box margins represent the 25th and 75th percentile with bars extending to the 5th and 95th percentiles. Where notches overlap between boxes then medians are assumed to be statistically non-significant ($p < 0.05$).

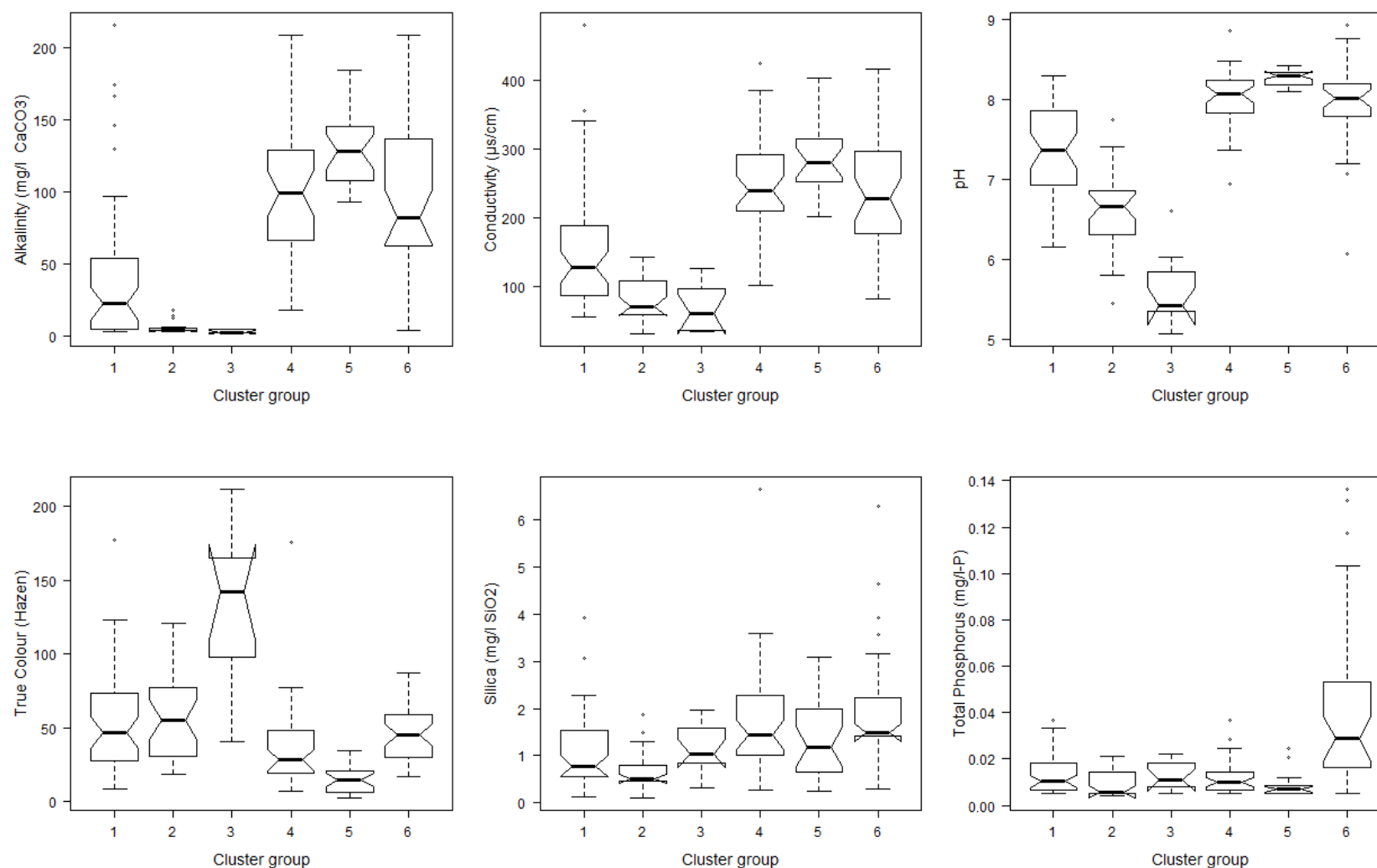


Figure G.1 (Continued) The distribution of physicochemical and landuse variables amongst the six clusters. The inner line is the median, the box margins represent the 25th and 75th percentile with bars extending to the 5th and 95th percentiles. Where notches overlap between boxes then medians are assumed to be statistically non-significant ($p < 0.05$).

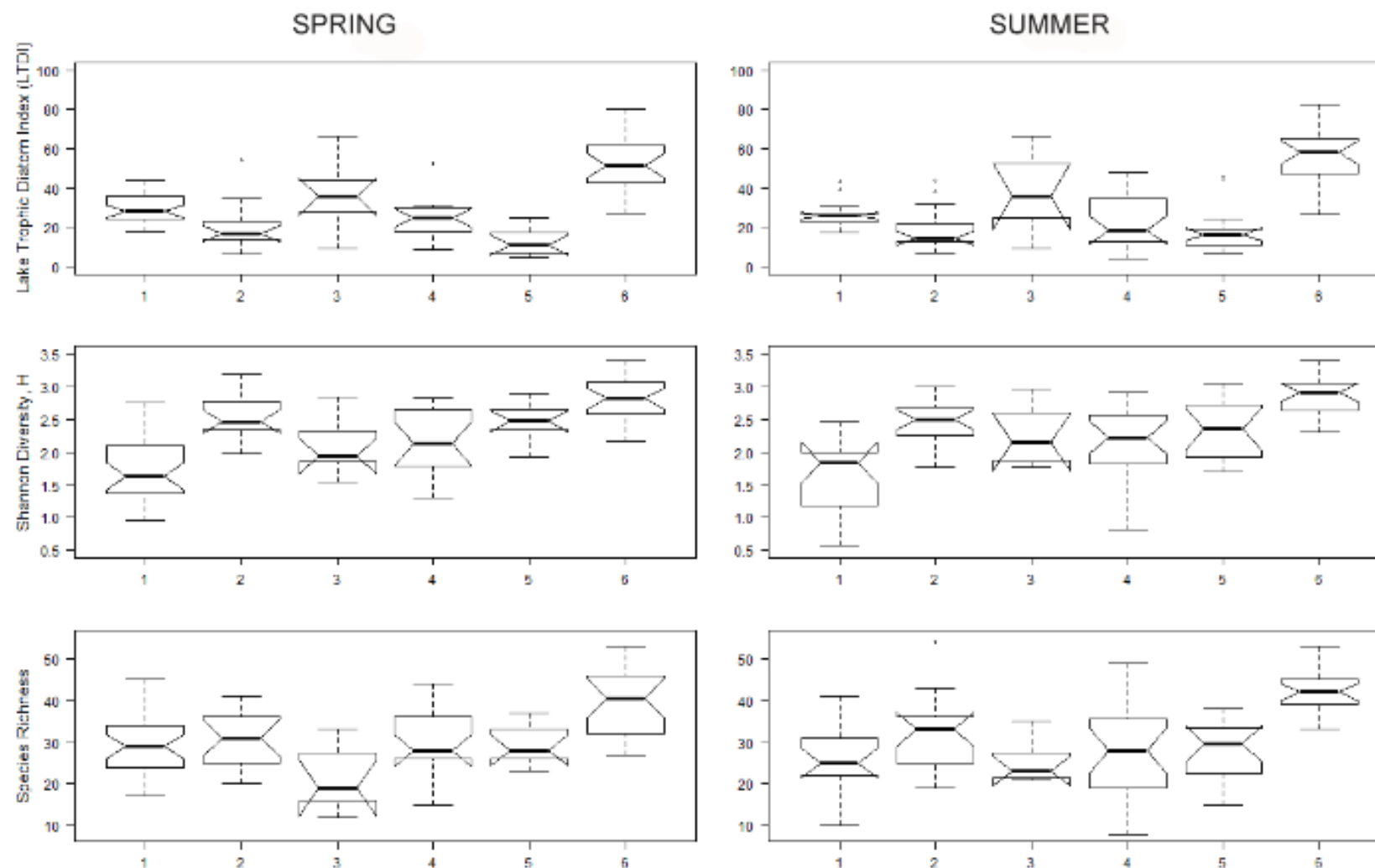
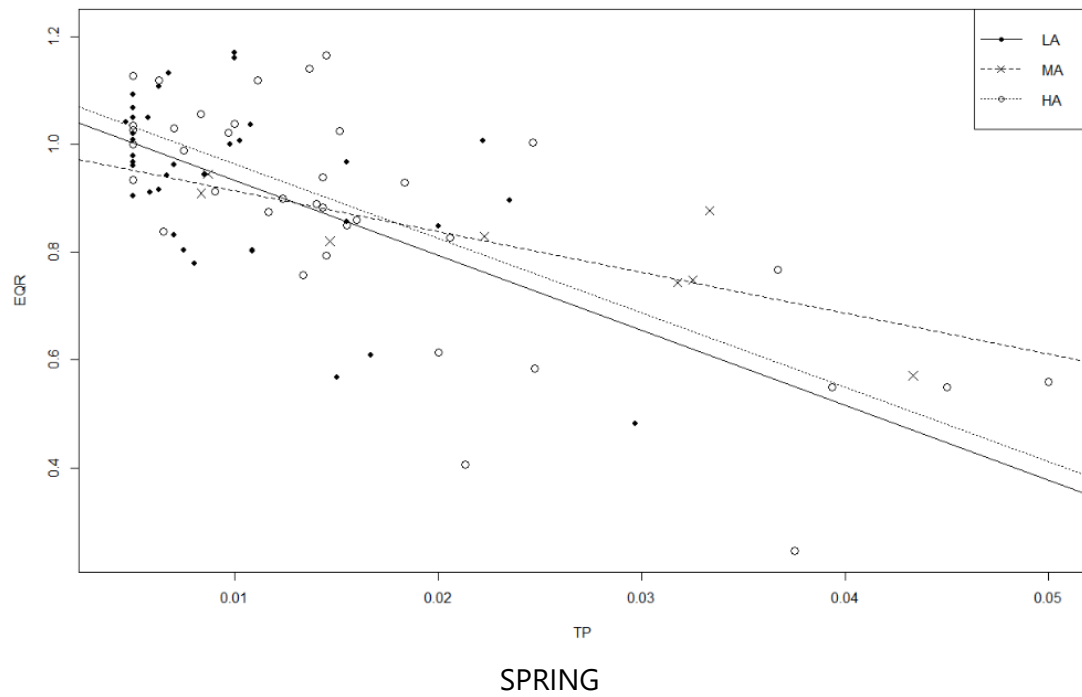


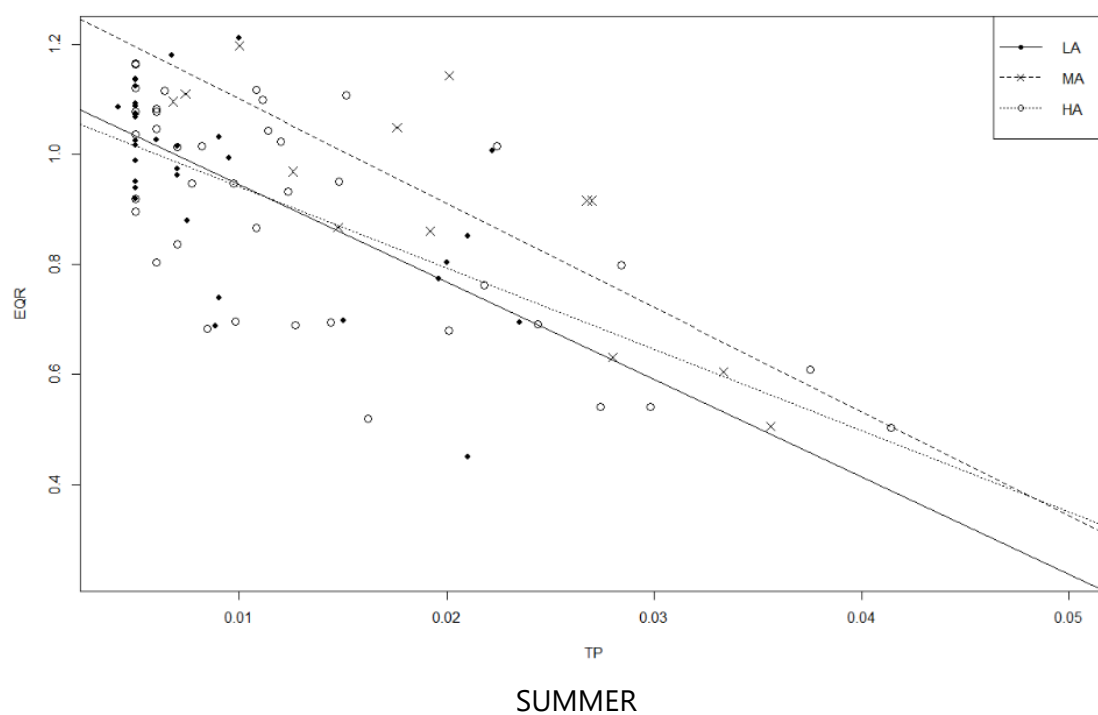
Figure G.2 The distribution of diatom diversity and LTDI metric scores in the six clusters. The inner line is the median, the box margins represent the 25th and 75th percentile with bars extending to the 5th and 95th percentiles. Where notches overlap between boxes then medians are assumed to be statistically non-significant ($p < 0.05$).

APPENDIX H



LA $EQR_{sp} = -13.91827 (TP) + 1.07331$	$r^2_{adj} = 0.28$	$p < 0.001$
MA $EQR_{sp} = -7.56126 (TP) + 0.98977$	$r^2_{adj} = 0.62$	$p = 0.012$
HA $EQR_{sp} = -13.79409 (TP) + 1.10144$	$r^2_{adj} = 0.53$	$p < 0.001$

Figure H.1 Relationships of Ecological Quality Ratio and total phosphorus in low (LA), moderate (MA) and high (HA) alkalinity lakes in spring for lakes with a restricted range of total phosphorus ≤ 0.05 mg/L-P.



$$\text{LA EQR}_{\text{su}} = -17.73213 (\text{TP}) + 1.12315 \quad r^2_{\text{adj}} = 0.40 \quad p < 0.001$$

$$\text{MA EQR}_{\text{su}} = -18.93716 (\text{TP}) + 1.29033 \quad r^2_{\text{adj}} = 0.66 \quad p < 0.001$$

$$\text{HA EQR}_{\text{su}} = -14.79173 (\text{TP}) + 1.008984 \quad r^2_{\text{adj}} = 0.46 \quad p < 0.001$$

Figure H.2 Relationships of Ecological Quality Ratio and total phosphorus in low (LA), moderate (MA) and high (HA) alkalinity lakes in summer for lakes with a restricted range of total phosphorus ≤ 0.05 mg/L-P.

APPENDIX I

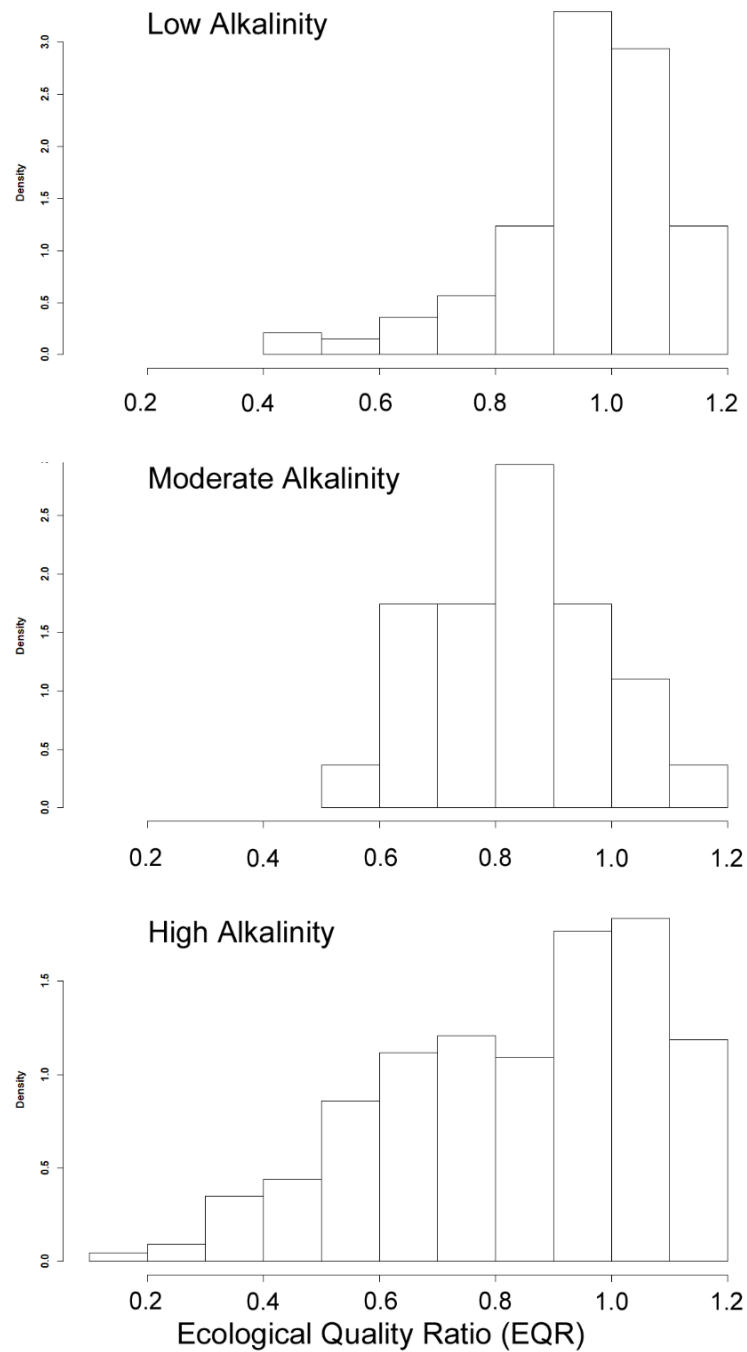


Figure I.1 Histograms showing the distribution of Ecological Quality Ratios (EQR) for epilithic diatom samples for three lake alkalinity types based on an expected value of the LTDI = 20.

CHAPTER 3

A review of the genus *Brachysira* in Ireland with the description of *Brachysira praegeri* and *Brachysira conamarae*, new raphid diatoms (Bacillariophyceae) from high status waterbodies

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Phytotaxa 326, 1-27.

<https://doi.org/10.11646/phytotaxa.326.1.1>

3.1. ABSTRACT

The genus *Brachysira* is frequently encountered in the algal flora of oligotrophic freshwaters. In order to re-evaluate its diversity in Irish waters, light and scanning electron microscopy was used to examine populations at 209 river sites and in 85 lakes. The full range of morphological variation of all common and sometimes challenging *Brachysira* populations was investigated. The study revealed two new species, *Brachysira praegeri* sp. nov. and *Brachysira conamarae* sp. nov. and compared them to all closely related taxa. The study also recorded *B. intermedia* and *B. neglectissima* which are new records for Ireland. Further taxonomic clarification is required for a diatom resembling *B. intermedia* and the complex of taxa around the polymorphic *B. microcephala*. Individual *Brachysira* taxa differed with respect to their

optima along an alkalinity-pH gradient, but the trophic requirements of all forms were comparable, suggesting that species may be merged for routine water quality assessment. Nevertheless, it is recommended that *Brachysira* morphotypes be enumerated separately until their taxonomy is more fully resolved and their significance for the biodiversity of epilithic communities has been clearly established.

Key Words: Bacillariophyta, taxonomy, morphology, ecology, Ireland, new diatom species, *Brachysira praegeri*, *Brachysira conamarae*.

3.2. INTRODUCTION

The genus *Brachysira* Kützing (1836: 153) was emended by Round & Mann (1981) and expanded significantly with the description of many new species following the publication of Lange-Bertalot & Moser (1994). Since then, the subsequent increase in numbers of species can be attributed to investigations of less well studied regions, as many taxa in the genus have a restricted range or are endemic (Moser *et al.* 1995, 1998, Metzeltin *et al.* 1998, Metzeltin & Lange-Bertalot 2007). Higher resolution scanning electron microscopy and greater appreciation of dissimilarities in ultrastructure now used to define taxa have also helped differentiate species within former complexes (Van de Vijver 2014). Species such as *Brachysira garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot in Lange-Bertalot & Moser (1994: 29) and *Brachysira neglectissima* Lange-Bertalot in Werum & Lange-Bertalot (2004: 128) were historically overlooked because of their superficial similarity to the cosmopolitan species *Brachysira microcephala* (Grunow) Compère (1986: 26). Apparent taxonomic aggregates such as *Brachysira brebissonii* agg. R. Ross (in Hartley 1986: 607), which had formerly shown relatively large variability, have now been more critically

examined and subsequently demonstrated to consist of morphologically comparable, yet distinct taxa (Lange-Bertalot & Moser 1994, Wolfe & Kling 2001, Van de Vijver 2014). In many cases taxonomic force fitting and the resulting taxonomic drift that follows from this practice (Van de Vijver & Kopalová 2014) has culminated in confusion, requiring additional taxonomic re-evaluations of the genus (Vouilloud *et al.* 2014).

Brachysira taxa are common constituents of the diatom flora in freshwater systems including bogs, springs, lakes and rivers (Van Dam *et al.* 1994, Tolotti 2001, Werum & Lange-Bertalot 2004, Kulikovskiy *et al.* 2010, Vidakovic *et al.* 2016, Juggins *et al.* 2016). Located on the western Atlantic periphery, Ireland has a large number of humic lakes and rivers in peatland catchments which experience low levels of acidifying and other atmospheric pollutants relative to those in mainland Europe (Aherne & Farrell 2002, Burton & Aherne 2012). Populations of *Brachysira* can dominate the epilithic communities in these waterbodies and are often numerically greater than the more prevalent indicator *Achnanthes* Kützinger (1844: 75) in soft waters. The most recent taxonomic evaluation of the national diatom flora in Ireland was provided by Foged (1977) who credits O'Meara with providing the preceding treatise a century earlier (O'Meara 1875–77). Foged (1977) predates important advances in taxonomy and many newly defined species. Therefore, knowledge of the occurrence and distribution of members of this genus is limited, notwithstanding their ecological importance and application in monitoring programs.

The objective of this study is to improve our understanding of the taxonomy, ecology and distribution of *Brachysira* in lotic and lentic habitats in Ireland. We investigate the morphology of some previously undescribed species to compare them to closely

related taxa. We re-examine *Brachysira* populations to establish the limits of their morphological diversity so their ecological preferences can be evaluated, including the cosmopolitan yet taxonomically challenging *B. microcephala* aggregate. We briefly discuss these findings in the context of water quality assessment using epilithic diatoms in Ireland.

3.3. METHODOLOGY

3.3.1. MATERIALS AND METHODS

Epilithic diatoms were sampled from 85 lakes and 209 rivers between 2007 and 2015 (Figure 3.1). Diatoms were sampled according to European Committee for Standardisation (CEN) protocols by brushing the biofilm from the top surface of at least five cobbles in a tray with water and then pouring the collected slurry into glass bottles with sufficient Lugol's Iodine for preservation (CEN, 2003). Samples were digested to remove organic material using concentrated sulphuric acid, potassium permanganate and oxalic acid, after a preliminary removal of carbonates using concentrated hydrochloric acid (CEN, 2003). Following rinsing in deionised water, a dilute suspension of the oxidised material was air-dried onto glass coverslips for 24 hours. Permanent glass slides were prepared by mounting the coverslip on the slide using Naphrax® (refractive index=1.74; Brunel Microscopes Ltd., UK).

The relative abundance of diatoms was calculated by counting single or multiple non-overlapping transects through the centre of each coverslip, until typically 300–500 random non planktonic valves were enumerated at x1000 with a Nikon E80i microscope (Plan Fluor x100 oil immersion objective n.a.=1.3) equipped with phase contrast (CEN, 2004). Light microscope micrographs were obtained using a Nikon Ni-

U (Plan Fluor objective n.a.=1.45) equipped with Differential Interference Contrast (DIC) and a CoolPix DS Ri-2 digital camera.

Samples were prepared for viewing using scanning electron microscopy (SEM) by air-drying digested material on glass coverslips or filtering a suspension through 0.2 μm IsoporeTM membrane filters, before fixing to aluminium stubs with carbon tabs, sputter-coating with gold and grounding with silver dag. Microscopic observations were performed at the CRANN (Centre for Research on Adaptive Nanostructures and Nanodevices) Advanced Microscope Laboratory (AML) in Trinity College Dublin using a Zeiss Ultra SEM microscope operated at an acceleration voltage of 5 kV and working distance of ~ 5.0 mm. All measurements were completed during SEM sessions or subsequently using the open source software ImageJ V1.48 (Rasband 1997–2016) calibrated to scale bars on TIFF files. Digital microscopic images were assembled into plates using Photoshop Elements V9. Distribution maps for the species were prepared using Arc GIS 10.2. Samples and slides are stored at the Trinity College Dublin (TCD) herbarium and Alfred Wegener Institute (AWH).

Chemical data was accessed from AQUARIUS, an environmental database for the national water quality monitoring program in Ireland (EPA 2006). Taxon environmental optima and tolerance values for pH were calculated from a weighted average of their unimodal distributions using the Analogue package based on the theoretical assumption that species occur in highest abundance near their ecological optima (Birks *et al.*, 1990; Simpson & Oksanen 2016) in R 3.1.2 (R Core Team 2014). The range in concentration for additional chemical parameters was determined from samples which exceeded 1.0% abundance for the relevant *Brachysira* taxon.

The morphology of the new taxa was compared with species and morphotypes reported or published for the genus (Compère, 1986, 1988, Lange-Bertalot & Moser 1994, Moser *et al.* 1995, 1998, Lange-Bertalot & Metzeltin 1996, Rumrich *et al.* 2000, Wolfe & Kling 2001, Metzeltin & Lange-Bertalot 2002, 2007, Shalyer & Siver 2004a,b, Werum & Lange-Bertalot 2004, Flower, 2005, Alfinito & Lange-Bertalot 2013, Anderson *et al.* 2013, Potapova *et al.* 2014, Van de Vijver 2014, Vouilloud *et al.* 2014, Guiry & Guiry 2016). Morphometric data and selected features of relevance in published accounts for some of the more closely related *Brachysira* taxa referred to in the text are given in table 3.1. Diatom terminology and description of morphology for new taxa follow Ross *et al.* (1979), Round & Mann (1981), Round *et al.* (1990) and Van de Vijver (2014).

Taxon	Length (µm)	Width (µm)	L:W (range)	Striae in 10 µm	Central area	Apices	Remarks
<i>B. ontonageniana</i>	16–40	4.5–5.5	-	28–31	Indistinct narrow lanceolate	Rounded not protracted	Heteropolar outline
<i>B. gatesii</i>	16–32	4–5	-	29–32	Small but distinctly rounded	Headpole rounded and footpole protracted	Slightly asymmetrical to transapical axis
<i>B. brebissonii</i>	18.2–27.7	4.9–5.8	-	24–27	Very small rounded to rhombic	Acutely rounded	Examination of original type material Van de Vijver (2014)
<i>B. sandrae</i>	21–40	5.4–7.1	-	29–31	Small symmetric rounded to elliptical	Clearly protracted rostrate	Larger valves with coarser striae and rostrate ends Van de Vijver (2014)
<i>B. sp.</i> (cf. <i>B. brebissonii</i>)	20–24	6–7	-	35–40	Small rounded to transapically widened	Small rounded to narrowly rounded	Alfinito & Lange-Bertalot (2013)

Table 3.1 Comparison of morphometric data and selected features of relevance in published accounts for some of the more closely related *Brachysira* taxa referred to in the text.

Taxon	Length (μm)	Width (μm)	L:W (range)	Striae in 10 μm	Central area	Apices	Remarks
<i>B. sp.</i> (aff. <i>B. brebissonii</i>)	17–20	4.5–5	3.9–4.1	27–30	Asymmetrical rhombic	Rounded (narrowly)	Vouilloud <i>et al.</i> (2014)
<i>B. huitotarum</i>	17.0–28.5	5.0–7.5	2.7–3.8	25–30	Rhomboid & slightly irregular to asymmetrical	Rounded to subrostrate	Vouilloud <i>et al.</i> (2014)
<i>B. procera</i>	25–60	4.5–6	-	27–30	Barely transversely dilated but apically elongate in larger valves	Non-protracted, obtuse- rounded	Lange-Bertalot & Moser (1994)
<i>B. microcephala</i>	19.0–22.5	4–5	-	35–36	Rounded	Rostrate	Compère (1986)
<i>B. microcephala</i>	13–28	4.0–5.2	-	35–39	Distinctly rounded or lanceolate appearing	Rostrate to subcapitate	Compère (1988)
<i>B. neoexilis</i>	12–34 (36)	3–5 (7)	-	30–36	Small circular to moderately rhombic	Mostly distinctly rostrate or subcapitate	Lange-Bertalot & Moser (1994)
<i>B. garrensis</i>	8.3–32.0	3.0–6.5	-	36–40	Expanded to almost completely absent	Protracted to non- protracted	Lange-Bertalot & Moser (1994)

Table 3.1 (Continued) Comparison of morphometric data and selected features of relevance in published accounts for some of the more closely related *Brachysira* taxa referred to in the text.

Taxon	Length (µm)	Width (µm)	L:W (range)	Striae in 10 µm	Central area	Apices	Remarks
<i>B. neglectissima</i>	18–30	4.3–5.4	-	36–40	Variable, indistinct to very small	More or less clearly drawn out, rostrate to subcapitate	Werum & Lange- Bertalot (2004)
<i>B. vitrea</i>	16–40	5.5–9	-	30–35	Not or only slightly expanded	Predominantly distinctly capitate to occasionally rostrate	Lange-Bertalot & Moser (1994)

Table 3.1 (Continued) Comparison of morphometric data and selected features of relevance in published accounts for some of the more closely related *Brachysira* taxa referred to in the text.

3.4. RESULTS

3.4.1. *BRACHYSIRA* DISTRIBUTION

A total of 12 morphospecies were reported with greater than half of lake waterbodies (n=50) and almost one-fifth of river stations (n=39) containing populations of *Brachysira*. For waterbodies where *Brachysira* was encountered, the average and maximum taxa number was higher for lakes (4, 10) than rivers (2, 6). The largest populations were found in catchments with acid bedrock and peatlands, but the genus was absent from waterbodies having an annual average pH below 5.1 or minimum pH below 4.6. The dominance of *Brachysira* in naturally acidic waterbodies declines with increasing alkalinity and it is replaced by other taxa like *Achnantheidium minutissimum* (Kützing) Czarnecki (1994: 157). However, selected *Brachysira* taxa were found in certain calcareous lakes in the midlands and the Burren (Figure 3.1). *Brachysira* was replaced in epilithic communities by *Eunotia* and *Pinnularia* spp. in sites with lower pH values.

Two new *Brachysira* species are proposed including *B. praegeri* which reached maximum abundance in two reference low alkalinity lakes in the west. *B. praegeri* was also recorded in lotic waters but always at densities <1.0% and was restricted to the most pristine oligotrophic and naturally acidic watercourses. The other new species, *B. conamarae* is a rare diatom, found in the epilithon from two reference lakes in the Connemara region.

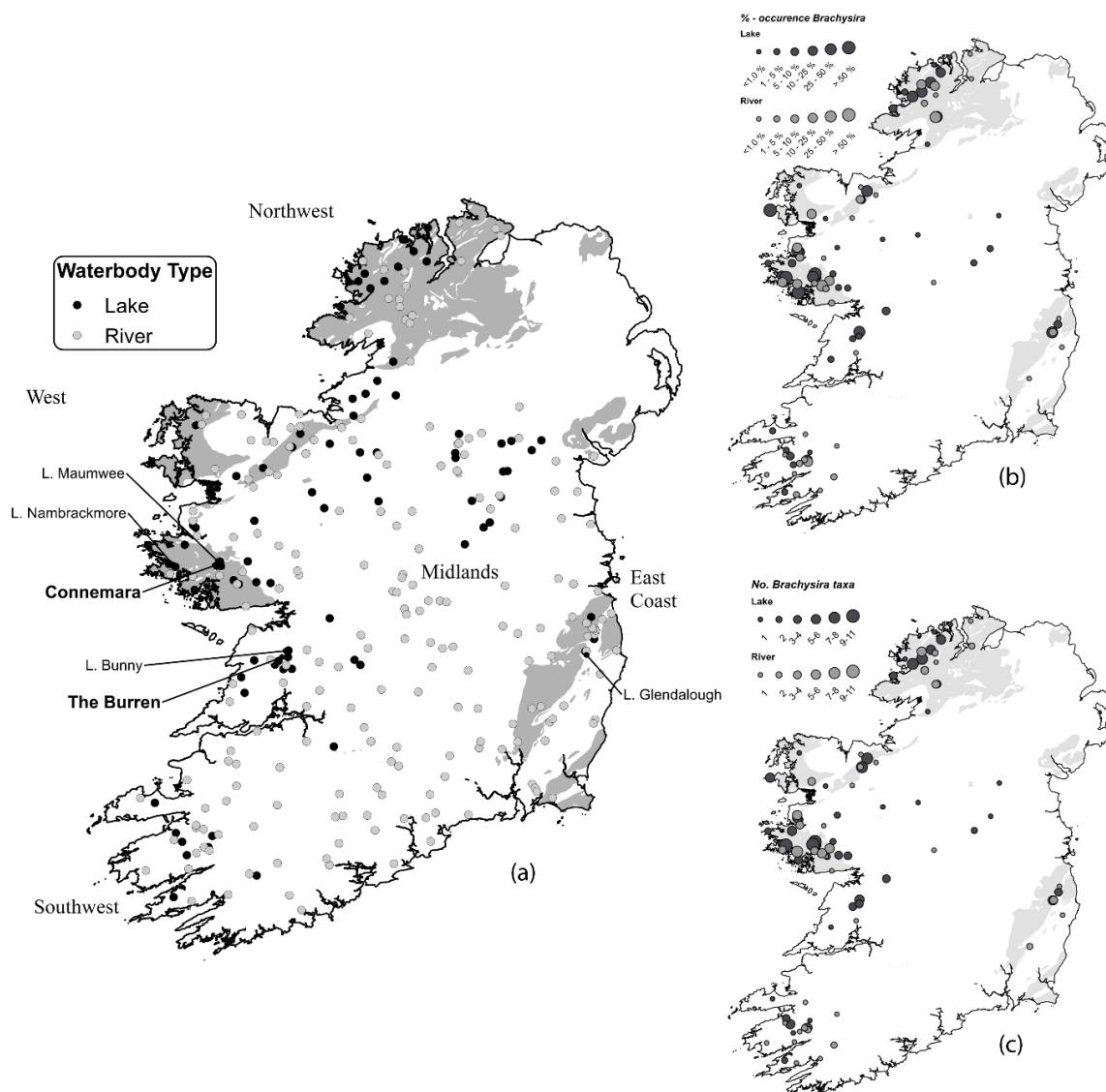


Figure 3.1 Ireland. (a) Map showing the location of the sampling sites and the distribution of the primary acidic bedrock geology. The regions and lakes indicated are referred to in the text. (b) *Brachysira* species as % of all diatom species at sites. (c) number of *Brachysira* species at sites. Light grey refers to river sites and dark grey refers to lakes in (b) and (c). Rivers and lakes are represented by light grey and grey dots, respectively.

Several cosmopolitan taxa were rare in epilithic samples and only encountered as isolated valves in the west of Ireland, including the acidobiontic *Brachysira serians* (Brébisson in Kützing) Round & D.G. Mann (1981: 227), *Brachysira styriaca* (Grunow) Ross (in Hartley 1986: 607) and *Brachysira zellensis* (Grunow) Round & D.G. Mann (1981: 227). The genus was mostly absent from catchments draining calcareous bedrock, but with some important exceptions including *Brachysira vitrea* (Grunow) Ross (in Hartley 1986: 607) and *Brachysira liliana* Lange-Bertalot & Moser (1994: 41) which were found predominately in lakes in the central lowlands and the Burren region (Figure 3.1). *B. neglectissima* was more widespread and reached a maximum relative abundance in Lough Bunny. Reduced densities were also encountered in some oligotrophic calcareous lakes in the midlands and the west (Figure 3.2). These alkaliphilous species were almost exclusively restricted to lotic habitats with the exception of a single river station which had a diverse *Brachysira* community. Their exceptional occurrence may be owing to populations flushed from a small lake upstream of the sampling location, or from epilithic communities established in lower velocity areas of the cascade-step-pool habitat.

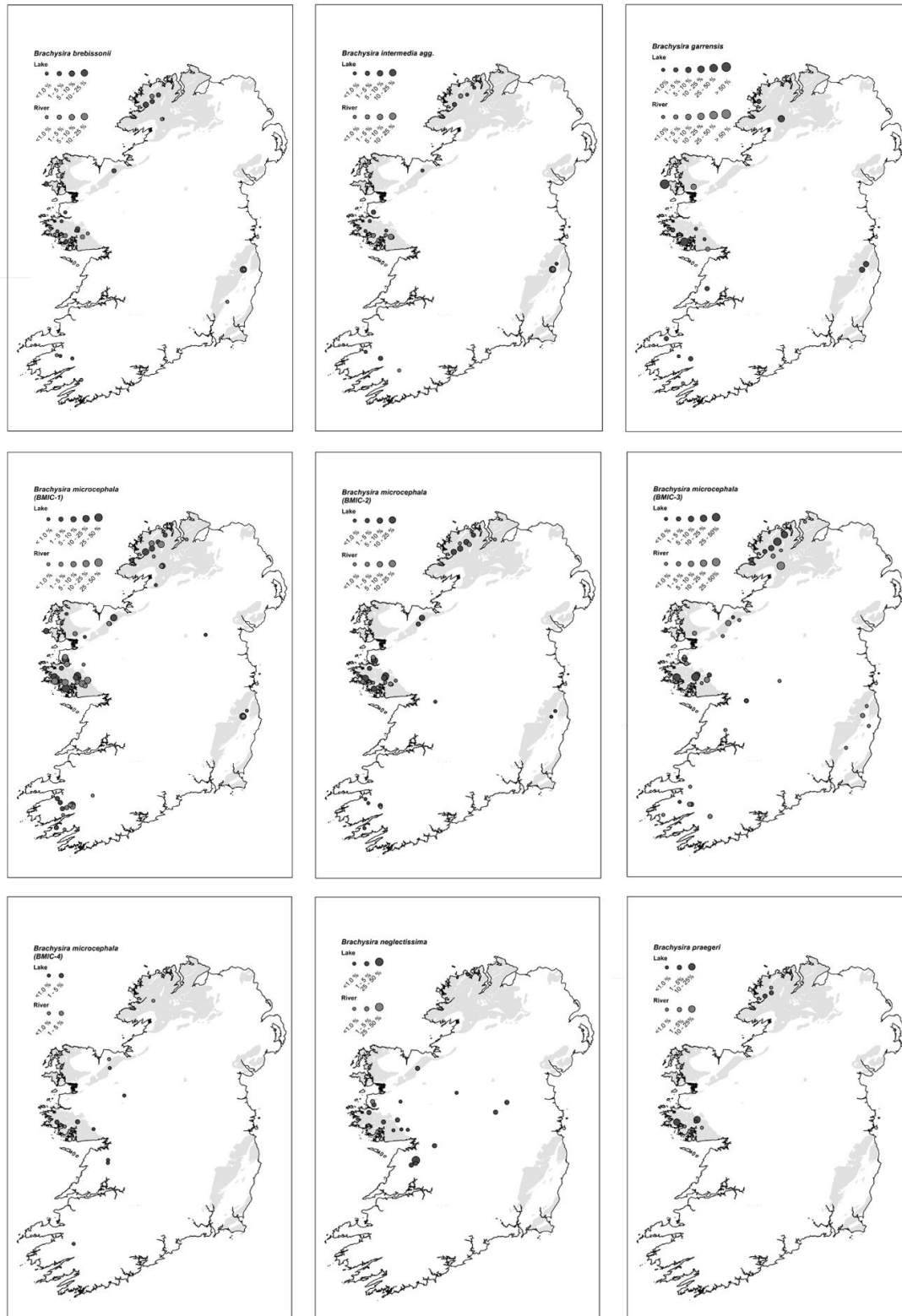


Figure 3.2 The distribution of the more abundant *Brachysira* taxa in Irish lakes and rivers. Rivers and lakes are represented by light grey and grey dots, respectively.

The ecology and morphology of many of these taxa conform to the typical range reported for their type specimens and in published accounts. Some differences in the morphology of *B. neglectissima* relative to the type description are apparent, while the morphology of *Brachysira intermedia* (Østrup) Lange-Bertalot in Lange-Bertalot & Moser (1994: 34) is discussed in further detail in the species accounts provided below. *B. garrensis*, *B. brebissonii* and *B. intermedia* had slightly lower optima for pH (Table 3.3) and very large abundances of *B. garrensis* (approximately 50% abundance) were observed in samples from two impounded coastal lakes.

The ubiquitous *B. microcephala* complex was the most commonly encountered and abundant in both low alkalinity lakes and rivers (Figure 3.2). It was also the most morphologically heterogeneous group, with many morphotypes co-occurring in the same samples. The polymorphic valve outline fits the general concept for this taxon reported elsewhere (Lange-Bertalot & Metzeltin 1996, Wolfe & Kling 2001, Shalyer & Siver 2004a, Hamilton 2010). The categories BMIC-1, BMIC-3 and BMIC-4 were similar in dimension and although differences in their outline and the shape of their poles usually enabled differentiation among them, a continuum between some forms was apparent. Form BMIC-2 had a maximum frustule length exceeding that reported for *B. microcephala* and had a close affinity with *Brachysira procera* Lange-Bertalot & Moser (1994: 55) and some of the comparable unidentified taxa illustrated in the same monograph (Tafel 46, Fig 19–25). Its stria density was higher and never approached the published range reported for *B. procera* (Table 3.2). Because of their similar morphology, smaller valves of this form were difficult to separate from non-protracted frustules of BMIC-1. However, the greater maximum valve length, non-protracted poles, slightly coarser stria density and elongate central area were distinctive in larger

valves. The environmental optima calculated for *B. microcephala* morphotypes were similar (Table 3.3). The broadly capitate BMIC-4 had a higher optima for pH but populations were encountered less frequently relative to the other more widespread forms and it never reached a high abundance.

3.4.2. DIATOM MORPHOLOGY

New Taxa

Division Bacillariophyta

Class Bacillariophyceae Haeckel [emend. Medlin & Kaczmarska 2004]

Subclass Bacillariophycidae D.G. Mann in Round *et al.* 1990

Order Naviculales Bessy 1907

Family Brachysiraceae D.G. Mann in Round *et al.* 1990

Genus *Brachysira* Kützing 1836

Brachysira praegeri Kennedy & Allott, sp. nov. (Figures 3.3–3.24)

Type: IRELAND. Co. Galway: Lough Nambrackmore, 53.4450215 N, 9.93394597 W, elev. 21.9 m, coll. B. Kennedy, coll. date 15 August 2013. (Holotype: specimen here designated Finder: P41 (The England Finder™ Graticule) TCD0018137, illustrated in Figure 3.8; type, slide ZU 10/78, Hustedt Collection, Alfred Wegener Institute).

LM (Figures 3.3–3.18): Cells solitary. Valves lanceolate with convex margins tapering evenly to non-protracted, rounded to occasionally moderately rounded blunt apices. Valve dimensions (n=50): length 16–22.5 µm, width 4–5.5 µm. Axial area narrow, linear. Central area very narrow due to slightly shortened striae, symmetrical to asymmetrical, typically rounded-elliptical to barely discernible from axial area. Raphe straight, filiform with simple, straight, closely located proximal endings. Distal raphe endings not visible in LM. Striae lineate, uniseriate, radiate at center changing to parallel and slightly convergent at apices; predominately 34 striae ranging from 32–36 in 10 µm, often denser at apices. Each stria composed of 3–4 areolae, discernible with LM.

SEM (Figures 3.19–3.24): Valves surrounded by an elevated ridge, marginally thicker near valve apices (Figure 3.19). Striae uniseriate, composed of 3–4 transapically elongated areolae, decreasing in number at valve centre and apices. Areolae linear of similar length, or sometimes longer in the outer row, with small gaps between individual areolae producing narrow apically running hyaline undulations on valve surface. Small papillae present on interstriae, usually 4–5 per interstriae at valve centre, reducing to single papillae at apices. Irregular papillae often well dispersed over or around central area (Figures 3.19, 3.22–3.24). Voigt discontinuity not observed. Mantle slopes away at a shallow angle from valve face to reveal striae composed of single elongated bacilliform areola with 1–2 papillae present between them. Areolae foramina with small indentations. Around apices, mantle striae closer together. Raphe branches bordered by an elevated ridge running from central area to apices, never fusing with marginal ridge. Raphe straight, with weakly expanded straight proximal endings (Figure 3.19). T-shaped distal raphe endings present with curved branches. Internally, central area symmetrical to asymmetrical, distal raphe endings terminate on weakly raised helictoglossae (Figure 3.20). Striae internally covered by subdivided hymenes.

Etymology: The specific epithet "*praegeri*" commemorates the Irish naturalist Robert Lloyd Praeger for his diverse investigations on the natural history of Ireland. His search for biological endemism, a characteristic apparent in the genus, culminated in the publication of the Clare Island Survey which was undertaken near the region where this species was discovered.

Similar species: The combination of morphological characteristics including, the overall valve size and symmetric outline, shape of the apices, structure and density of

the striae, and narrow central area with closely positioned proximal raphe ends allow differentiation from similar taxa using LM alone (Figures 3.3–3.18).

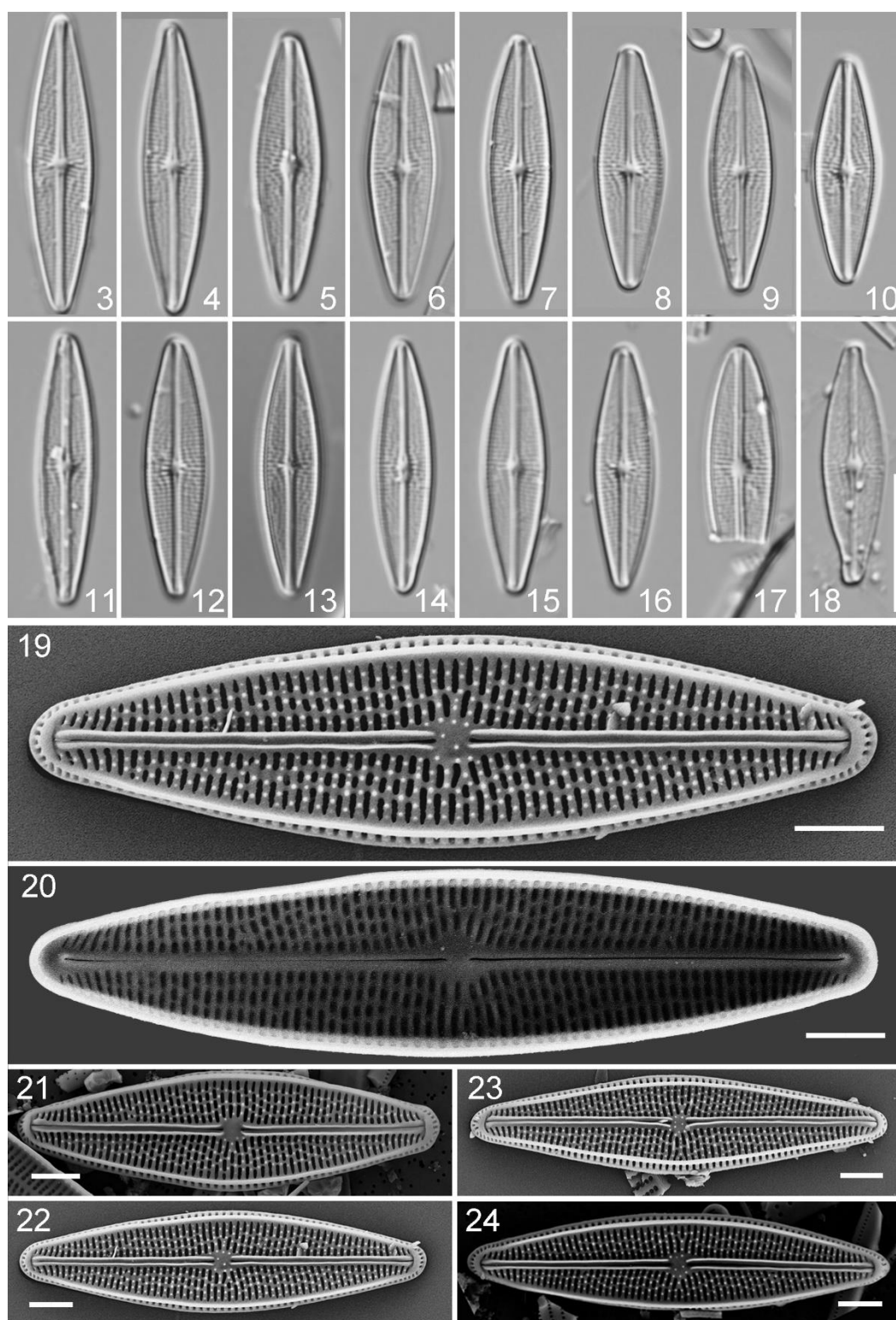
Brachysira praegeri has morphological features in common mostly with *Brachysira brebissonii* or associates of the *brebissonii*-complex (*sensu* Van de Vijver 2014) having lanceolate valves with rounded apices. However, *B. praegeri* has a stria density approaching the upper end of the published range for this taxonomic aggregate. Conspecificity with taxa having obviously widened rhombic outlines, coarser striae or broadly lanceolate valves tapering to acutely rounded apices can easily be ruled out including, *Brachysira manfredii* Lange-Bertalot (in Lange-Bertalot & Moser 1994: 44), *Brachysira metzeltinii* Lange-Bertalot (ibid: 45), and *Brachysira sandrae* (Van de Vijver 2014: 141).

In common with *B. brebissonii* and *B. sp.* (cf. *B. brebissonii*, Alfinito & Lange-Bertalot 2013: 140) from Sierra Leone, the apices of *B. praegeri* are never protracted but tend towards more obviously rounded poles. There is some overlap in valve dimensions with *B. brebissonii*, but *B. praegeri* is smaller in dimension (Table 3.2). Stria density is also higher (32–36 in 10 µm) and the structure of the striae and areolae differs to that found in the type material of *B. brebissonii* (29–31 in 10 µm) which also possesses much more easily discernible apically running hyaline undulations (Figures 3.81–3.91). The central area is also less well defined and the proximal raphe ends are more narrowly disposed in *B. praegeri*. Stria density is higher in the *B. sp.* (cf. *B. brebissonii* morph) from Sierra Leone (35–40 in 10 µm) and valve widths do not overlap. Ultrastructural differences, including the papillae shape and their higher number on the mantle differ considerably. Separation from *B. sp.* (aff. *B. brebissonii* (Vouilloud *et al.* 2014: 148) which has a similar size range can also be determined from its coarser

striae (27–30 in 10 μm), presence of well delimited wider hyaline lines and the presence of a clearly rhombic central area in this species. *Brachysira subtile* (Potapova *et al.* 2014: 103) can also be separated easily based on the longer more linear-lanceolate valve outline and its apically elongated central area. *B. praegeri* overlaps in size and also has a narrow reduced central area similar to *Brachysira ontonageniana* Kociolek & Lowe (in Anderson *et al.* 2013: 15) and *Brachysira gatesii* Kociolek & Lowe (*ibid.*). However, *B. praegeri* never displays their transapical asymmetry and also differs from the valve outline found in these taxa. Their coarser stria density (28–31 & 29–32 in 10 μm , respectively) and the protracted apices in *B. gatesii* are also distinctly different.

Although stria density sometimes overlap, the valve outline of *Brachysira microcephala* often has clearly protracted and elongated ends while the apices of *B. praegeri* are less acutely rounded to moderately rounded and never protracted. Comparison with non-protracted forms of *B. microcephala* is also warranted (see *Brachysira neoexilis* Lange-Bertalot Morphotype I in Lange-Bertalot & Metzeltin 1996 TFL 36 figs 27–28; *B. sp.* (cf. *B. neoexilis* Lange-Bertalot & Moser 1994 TFL 46 figs 19–22)) but conspecificity with this morphotype can also be excluded based on the narrower lanceolate outline and more acutely rounded elongate apices of *B. microcephala*. Ultrastructurally, the sloping angle of the mantle visible with SEM in *B. praegeri* contrasts with that found for *B. microcephala* (Figures 3.168–3.172) which also lack papillae between the mantle aerolae. Based on these clearly different combination of features used in the characterisation of the genus (Round & Mann 1981), separation of *B. praegeri* from other taxa is warranted.

Distribution & Ecology: *Brachysira praegeri* was observed in five lakes and three rivers in west and northwest Ireland (Figure 3.2) reaching a maximum relative abundance of 17.4% in L. Maumwee. The diatom assemblage there is dominated by *Brachysira microcephala*, with *Achnantheidium caledonicum* (Lange-Bertalot in Lange-Bertalot & Moser) H. Lange-Bertalot (1999: 277) and *Encyonopsis neerlandica* Van de Vijver *et al.* (2012: 44) of secondary importance. It is a species of low alkalinity (4.0–5.0 mg/l CaCO_3), slightly acidic waters (pH: 6.0–6.8) and relatively low colour (29–83 Hazen) with nutrients at low concentration below the limit of detection (Table 3.3).



Figures 3.3–3.24 *Brachysira praegeri* sp. nov. Figs 3–18. LM. Fig. 8. Holotype specimen. Figs 7–10. Range of valves from the holotype population (TCD0018137) L. Nambrackmore. Figs 3–6, 11–17. Valves showing morphological range and size variation in other populations. Fig. 18. A teratological valve giving the abnormal impression of rostrate ends. Figs 19–24. SEM. Fig. 19. External view. Fig. 20. Internal view. Figs 21–24. Variation in valve outline and occurrence of papillae in the central area. Scale bar in Fig. 18 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

Taxon	Length (μm)	Width (μm)	L:W (range)	Striae (in 10 μm)	*Dist.PRE (μm)	Central area	Apices	Remarks
<i>B. praegeri</i>	16.1–22.6 (19.6)	4.2–5.4 (4.8)	3.4–4.9 (4.1)	32–36 (33.5)	-	Indistinct rounded or absent often with papillae	Rounded and never protracted	Symmetrical outline and often with moderately broad ends
<i>B. conamarae</i>	22.9–40.0 (28.0)	4.8–5.8 (5.1)	4.5–7.5 (5.4)	34–37 (35)	1.1–2.2 (1.6)	Indistinctly lanceolate & only slightly wider than axial area	Slightly protracted rounded	Lacks siliceous ribs bordering the raphe
<i>B. brebissonii</i>	14.5–28.5 (21.6)	4.8–7.9 (6.2)	2.7–4.3 (3.5)	26–29(30) (27.5)	-	Small rounded to rhombic	Acutely rounded	Valves with narrowly rounded poles occasional
<i>B. sp.</i> (cf. <i>B. intermedia</i>)	17.9–24.8 (22.0)	4.8–6.3 (5.5)	3.2–5.0 (4.0)	25.5–27.5 (26)	-	Clearly rhombic	Acutely rounded or slightly protracted	Two rows of areolae clearly separated by clearly widened hyaline undulations

Table 3.2 Comparison of morphometric features of the common *Brachysira* in Ireland including new species observed during this study. Mean values are given in parenthesis under the range. *Dist.PRE indicates the distance between the proximal raphe endings.

Taxon	Length (µm)	Width (µm)	L:W (range)	Striae (in 10 µm)	*Dist.PRE (µm)	Central area	Apices	Remarks
B. <i>microcephala</i> agg. (BMIC-1)	12.0–27.0 (19.3)	3.6–5.0 (4.3)	3.0–5.6 (4.4)	33–38 (36)	0.6–1.7 (1.1)	Small rounded to transapically widened or almost absent	Usually rounded to slightly rostrate	Broadly rounded ends in smallest valves. Large overlap in outline with BMIC-2 for some populations
B. <i>microcephala</i> agg. (BMIC-2)	15.5–44.4 (29.7)	4.0–6.2 (4.8)	3.9–8.2 (6.1)	32–36 (34)	0.4–3.4 (1.7)	Apically elongate in larger valves – smaller valves as for BMIC	Not protracted and narrowly rounded	Intermediate characteristics of B. <i>microcephala</i> agg. & B. <i>procera</i> cf. Smaller valves may have a more undulating margin
B. <i>microcephala</i> agg. (BMIC-3)	16.0–33.5 (23.2)	4.0–5.0 (4.4)	3.8–7.1 (5.3)	33–37.5 (35)	0.6–1.7 (1.1)	Small symmetric rounded to elliptical or almost absent	Clearly protracted	Some heteropolarity or valves with elongate protracted ends

Table 3.2 (Continued) Comparison of morphometric features of the common *Brachysira* in Ireland including new species observed during this study. Mean values are given in parenthesis under the range. *Dist.PRE indicates the distance between the proximal raphe endings.

Taxon	Length (µm)	Width (µm)	L:W (range)	Striae (in 10 µm)	*Dist.PRE (µm)	Central area	Apices	Remarks
<i>B. microcephala</i> agg. (BMIC-4)	15.9–25.3 (18.0)	3.5–5.0 (4.3)	3.7–5.5 (4.2)	33–39 (36)	0.5–1.2 (0.8)	Small rounded to transapically widened or almost absent	Capitate	Small broadly elliptical & capitate valves. Up to 39 striae in 10 µm in some valves
<i>B. garrensis</i>	12.9–25.9 (17.1)	3.6–4.9 (4.1)	3.0–5.3 (4.2)	36–40 (38)	-	Small rounded to transapically widened or almost absent	Rounded, rostrate or subcapitate	Fully capitate valves not yet observed in SEM
<i>B. vitrea</i>	19.3–31.8 (26.4)	5.4–7.3 (6.4)	3.0–5.2 (4.1)	32–36 (34)	-	Usually absent	Broadly capitate	Occasional rostrate or subcapitate ends observed
<i>B. neglectissima</i>	15.9–36.5 (24.3)	3.8–5.1 (4.6)	3.9–6.9 (5.3)	35–40 (38)	-	Distinctly rounded or lanceolate appearing	Subcapitate, rostrate or not protracted	Non protracted outline also observed in some populations

Table 3.2 (Continued) Comparison of morphometric features of the common *Brachysira* in Ireland including new species observed during this study. Mean values are given in parenthesis under the range. *Dist.PRE indicates the distance between the proximal raphe endings.

Taxon	No.	Max %	pH		Alkalinity	Conductivity	True colour	Total Oxidised	Ammonium	ortho-phosphate
			opt.	tol.	(mg/l CaCO ₃)	(µS/cm ²)	(Hazen)	Nitrogen (mg/l-N)	(mg/l NH ₄ -N)	(mg/l-P)
					Range	Range	Range	Range	Range	Range
<i>B. brebissonii</i>	26	12.4	6.3	0.5	4–7	32–127	29–70	≤0.2–0.3	<0.02–0.03	<0.01
<i>B. intermedia</i>	19	12.8	6.5	0.4	2–7	32–107	25–83	≤0.2–0.3	<0.02–0.03	<0.01
<i>B. praegeri</i>	8	17.4	6.7	0.3	4–5	48–95	29–83	≤0.2	<0.02–0.03	<0.01
<i>B. microcephala</i> (BMIC–1)	55	32.9	6.6	0.5	2–175	37–398	5–170	≤0.2–1.5	<0.02–0.1	≤0.01
<i>B. microcephala</i> (BMIC–2)	34	14.9	6.7	0.4	4–68	48–171	11–106	≤0.2	<0.02–0.03	<0.01
<i>B. microcephala</i> (BMIC–3)	39	31.3	6.6	0.5	2–114	37–250	6–106	≤0.2–0.3	<0.02–0.03	<0.01
<i>B. microcephala</i> (BMIC–4)	11	4.4	7.1	0.7	4–130	80–90	35–106	<0.2	<0.02	<0.01
<i>B. garrensis</i>	16	55.6	6.0	0.8	3–8	32–134	28–153	≤0.2–0.3	<0.02–0.03	<0.01
<i>B. neglectissima</i>	18	32.2	8.0	0.6	4–175	62–398	5–32	≤0.2–0.3	<0.02–0.03	<0.01
<i>B. vitrea</i>	14	1.4	7.6	0.6	4–155	62–353	17–32	≤0.2–0.3	≤0.02	<0.01

Table 3.3 The number of occurrences, maximum relative abundance, environmental optima and tolerance (pH) and range for some parameters for common *Brachysira* taxa (for samples >1.0% abundance) determined in this study.

			pH		Alkalinity (mg/l CaCO ₃)	Conductivity (µS/cm ²)	True colour (Hazen)	Total Oxidised Nitrogen (mg/l-N)	Ammonium (mg/l NH ₄ -N)	ortho-phosphate (mg/l-P)
Taxon	No.	Max %	opt.	tol.	Range	Range	Range	Range	Range	Range
<i>B. liliana</i>	5	1.4	7.9	0.6	5–130	70–309	9–26	≤0.2	≤0.02	<0.01

Table 3.3 (Continued) The number of occurrences, maximum relative abundance, environmental optima and tolerance (pH) and range for some parameters for common *Brachysira* taxa (for samples >1.0% abundance) determined in this study.

Brachysira conamarae Kennedy & Allott, sp. nov. (Figures 3.25–3.33)

Type: IRELAND. Co. Galway: Lough Maumwee, degrees 53.47439656N, 9.54091316W, elev. 44.3 m, coll. B. Kennedy, coll. date 19 April 2007. (Holotype: specimen here designated Finder: L 60N2 (The England Finder™ Graticule) TCD0018138, Holotype illustrated in Figure 3.25; type, sample ZU 10/79, Hustedt Collection, Alfred Wegener Institute).

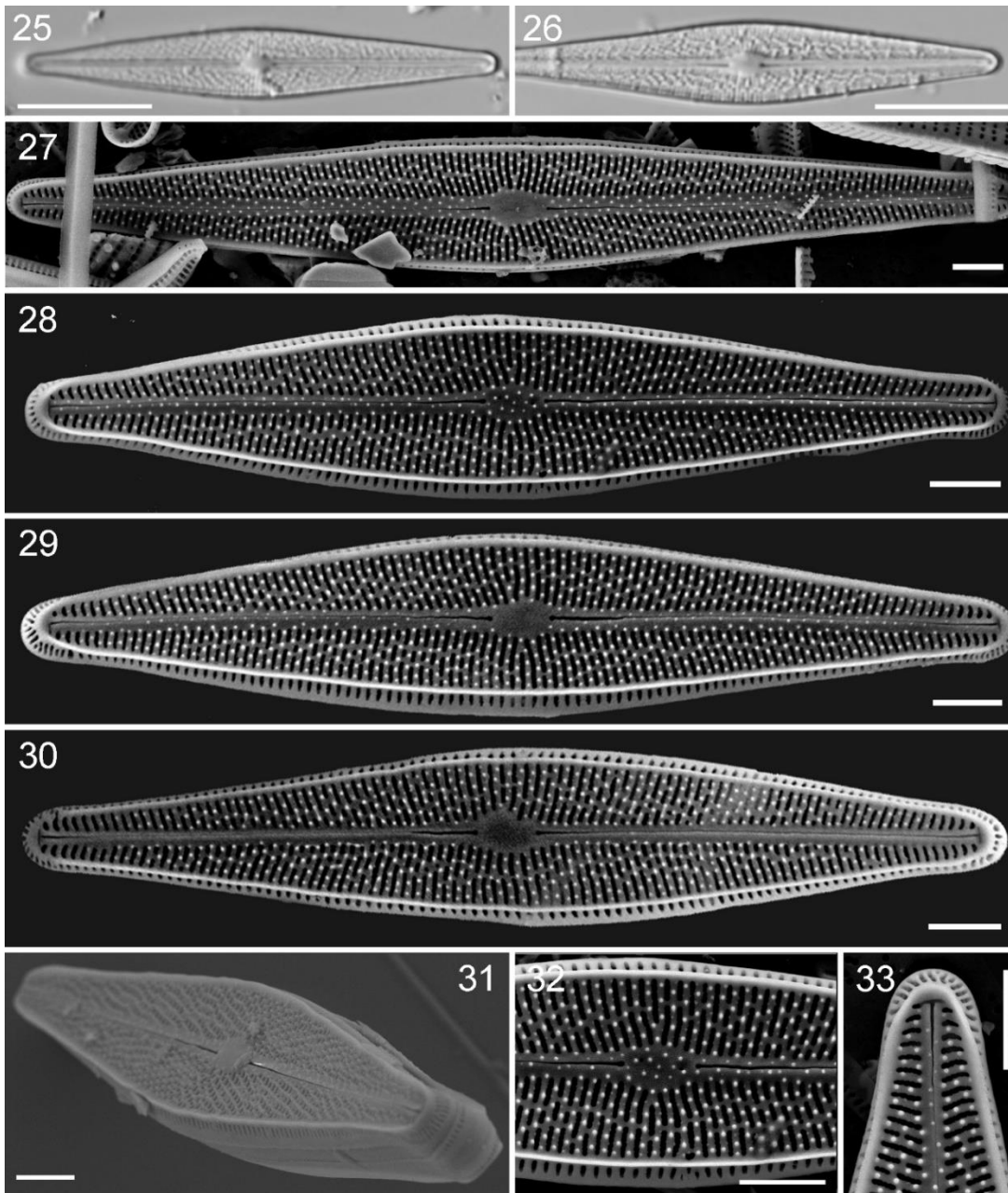
LM & SEM (Figures 3.25–3.33): Cells solitary. Valves lanceolate, tapering uniformly to weakly or hardly protracted rounded apices. Valve dimensions (n=15): length 23–40.0 µm, width 5–6 µm. Axial area narrow linear, sometimes appearing more prominent (Figures 3.26–3.27). Central area indistinctly lanceolate and only slightly wider than axial area. Valve face flat, surrounded by an elevated ridge, thickened near valve apices (Figures 3.28–3.30, 3.33). Raphe straight, filiform with simple, straight, slightly punctiform proximal raphe endings. Distal raphe endings T-shaped with straight branches. Raphe branches bordered by a thickening of the axial area, lacking clear ribs, only slightly more prominent towards apices (Figures 3.28–3.30). Striae lineate, uniseriate, radiate at centre, parallel to weakly convergent at apices, 34–37 in 10 µm. Striae composed of 2–3 transapically elongated areolae, similar or dissimilar in length, decreasing in number both at valve centre and apices. A single elongate areola per stria occasionally at valve centre. Voigt discontinuity not observed. Irregular gaps present between areolae producing discontinuous apically running hyaline undulations, especially prominent towards mid-valve. Small narrowly pointed papillae present on interstriae, usually 4–5 per interstriae at valve centre, reducing to a single papilla at apices. Papillae occasionally irregularly dispersed over central or axial area along full length of raphe and in close proximity to it (Figures 3.27–3.32). Single

elongate bacilliform areola, lacking papillae on mantle. Areolae foramina with small indentations. Around apices, mantle striae close together (Figures 3.28–3.30).

Etymology: The specific epithet refers to the original Irish spelling (*Conamara*) of Connemara, a mountainous region in Co. Galway in western Ireland where the species was discovered.

Similar species: The comparable size and stria density of larger valves of the *B. microcephala* morphotype reported here (BMIC-2; Figures 3.173–3.205) makes it difficult to separate *B. conamarae* with LM alone. An absence of siliceous ribs bordering the raphe is the principal ultrastructural characteristic separating it from this closely related taxon and is only discernible with SEM. The range in stria density overlaps with BMIC-2. Conspecificity with *B. procera* can be ruled out based on a coarser stria density (27–30 vs. 34–37 10 μm in *B. conamarae*). *B. garrensis* also has significantly reduced ribs bordering the raphe relative to other *Brachysira*, but the valves of *B. conamarae* are much larger and possess abundant papillae between the coarser striae which are lacking in the former species. *B. guarrerae* Vouilloud *et al.* (2014: 152) notably possesses only a single prominent rib with omission of the rib at the junction between the valve face and mantle. It also lacks papillae and has a more protracted outline. The absence of transapical ribs bordering the raphe is a feature of the morphology of *Brachysira lecohui* Lange-Bertalot (in Lange-Bertalot & Moser 1994: 39) but stria density is lower and a lack of papillae and other features of its ultrastructure prevent confusion with this taxon. Wang & Wu (2005) provide an SEM micrograph for the heteropolar *Brachysira steindorfiana* Moser, Lange-Bertalot & Metzeltin (1998: 101) with a similar absence of silica ribs bordering the raphe, but the morphology of this taxon does not otherwise resemble *B. conamarae*. While the

presence of conspicuous papillae along the axial area has been observed for other taxa (e.g. *B. australofollis*, *B. julio* and *B. nubigena* in Lange-Bertalot & Moser 1994: 17, 37, 54) they were also occasionally absent in *B. conamarae*. A difference in the valve outline and denser striae easily separates it from these species.



Figures 3.25 - 3.33 *Brachysira conamarae* sp. nov. Figs 25–26. LM. Fig. 25. Holotype specimen (TCD0018138). Figs 27–33. SEM. Fig. 27. External view of an elongate valve with slightly more prominent axial area. Figs 28–30. Variation in valve morphology apparent for the holotype population with varying pattern of occurrence of papillae over the valve face. Fig. 31. Oblique valve in tilt illustrating ultrastructural features. Fig. 32. Detail of the centre of the frustule with papillae over the entire valve face. Fig. 33. Detail of the valve end and T-shape distal raphe endings. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

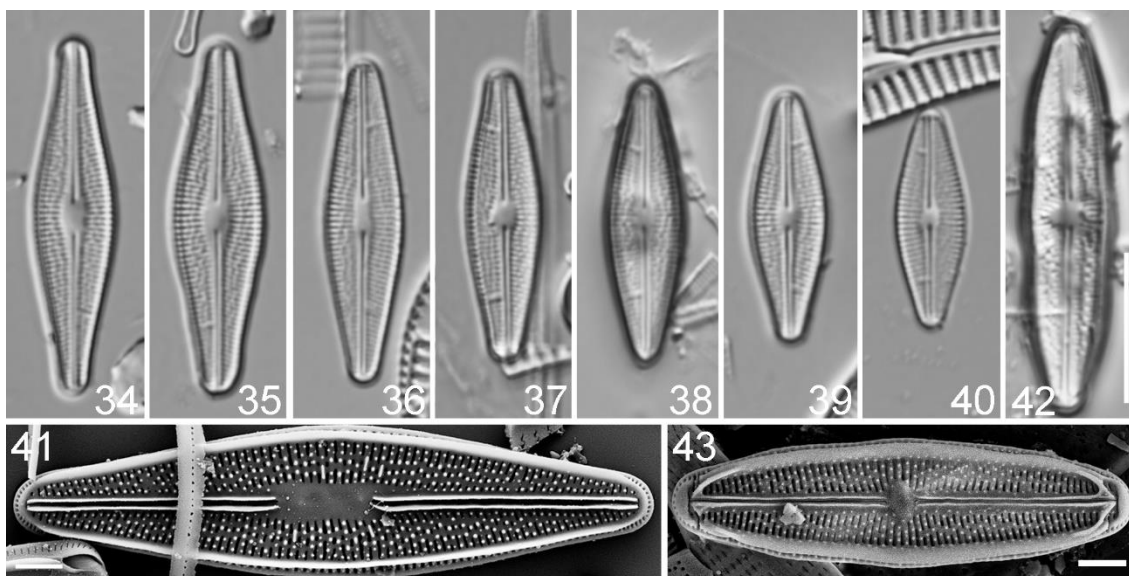
Distribution & Ecology: *Brachysira conamarae* has been found so far as occasional valves in two lakes in Connemara, Co. Galway. It co-occurs with *B. praegeri* and is associated with the same diatom flora as this species. It occurs in near-pristine waters of low alkalinity which are slightly acidic (alkalinity=5.0 mg/l CaCO₃, mean pH=6.8, min. pH=6.4) and low colour (Hazen=29) with nutrients below the limit of detection.

3.4.3. DIATOM MORPHOLOGY

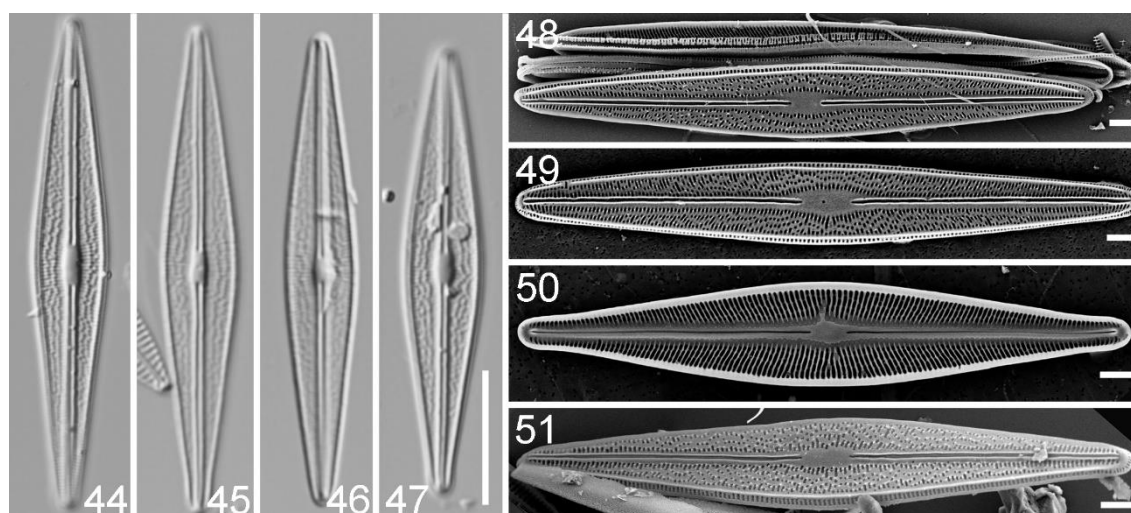
Other *Brachysira* Taxa

Rarely occurring species

B. zellensis (Figures 3.42–3.43) and *B. serians* were encountered only as isolated valves in two and three lake samples respectively, in the west of the country. *B. styriaca* (Figures 3.34–3.41) and *B. liliana* (Figures 3.44–3.51) were also uncommon, reaching a slightly higher maximum abundance (1.2% & 1.4%), but also occur infrequently in western lakes. *B. liliana* was restricted to calcareous lakes and often found in association with *B. neglectissima* (see below). The morphology and ecology of the small number of valves encountered for these less common taxa conform to that given for the type description and in published accounts elsewhere.

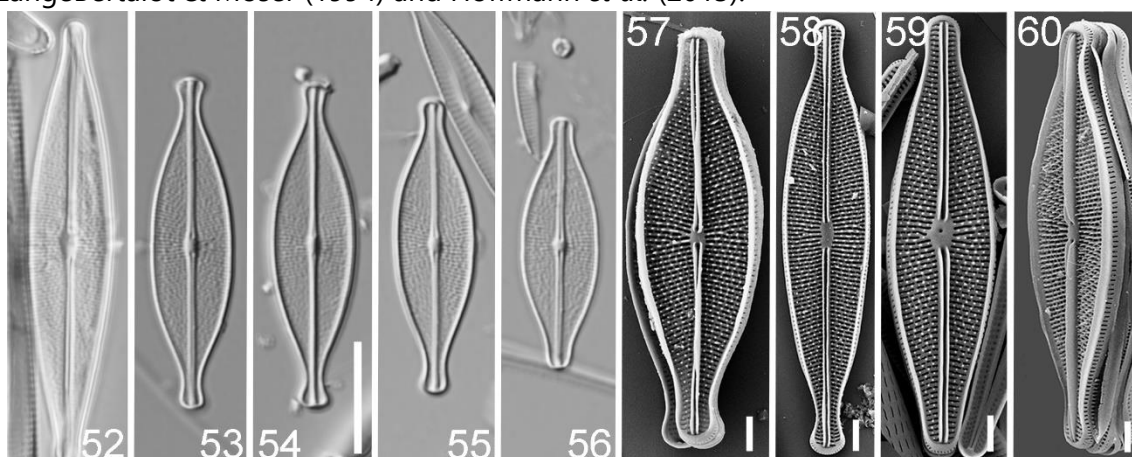


Figures 3.34–3.43 *Brachysira styriaca* and *Brachysira zellensis*. Figs 34–40. *B. styriaca*, LM. Fig. 41. *B. styriaca*, SEM. Fig. 42. *B. zellensis*, LM. Fig. 43. *B. zellensis*, SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .



Figures 3.44–3.51 *Brachysira liliana*. Figs 44–47. LM. Figs 48–51. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

Brachysira vitrea (Grunow) Ross in Hartley (Figures 3.52–3.60) Morphologically the outline is typically broadly elliptic-lanceolate with distinctly capitate poles. Valves with subcapitate ends (Figures 3.52 & 3.59) were occasionally encountered. Valve dimensions (n=29): length 19.5–32 μm , width 5.5–7.5 μm and striae density 32–36 in 10 μm . *B. vitrea* was found predominately in lakes. Although encountered across a broad alkalinity gradient, it occurred only at low densities (<0.7%) in near neutral lakes (pH=6.8) of low alkalinity (4.0 mg/l CaCO_3) and reached maximum abundance (1.4%) in higher alkaline calcareous lakes which were often marl precipitating (68–155 mg/l CaCO_3 ; pH: 8.0–8.1). Morphology and ecology conforms well to that outlined by LangeBertalot & Moser (1994) and Hoffmann *et al.* (2013).



Figures 3.52–3.60 *Brachysira vitrea*. Figs 52–56. LM. Figs 57–60. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

Brachysira neglectissima Lange-Bertalot (Figures 3.61–3.80)

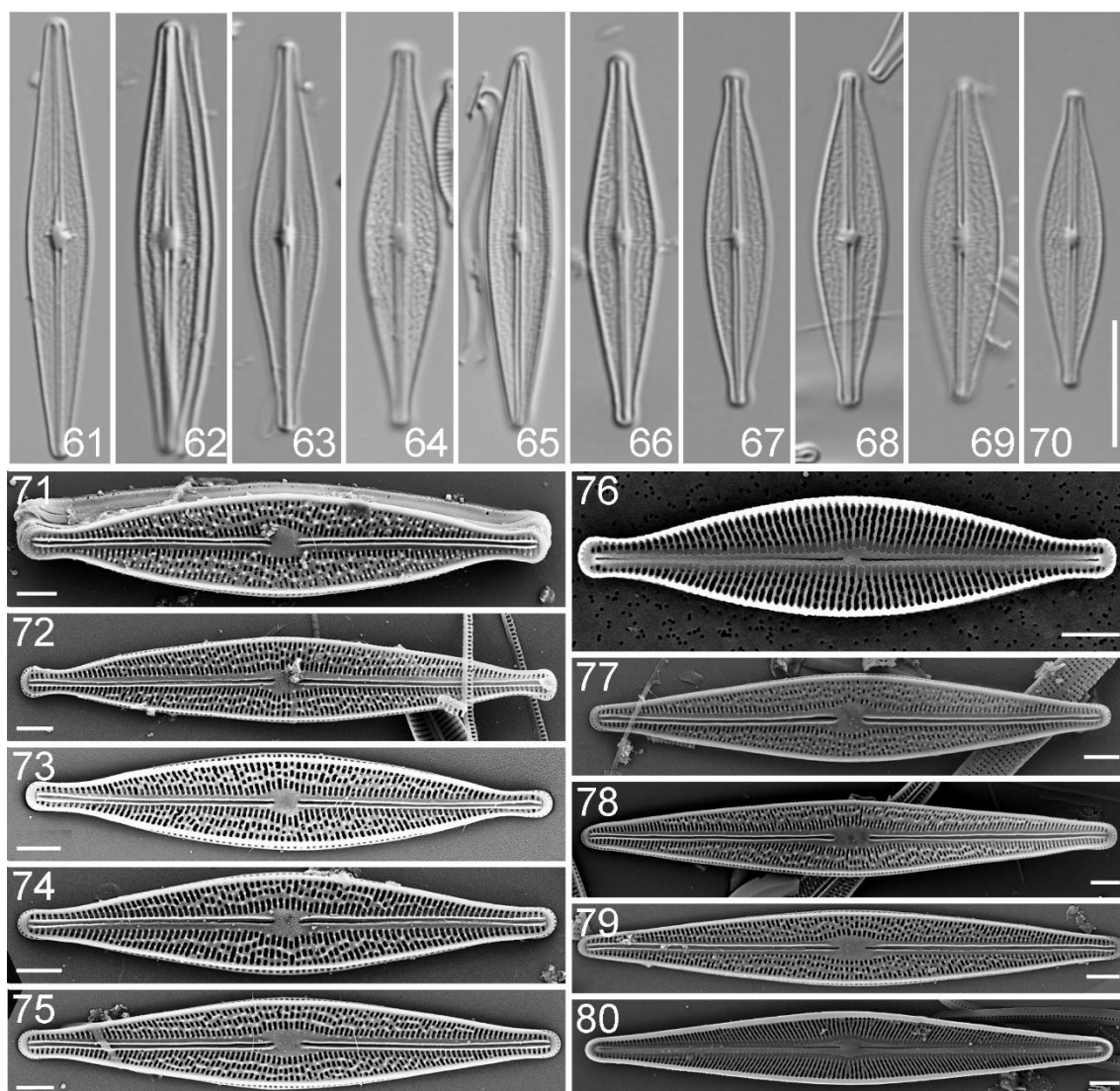
Valves are lanceolate with a continuum in outline observed from those that are clearly capitate, or gradually protracted, to those having distinctly narrow pointed ends (Figures 3.71–3.80). Valve dimensions (n=53): Length 16–36.5 μm , width 4–5 μm and striae density 35–40 in 10 μm . Caution is required when applying the character of protracted ends to help with its separation from *B. liliana* (Hoffmann *et al.* 2013); a

complete examination of the population is required. The greater width of *B. liliana* from the limited number of observations in our study was in agreement with guidance given in the same reference. A small number of frustules with a morphology comparable to *B. neglectissima* and co-occurring with it were more broadly elliptic in outline and possess an additional row of areolae (n=4 rows). Whether it represents a different taxon or merely further variation in *B. neglectissima* will require the study of more abundant populations (Figure 3.69). *B. neglectissima* occurred in 17 oligotrophic lakes and a single river station. It was widely distributed and found from the midlands to the Burren region and throughout the west and northwest. It reached maximum relative abundance (32.2%) in a high status calcareous lake (L. Bunny) but was also observed occasionally (1.3–3.4%) in low alkalinity lakes (4.0–8.0 mg/l CaCO₃) with near neutral pH (pH=6.8–7.1).

Brachysira brebissonii* ssp. *brebissonii Ross in Hartley, and ***Brachysira intermedia*** (Østrup) Lange-Bertalot (Figures 3.81–3.98)

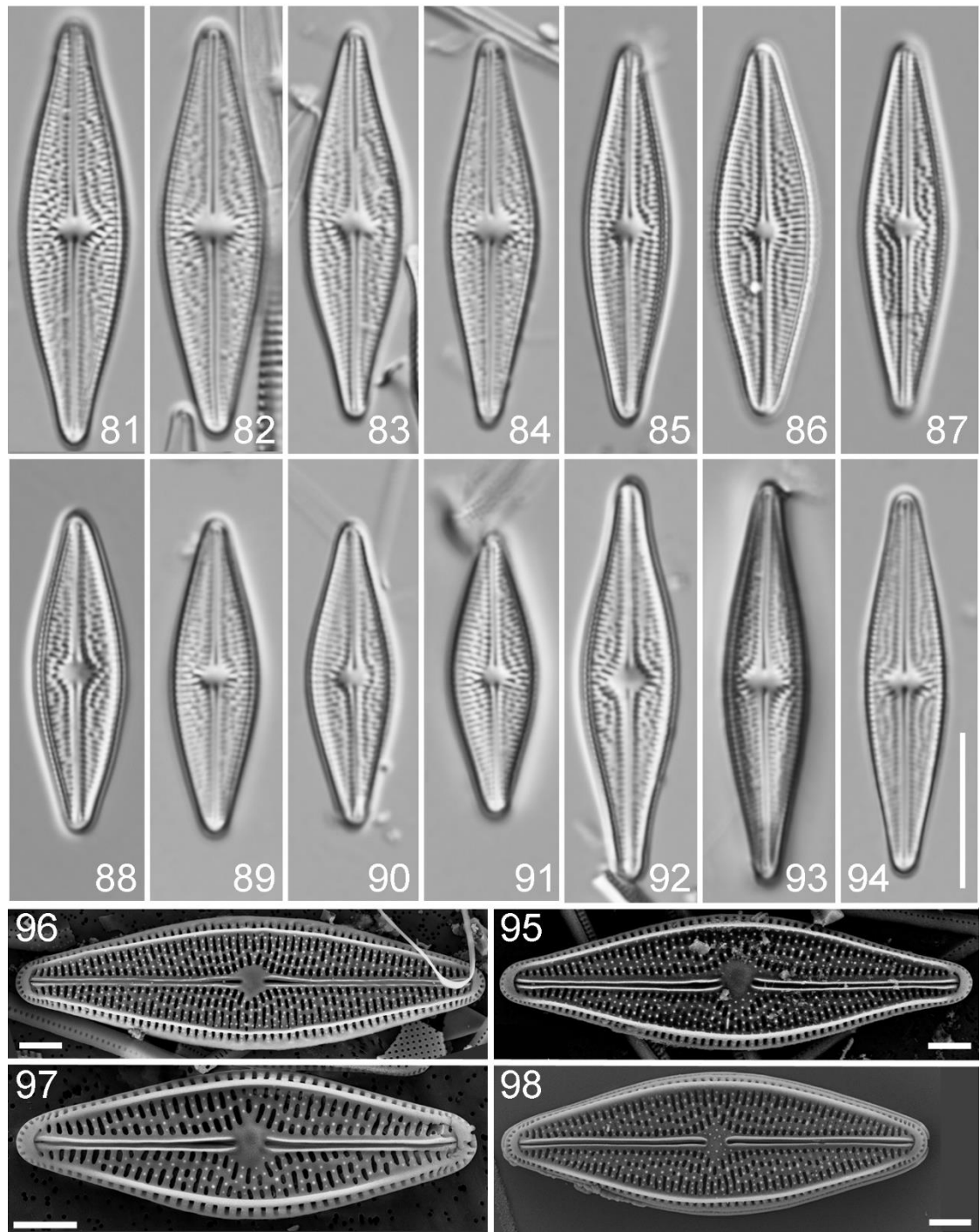
The morphology of *B. brebissonii* (Figures 3.81–3.91, 3.96–3.98) conforms to the recent analysis of what is considered to be the most likely type material for this species reported in Van de Vijver (2014). Non-protracted or almost non-protracted rhombic-valves, with acutely rounded apices dominate Irish populations. Although valves with more rounded ends were encountered occasionally, obtusely rounded valves were never observed. Valve dimensions (n=20): Length 14.5– 28.5 µm, width 5–8 µm and striae density 26–29(30) in 10 µm. *B. brebissonii* was found in 26 lakes and rivers draining siliceous peatland catchments on the periphery of the island and were absent from higher alkalinity regions in the Burren and midlands (Figure 3.2). Maximum

abundance (12.4%) was recorded in L. Glendalough on the east coast with lower densities (0.2–4.9%) recorded for rivers systems along the Atlantic seaboard.

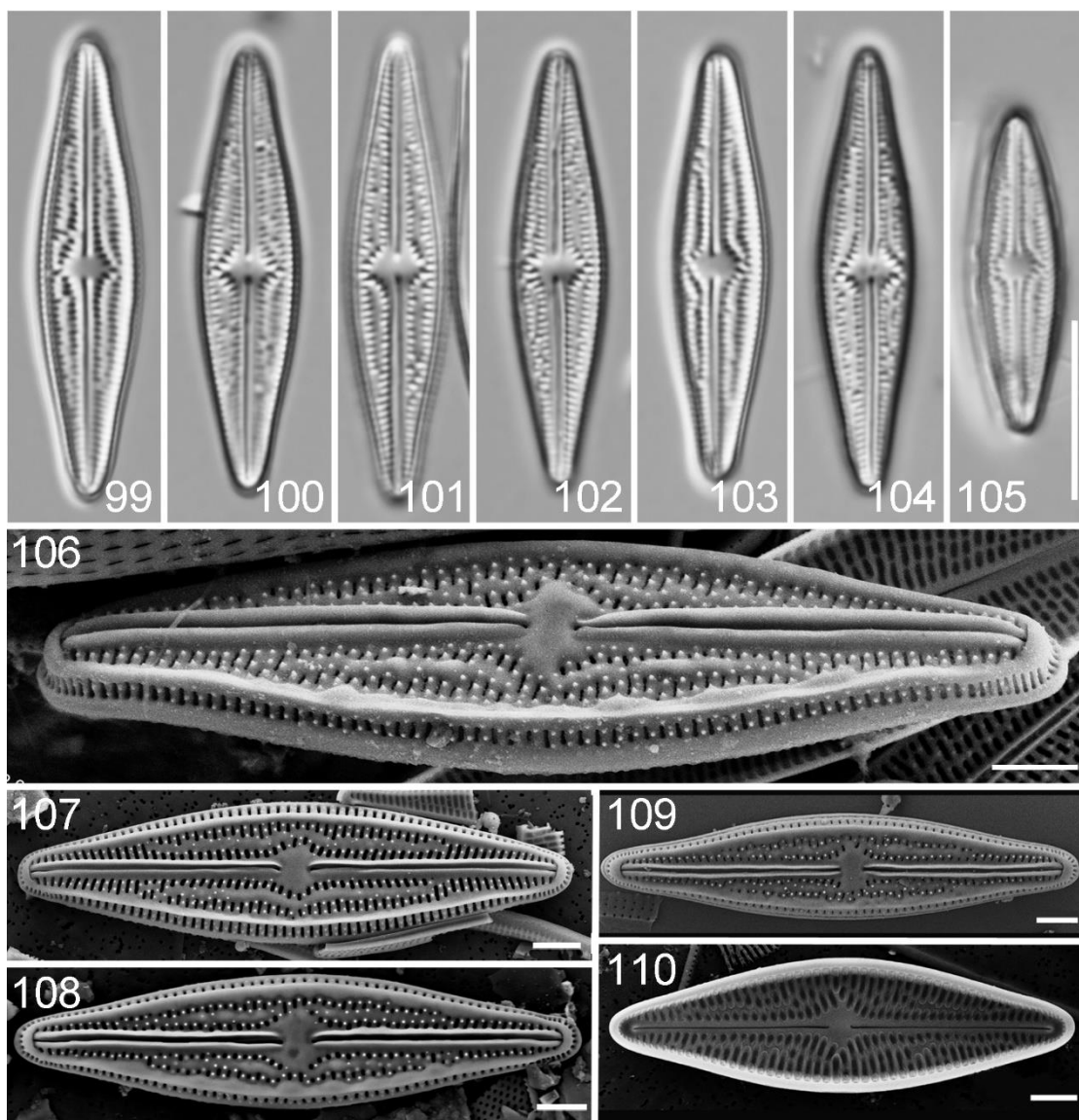


Figures 3.61–3.80 *Brachysira neglectissima*. Figs 61–70. LM. Figs 71–80. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

LM, separation of *B. intermedia* from *B. brebissonii* is via its more protracted outline (Figures 3.92–3.95) relative to the obviously rounded obtuse ends in the latter species. Their similar morphology and a lack of SEM micrographs probably contributed to confusion with their identification during routine monitoring in the past. Another unidentified form, closely resembling *B. intermedia* in morphology was commonly encountered in Irish samples (Figures 3.99–3.110). Its ultrastructure was dissimilar to *B. intermedia*, however differences in the structure of the striae, which always had 2 interstriae papillae and a greater relief between striae were distinctive (Figures 3.106–3.109). Valve dimensions (n=20): Length 18–25 µm, width 5–6.5 µm and stria density 25.5–27.5 in 10 µm. This unknown valve form co-occurs with *B. brebissonii*. It was observed in samples from 13 lakes and 6 rivers and also reached a maximum relative abundance (12.8%) in L. Glendalough. It was combined with *B. intermedia* for calculating the environmental optima reported in this study. This morphotype may also to be illustrated in Hoffmann *et al.* (2013; Tafel 59, figs 19–20) and possibly in Wolfe & Kling (2001; fig. 13) and its formal relationship with *B. intermedia* requires further investigation.



Figures 3.81–3.98 *Brachysira brebissonii* and *Brachysira intermedia*. Figs 81–91. *B. brebissonii*, LM. Figs 92–94. *B. intermedia*, LM. Fig. 95. SEM, *B. intermedia*. Figs 96–98. SEM, *B. brebissonii*. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

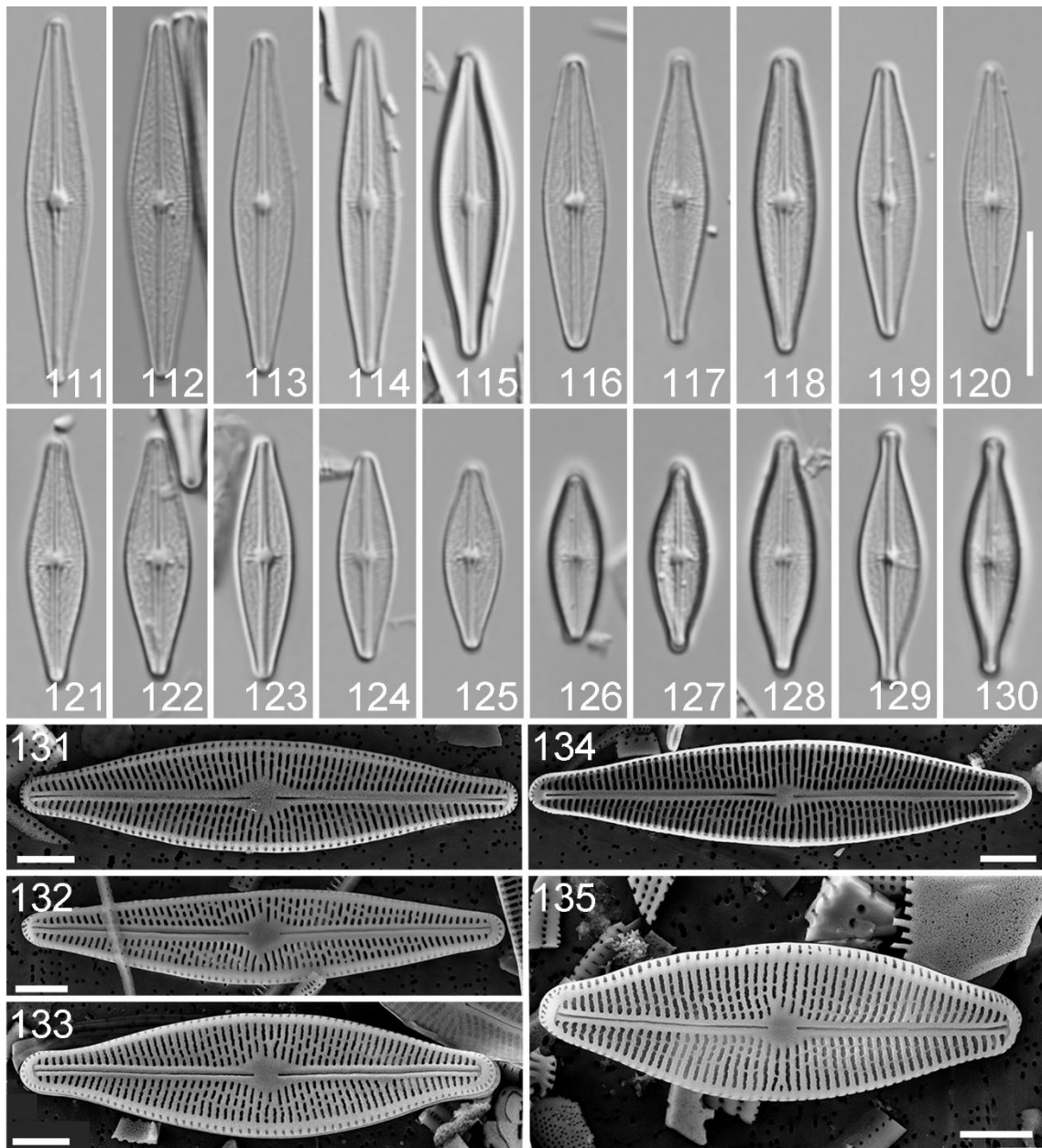


Figures 3.99–3.110 *Brachysira* sp. (aff. *B. intermedia*). Figs 99–105. LM. Figs 106–110. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

Brachysira garrensis (Lange-Bertalot & Krammer) Lange-Bertalot (Figures 3.111–3.135)

This taxon is distinguished from *B. microcephala* in LM by the denser striae. Krammer & Lange-Bertalot (2000) proposed that the finer structure, including stria density (≥ 36 in 10 μm) and lack of protracted apices can be used to differentiate *B. garrensis*. A higher stria density for some populations of *B. microcephala* reported in this study (see below, but also in Compère 1988) and the fact that rostrate-subcapitate valves

may occur in both taxa confirms a greater overlap in these characteristics for Irish populations at least (compare Figures 3.133 & 3.135 with 3.171 & 3.172). Nevertheless, the higher average stria density and the “finer-appearing pattern” of areolae, which were not observable or are more difficult to resolve in *B. garrensis* relative to *B. microcephala*, was sufficiently reliable to enable their separation. Some challenging material included diminutive rhombic forms of both species which often co-occur (compare Figures 3.125–3.126 & Figures 3.160–3.165) and smaller capitate valves of *B. microcephala* with dense striae (Figures 3.129, 3.244–3.246). Valve dimensions (n=20): Length 13–26 μm , width 3.5–5 μm and striae density 36–40 in 10 μm . *B. garrensis* was found in 13 lakes and reached a high relative abundance (max=55.6%) in two western coastal peatland lakes which have been dammed for water abstraction. It was confirmed in three river samples in the west (max=9.6%) and had a low optima for pH relative to other taxa in the genus which may be important for environmental assessment (Table 3.3).



Figures 3.111–3.135 *Brachysira garrensis*. Figs 111–130 LM. Fig. 135–SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

Brachysira microcephala (Grunow) Compère (Figures 3.136–3.252)

Varying taxonomic concepts contributed to the historical confusion and ensuing divergence of opinion around the most correct specific epithet applicable to this taxon (Wolf & Kling 2001, Shayler & Siver 2004a). The name *Brachysira neoexilis* Lange-Bertalot in Lange Bertalot & Moser 1994 is still retained in some floras (e.g. Hoffmann *et al.* 2013). A large variation in its morphology may contribute to the lack of consistency in the identifications and the species is often documented with reference to its capitate or non-capitate morphotypes (Lange-Bertalot & Metzeltin 1996, Fallu *et al.* 2000, Shayler & Siver 2004a). Although Wolf & Kling (2001) discount this as merely intra-specific variability an equivalent approach is followed here to help assess and illustrate the variability for four dominant forms.

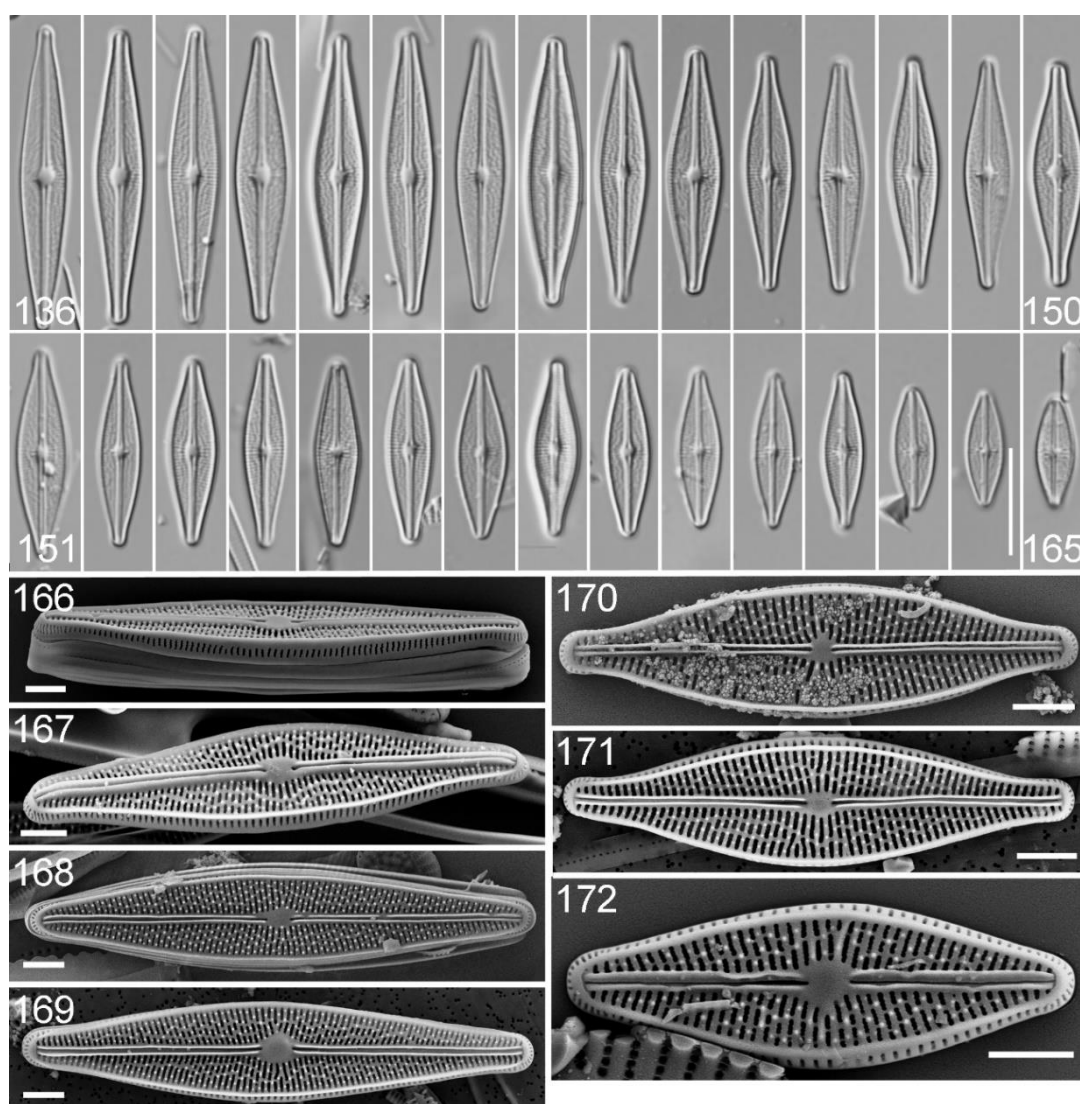
BMIC-1: Morphotype with rounded or rostrate poles (Figures 3.136–3.172)

This category conforms to the non-capitate and rostrate valve outline illustrated in Compère (1988) and has the equivalent higher range in stria density compared to other forms of *B. microcephala*. Valve dimensions (n=35): Length 12.0–27.0 µm, width 3.5–5.0 µm and striae density 33–38 in 10 µm. This morphotype is well dispersed in the periphery in peatland catchments (Figure. 3.2) and was found equally abundant in rivers and lakes from a total of 55 samples (max=32.9%).

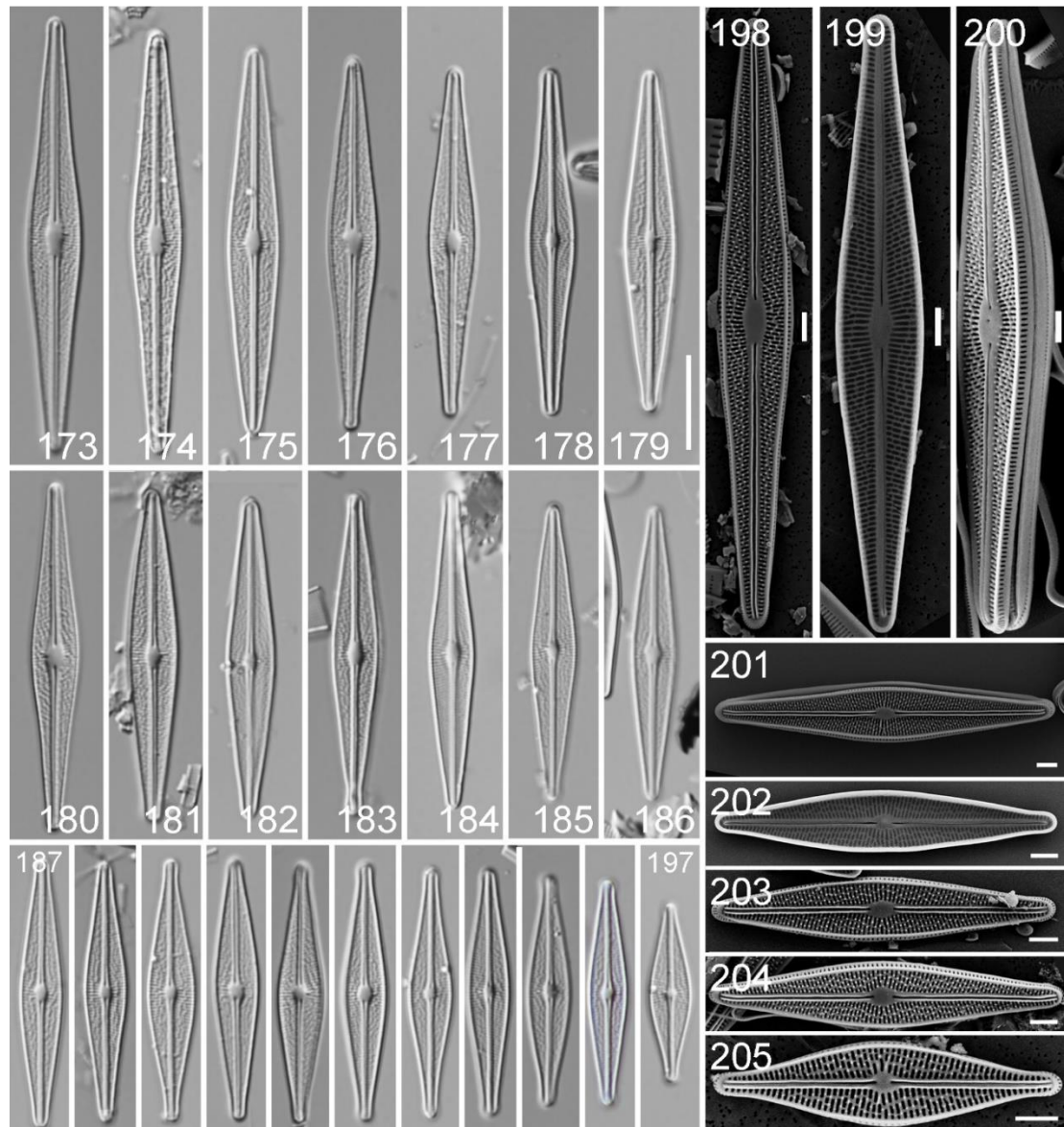
BMIC-2: Non-protracted morphotype aff. *B. procera* (Figures 3.173–3.205)

This morphotype has an affinity with *B. procera* with its non-protracted outline, larger maximum valve length and a propensity for widening of the central area apically in larger specimens. There was a statistically significant linear relationship ($r^2 = 0.86$, $p < 0.001$) between valve length and distance between the proximal raphe endings,

with a continuum in the latter character from the largest to the smallest valves examined. The higher stria density and a lower minimum valve length do not fit the existing concept for *B. procera*. Valve dimensions (n=88): Length 15.5–44.5 μm , width 4.0–6.0 μm and striae density 32–36 in 10 μm . This morphotype was widespread and occurred in 34 samples in association with BMIC-1. It occurred in both lotic and lentic habitats (max=14.9%) but was more prevalent and reached a higher density in low alkalinity oligotrophic lakes (Figure 3.2).



Figures 3.136–3.172 *Brachysira microcephala* morphotype BMIC-1. Figs 136–165. LM. Figs 166–172. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

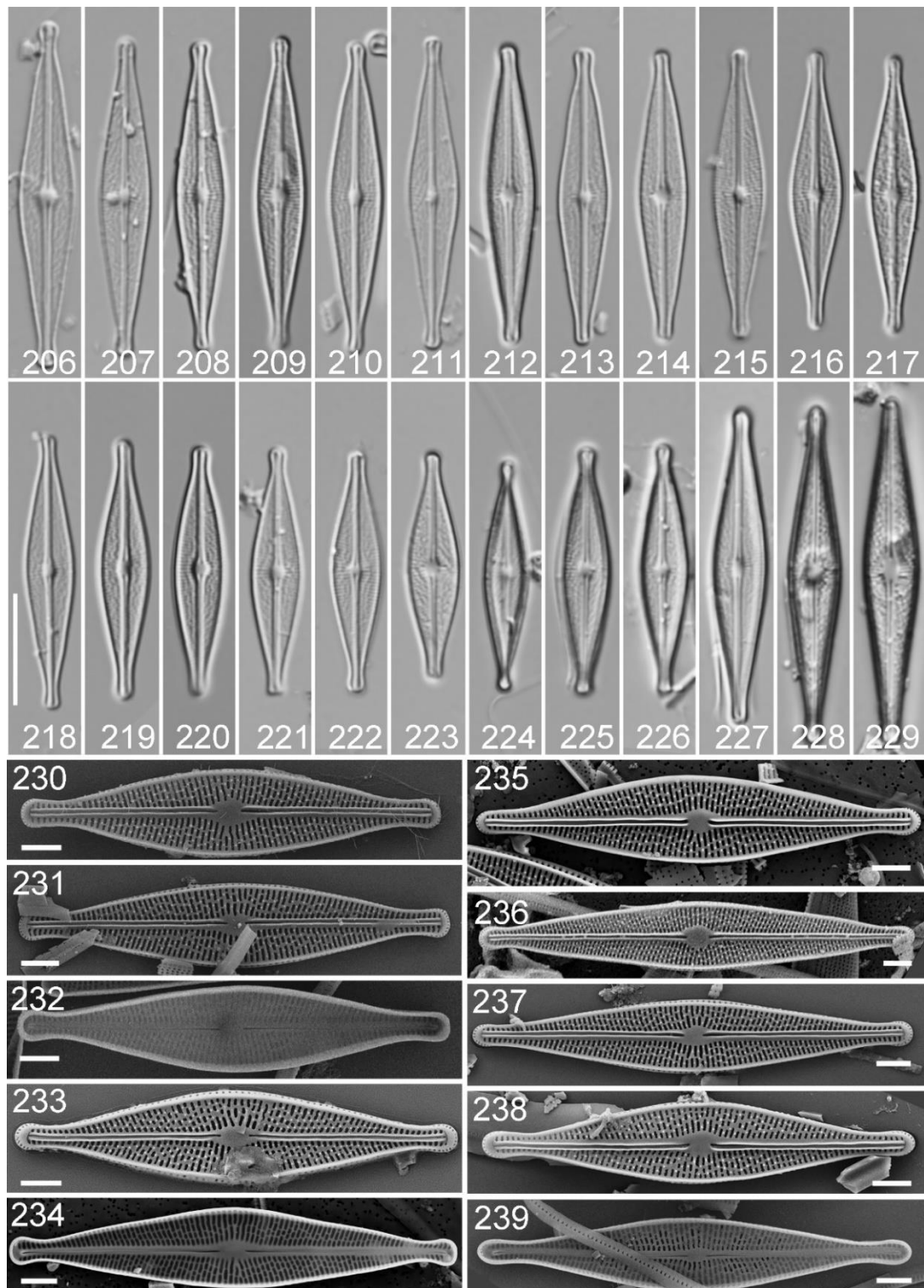


Figures 3.173–3.205 *Brachysira microcephala* morphotype BMIC-2. Figs 173–197. LM. Figs. 198–205. SEM. Scale bar = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 2 μ m.

BMIC-3: Lanceolate to linear-lanceolate outline with protracted poles (Figures 3.206–3.239)

Variability in the form of the protracted ends in this morphotype was obvious while a continuum of smaller valves with BMIC-4 was also apparent. Occasional heteropolar specimens with clearly pointed and capitate ends on the same valve were observed. A less numerous form with more produced or elongate capitate apices (Figures 3.224–

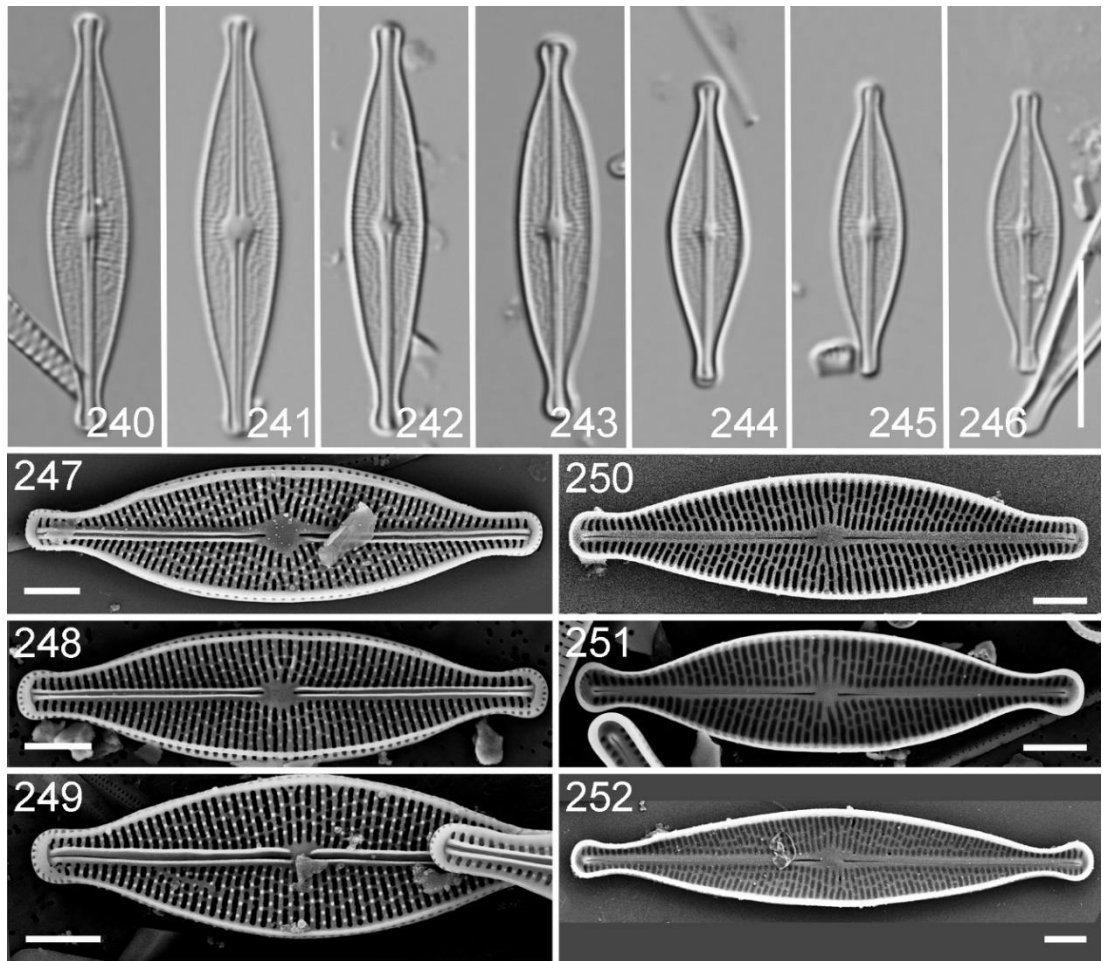
3.229, 3.238–3.239) was occasionally encountered in several lake samples. Valve dimensions (n=30): Length 16.0–33.5 μm , width 4.0–5.0 μm and striae density 33–37.5 in 10 μm . This category was abundant (max=31.3%) and widespread in lakes and rivers (n=39 samples) in the periphery.



Figures 3.206–3.239 *Brachysira microcephala* morphotype BMIC-3. Figs 206–229. LM. Figs 230–239. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

BMIC-4: Elliptic-lanceolate outline with clearly capitate poles (Figures 3.240–3.252)

Populations of this clearly capitate elliptic-lanceolate form were found in lakes at the extreme end of the alkalinity gradient. Smaller valves with a high maximum stria density (Figures 3.224–3.246, 3.248–3.249, 3.251) were found in low alkalinity lakes and probably represent no more than a size reduction of BMIC-3. A further small population of larger specimens (Figures 3.240–3.242, 3.247, 3.252) with a lower stria density was found in a highly calcareous marl precipitating lake. This is an unusual habitat for *B. microcephala* and was sufficient to result in higher optima for pH for this morphotype (Table 3.3). Its trophic preference was equivalent to the other forms. Valve dimensions (n=15): Length 16.0–25.5 μm , width 3.5–5.0 μm and striae density 33–39 in 10 μm . This less common form (n=11, max=4.4%) was restricted to the west coast.



Figures 3.240–3.252 *Brachysira microcephala* morphotype BMIC-4. Figs 240–246. LM. Figs 247–252. SEM. Scale bar = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

3.5. DISCUSSION

This study increases the number of *Brachysira* species recorded in Ireland to at least 16 taxa, including first records for *B. neglectissima* and *B. intermedia*. However, several species reported previously namely, *Brachysira aponina* Kützing (1836: 3) in Sims (2007), *Brachysira follis* (Ehrenberg) Ross in Hartley (1986: 607), *Brachysira vitrea* f. *lanceolata* (Mayer) F.R. Schoeman & R.E.M. Archibald (1988: 230) and *Brachysira zellensis* f. *difficilis* (Grunow) P.B. Hamilton in Hamilton, Poulin, Charles & Angell (1992: 29), compiled in Wolnik & Carter (2014) were not encountered in our survey. We describe two new diatom species; *B. praegeri* formerly of the *B. brebissonii*-complex and *B. conamarae* which has an affinity with *B. microcephala*. This latter new species has probably been overlooked in the past because it has so far only been reliably identified with SEM, which is necessary for confirmation of the absence of silica ribs bordering the raphe. Further *B. conamarae* is rare. Both taxa occur in weakly acidic near-pristine catchments in western Ireland.

As illustrated and reported in other studies (Lange-Bertalot & Moser 1994, Wolfe & Kling 2001) *B. microcephala* is highly polymorphic with respect to valve outline. Stria density in the populations examined is typically higher, with a median density equivalent to the upper range usually reported in published accounts, but is comparable to that in Compère (1988). A difficulty with separating some populations from *B. garrensis* in LM is apparent however. During routine monitoring, the diatom *B. microcephala* was often merged with or misidentified as *B. vitrea*. Our data confirm that there is no empirical basis to support the assumption of an overlap in the morphological or ecological criteria for these taxa in Irish populations. Both taxa occasionally occur together in the same habitat but *B. vitrea* is always greater in width

than the most similar form of *B. microcephala* (see BMIC-4). Another problematic taxon formerly reported from Ireland is *B. procera*. Chen *et al.* (2008) assigned an environmental optima to this species based on its occurrence in 14 lakes across Ireland. These records are most likely equivalent to the morphotype BMIC2 presented here. However, stria density (32–36 in 10 μm) conflicts with the lower stria density published elsewhere for *B. procera* (27–30 in 10 μm) and its size range is also different. When Lange-Bertalot & Moser defined this species, representatives from Irish samples were also illustrated in the plates (Lange-Bertalot & Moser 1994: page 116, figs 20–24) but we have not encountered frustules having a stria density equivalent to that given with the type description. Whether the morphotype represented here is a different taxon, a divergent form of *B. procera*, or whether the description of this taxon requires an amendment will require further clarification. Its morphology and ecology are very similar. The lake trophic diatom metric utilised in Ireland (Kelly *et al.* 2014) assigns a higher nutrient sensitivity score to *B. procera* ($s=2$) than for other taxa in the genus ($s=1$). Our data suggest that BMIC-2 has an environmental niche similar to the other forms of *B. microcephala* and we recommend that this morphotype be assigned an equivalent score for ecological status assessments. We cannot improve on the uncertainty around the limits of diversity apparent for morphotypes in this species complex, but illustrate the full range for the dominant forms encountered in Ireland. Molecular investigation and culturing studies would be useful to provide additional information and to explore the possibility of cryptic species (Vanormelingen *et al.* 2013) for this proposed morphotype.

The rostrate poles and narrower valves of *B. neglectissima* typically allow its separation from the closely related and sometimes co-occurring *B. liliana*. The Irish populations

conform to this general concept allowing its reliable identification in many cases, but the valve outline does form a continuum from valves with distinctly capitate endings to those possessing narrowly pointed and non-protracted poles. We did not find a sufficient number of valves to report the full range in morphology for *B. liliana* but the specimens encountered conform to the type description and their greater width allowed for separation from the former species.

Morphological characteristics that define *Brachysira* are the presence of papillae (sometimes referred to as spines or warts) or ridges of silica ornamenting the valve face (Round & Mann 1981). The tentative occurrence of additional small papillae in the central area of *B. praegeri* was a distinct feature observed with SEM, although these papillae were often reduced in number or absent on frustules within the same population. Their absence was not due to taphonomic processes as no other evidence for dissolution of silica was observed on the frustules that were examined. This changing morphological character was consistently observed in populations sampled over a 9-year monitoring period for L. Maumwee and L. Nambrackmore. The inconsistent occurrence of papillae was also identified along the axial area of *B. conamarae* from these same lakes. In an intensive search of a large number of additional valves in these samples, a single frustule of *B. brebissonii* (Figure 3.98) from L. Maumwee was observed with papillae in the central area. Populations of the more abundant *Brachysira* taxa occurring in the same samples never displayed the tendency and *B. garrensis* which lacks these silica outgrowths altogether was observed at low density in the same sample. A review of images of *Brachysira* in the literature reveals a scarcity of further published examples of frustules possessing such variability in the presence of central papillae. Both Wolfe & Kling (2001) and Shayler & Siver (2004b)

report a lack of papillae for *B. brebissonii* but frustules displaying an increased density of papillae are rare in the literature. Flower (2005: 52 fig. 83) illustrates a valve of *Brachysira actoborealis* Wolf & Kling (2001: 252) from lakes in The Falklands also displaying additional papillae in the central area. The reason why these lakes have some diatom taxa with a greater tendency to produce valves having this additional ornamentation, or what its significance is in terms of phenotypic adaptation, is not known at this time.

From a water quality perspective the genus is generally indicative of slightly acidic to circumneutral watercourses that are low in nutrients (Hoffmann *et al.* 2013, Vouilloud *et al.* 2014). In a review of several European diatom trophic indices, Besse-Lototskaya *et al.* (2011) acknowledged some of the cosmopolitan *Brachysira* as reliable indicators of oligotrophic or oligo-mesotrophic conditions. This study also confirms the importance of *Brachysira* in the epilithic community of weakly acidic rivers and lakes, and their association with underlying siliceous geology. These acidophilous *Brachysira* communities occupy a narrow niche along an alkalinity-pH gradient becoming subordinate to more circumneutral taxa with increasing alkalinity and were replaced completely by acidobiontic taxa below pH 5.1. This is similar to results reported by Siver *et al.* (2002) showing a narrow pH optima for *Brachysira* at 6.0. The absence of *Brachysira* taxa was notable in some rivers along the east coast and also in some extensively afforested peatland catchments in the west. Many paleolimnological studies confirm a decline in their abundance with nutrient enrichment and document their replacement in the community by more tolerant taxa with acidification (Leira *et al.* 2007, Enache *et al.* 2011, Battarbee *et al.* 2011). Larsen (2000) and Camburn & Charles (2000) report an increased abundance of the acidobiontic *B. seriens* in

response to acidification, but this species is never abundant in Irish samples (DeNicola *et al.* 2004, Chen *et al.* 2008). Species such as *B. liliana*, *B. styriaca* and *B. zellensis* were rare in the epilithic community and restricted to a small number of the higher ecological status lakes. *B. neglectissima* had a spatially restricted distribution, however, a large population was found in the epilithic community of a high quality calcareous lake where colour was low. *B. vitrea* also occurred in the benthic flora of high alkalinity systems but never reached high densities. The *Brachysira* community has almost certainly become less widespread, owing to nutrient enrichment and acidification.

It has been suggested that greater taxonomic resolution will increase the precision of applied diatom methods (Round 2004, Besse-Lototskaya *et al.* 2011, Morales *et al.* 2012) and improve our understanding of larger scale processes e.g. diatom biogeography (Novais *et al.* 2015, Kopalová *et al.* 2016). In our evaluation of the trophic optima of some closely related morphotypes we found that their environmental requirements were similar. All taxa in our dataset reached maximum abundance when molybdate reactive phosphorus was below the limit of detection (10 µg/IP) and when concentrations of nitrogen and ammonia were low. Nevertheless, the highest diversity of *Brachysira* was encountered in some reference waterbodies in near-pristine catchments, even though many of these species were rarely abundant. Few diatom-based ecological indices incorporate diversity of reference taxa explicitly in their calculations, even though metrics for the protection of endemic, red-list or taxa with a restricted regional distribution are feasible. The approach adopted by Schaumburg *et al.* (2012) is an exception and may potentially offer the opportunity to detect the earlier effects of anthropogenic stressors on vulnerable reference waterbodies (Cantonati & Lange-Bertalot 2011). Therefore, we suggest that for routine

environmental assessment, all species of *Brachysira* including the morphotypes illustrated here should continue to be enumerated separately until such time as their taxonomy and function in the ecology of oligotrophic ecosystems has been more fully resolved.

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CHAPTER 4

Taxonomy, ecology and analysis of type material of some small *Encyonopsis* with description of new species in Ireland

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4.1. ABSTRACT

The *Encyonopsis microcephala*-complex occurs in oligo-mesotrophic freshwaters and is often abundant in the periphyton of low nutrient lakes of high conservation value. It is globally distributed and increasing in apparent diversity with the discovery of additional species. To better differentiate this taxonomic aggregate, we re-examined the original type material of *E. alpina*, *E. angusta* and *E. tavirana* with light (LM) and scanning electron (SEM) microscopy. We investigated the morphology, ultrastructure and ecology of *Encyonopsis* populations from Ireland, which included previously unreported taxa and morphodemes of *E. krammeri*, *E. neerlandica* and *E. subminuta*. The apparently cosmopolitan *E. microcephala* was not found in collections from Ireland but two new species were described and compared to others in the complex. *Encyonopsis carraensis* sp. nov. was associated with a marl-precipitating community in low nutrient lakes, while *Encyonopsis hibernica* sp. nov. occurred in more productive

waterbodies. Several *Encyonopsis* species had environmental optima near the oligomesotrophic boundary characteristic of the *microcephala*-complex. However, the niches of others were distributed along a wider gradient of water quality. Therefore, when *Encyonopsis* populations are prevalent in epilithic communities, the use of a more refined taxonomy is recommended for lake ecological assessment.

Keywords: Bacillariophyta, taxonomy, morphology, ecology, Ireland, new diatom species, *Encyonopsis carraensis*, *Encyonopsis Hibernica*

4.2. INTRODUCTION

The genus *Encyonopsis* Krammer (1997a: 156) was established concurrent with a significant revision of *Cymbella* C. Agardh (1830: 1), and more recently its classification has been further refined with separation of the genus *Kurtkrammeria* Bahls (2015: 170). The former taxonomic aggregate *Cymbella microcephala* Grunow in Van Heurck (1885: 63) was examined in detail by Krammer and many independent taxa in this complex were diagnosed or assigned to the new genus (Krammer 1997b). These included the cosmopolitan *E. microcephala* (Grunow) Krammer (1997b: 91), *E. minuta* Krammer (1997b: 195), *E. subminuta* Krammer & Reichardt (Krammer 1997b: 96), *E. krammeri* Reichardt (1997: 61) and *E. alpina* Krammer & Lange-Bertalot (Krammer 1997b: 97). In the same monograph *E. angusta* Krammer & Lange-Bertalot (Krammer 1997b: 98), *E. tavirana* Krammer (1997b: 96) and others were also described, but apparently have more limited distributions outside their type localities. A restricted availability of the original monograph and a deficiency of high quality images to accompany species descriptions may be responsible in part for the lack of additional

records. Therefore, the original type material of *E. alpina*, *E. angusta* and *E. tavrana* is re-examined to better document the morphology of these similar taxa.

Since the publication of Krammer's monograph, appreciation of the taxonomic significance of subtle morphological differences has revealed even further diversity in the *microcephala*-complex (Bahls 2013, Graeff & Kociolek 2013). It now incorporates a much larger number of species, including *Encyonopsis krammerioides* Lange-Bertalot & Rumrich in Rumrich *et al.* (2000: 117), *E. lange-bertalotii* Krammer (2003: 148), *E. catharinae* Lange-Bertalot & Krammer (Krammer 2003: 148), *E. horticola* Van de Vijver & Compère in Van de Vijver *et al.* (2009: 199), *E. neerlandica* Van de Vijver, Verweij, Van der Wal & Mertens (2012: 44), *E. albertana* Bahls (2013: 10), *E. anacondae* Bahls (2013: 12), *E. bobmarshallensis* Bahls (2013: 13), *E. hustedtii* Bahls (2013: 19), *E. kutenaiaorum* Bahls (2013: 21), *E. lacusalpini* Bahls (2013: 22), *E. robusta* (Hustedt) Bahls (2013: 29), *E. coloradensis* C.L.Graeff & J.P.Kociolek (2013: 94), *E. rockiensis* C.L.Graeff & J.P.Kociolek (2013: 99), *E. riflensis* C.L.Graeff & J.P.Kociolek (2013: 101), *E. cilaosensis* Le Cohu & Gassiole in Le Cohu *et al.* (2014: 124) and *E. palmeti* Gassiole & Le Cohu in Le Cohu *et al.* (2014: 126). While many of these species are known only from their type localities, others are probably more widespread. Furthermore, other populations await formal evaluation or taxonomic clarification (Gottschalk & Kahlert 2012, Bahls 2013, Delgado *et al.* 2013, Graeff & Kociolek 2013, Solak *et al.* 2016, Rivera-Rondón & Catalan 2017). Two such forms, frequently encountered in Irish lakes are described here as new species and compared to similar taxa.

Encyonopsis is reported from cooler, well oxygenated, oligo-mesotrophic alkaline waters in alpine regions and equivalent habitats worldwide (Krammer 1997, Bahls 2013). More detailed assessment of the autecology of individual species has hardly

progressed past that given with Krammer's original observations (Lange-Bertalot *et al.* 2017). A single indicator value for a merged aggregate of the *microcephala*-complex currently provides a pragmatic solution for applied monitoring of lake epilithic communities (Schönfelder *et al.* 2002, Kelly *et al.* 2007). However, while some taxa are prevalent in high status marl lakes, which are significantly threatened across Europe (Pentecost 2009, Wiik *et al.* 2015), *E. microcephala* is recognized to be less demanding in terms of its water quality requirements (Bahls 2013). The ecological niche of the group has broadened more recently with the description of *E. neerlandica* from moorland pool systems (Van de Vijver *et al.* 2012) and the relatively higher trophic optimum reported for *E. horticola* by Van de Vijver & Compère (2009). Owing to significant challenges with their reliable identification (Kahlert *et al.* 2016), variation in reported optima for even some well-known taxa is apparent (Sgro *et al.* 2007, Slate & Stevenson 2007, Chen *et al.* 2008) potentially reducing the precision of ecological studies. Given their high diversity in lakes, we hypothesised that a more discriminating taxonomy of small *Encyonopsis* species would improve their indicator value for bioassessment. Therefore, after establishing the morphological limits and clarifying the taxonomy of the dominant taxa, we also evaluate their ecology.

4.3. MATERIALS AND METHODS

4.3.1. COLLECTION AND PREPARATION OF SAMPLES

A total of 210 epilithic diatom samples from 90 lakes (2007–2015) across a range of physio-chemical types was assessed to investigate the distribution of *Encyonopsis* in Ireland (Figure 4.1). Diatom assemblages from 203 rivers were also reviewed for their occurrence. Diatoms were sampled by brushing the biofilm from the top surface of at least five cobbles in a tray with water and then pouring the collected slurry into glass bottles with unacidified Lugol's Iodine used for preservation (CEN, 2003). Samples were digested to remove organic material using concentrated sulphuric acid, potassium permanganate and oxalic acid, after a preliminary removal of carbonates using concentrated hydrochloric acid (CEN, 2003). Following rinsing in deionised water, a dilute suspension of the oxidised material was air-dried onto glass coverslips for 24 hours. Permanent glass slides were prepared by mounting the coverslip on the slide using Naphrax® (refractive index=1.74; Brunel Microscopes Ltd., UK). Samples were prepared for viewing with scanning electron microscopy (SEM) by air-drying digested material on glass coverslips or filtering a suspension through 0.2 µm Isopore™ membrane filters, before fixing to aluminium stubs with carbon tabs, sputter-coating with gold and grounding with conductive silver paint.

4.3.2. MICROSCOPIC EXAMINATION

Light microscopy was performed with a Nikon E80i microscope equipped with phase contrast and a Plan Fluor x100 oil immersion objective (n.a.=1.3) (CEN, 2004). The proportional abundance of diatoms was calculated by counting single or multiple non-overlapping transects through the centre of each coverslip until 400–500 random

non-planktonic valves were enumerated at $\times 1000$ – 1500 . Images were obtained using a Nikon Ni-U (Plan Fluor objective n.a.=1.45) equipped with Differential Interference Contrast (DIC) and a CoolPix DS Ri-2 digital camera. SEM observations were performed at the Centre for Research on Adaptive Nanostructures and Nanodevices Advanced Microscope Laboratory in Trinity College Dublin using a Zeiss Ultra SEM microscope operated at an acceleration voltage of 5 kV and working distance of ~ 5.0 mm. All measurements were completed during SEM sessions or subsequently using the open source software ImageJ V1.48 (Rasband 1997–2016) calibrated to scale bars on TIFF files. Diatom images assembled in LM plates were similarly measured to maximise the reported range for the morphological criteria. The number of striae and areolae were counted for fixed distances ($1\text{ }\mu\text{m}$, $2.5\text{ }\mu\text{m}$ or $5.0\text{ }\mu\text{m}$), measured adjacent to the central area for both dorsal and ventral sides of the frustule (Figure 4.2). Stria density was reported as the number of striae occurring in $10\text{ }\mu\text{m}$ lengths, to the nearest 0.5 stria. Digital microscopic images were assembled into plates using Photoshop Elements V9.

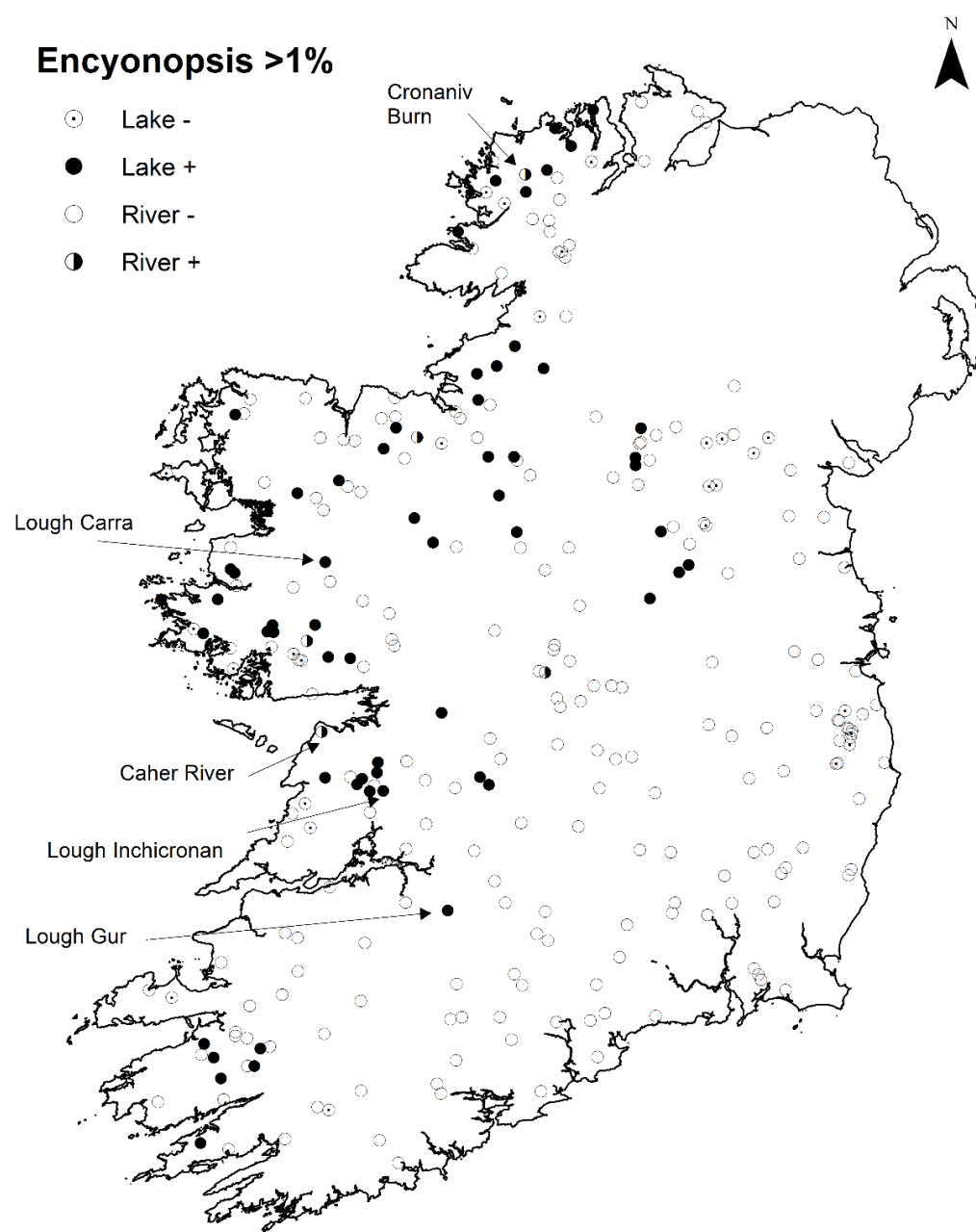


Figure 4.1 Ireland. Map illustrating locations where *Encyonopsis* was present (+) or absent (-) at $\geq 1\%$ abundance in lake and rivers. Named waterbodies refer to the location of type lakes or rivers referred to in the text.

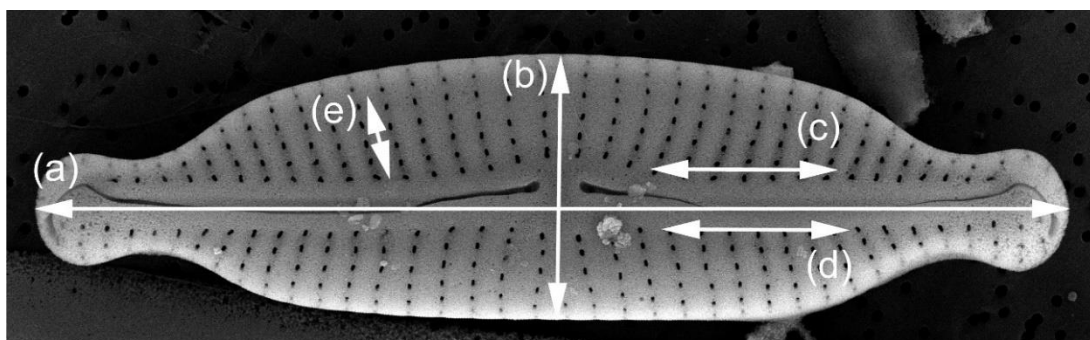


Figure 4.2 SEM of *Encyonopsis* external valve view indicating location where measurement of valve length (a) valve width (b) and counting of dorsal (c) and ventral (d) striae and areolae density (e) was determined.

4.3.3. MORPHOLOGICAL ANALYSIS

The distributions of morphometric characters measured in SEM (valve length and width, length/width ratio and dorsal and ventral stria density) were compared for all abundant forms with boxplots and a Principal Components Analysis (PCA) on variables scaled to zero mean and unit standard deviation. PCA biplots were presented to display the variability and extent of overlap in morphology with data fitted to convex hulls to help with visualisation. Analysis and illustrations were done with the R statistical computing and graphics software (R Core Team 2014) and “vegan” package (Oksanen *et al.* 2018).

4.3.4. ENVIRONMENTAL ANALYSIS

To compare species distribution in relation to environmental gradients, chemical data were obtained from AQUARIUS, an environmental database for the national Water Framework Monitoring Program in Ireland (EPA 2006). Species trophic optima for all commonly occurring taxa were assessed with simple weighted averaging (ter Braak & Barendregt 1986) using log transformed environmental variables. An optima U_{wa} for species j was calculated as the mean of measured environmental variable weighted by the abundance of this taxon at all sites according to the WA formula:

$$U_{wa\ j} = \sum_{i=1}^N Y_{ij} x_i / \sum_{i=1}^N Y_{ij}$$

where N is the total number of sites, x_i is the environmental variable of interest at site i and Y_{ij} is the abundance of species j at site i . To assess if lakes could be more usefully separated for ecological assessment based on the relative abundance of their constituent *Encyonopsis* taxa, a nonmetric multi-dimensional scaling (nMDS) analysis was run to find an optimal configuration that best preserved their rank ordering of pair-wise between site distances using Bray Curtis dissimilarity matrix. The vegan function 'envfit' was used to passively project environmental variables *a posteriori* to the ordination plot, to evaluate correlative associations between ordination scores and explanatory variables.

4.3.5. TAXON DESCRIPTION & TYPE SPECIMENS

The morphology of new species is compared with taxa and morphotypes published for the microcephala-complex compiled in the introduction. Terminology of valve morphology is based on Round *et al.* (1990), Krammer (1997b) and Graeff & Kociolek (2013) and species description follows that in Van de Vijver *et al.* (2012). Holotype samples and slides are stored at the Trinity College Dublin (TCD) herbarium. Type material was provided by the Alfred Wegener Institute, Germany, where isotype slides are also stored.

Type material

Encyonopsis angusta Krammer & Lange-Bertalot

Locus typicus: Lustsee, Oberbayern, Belag auf Steinen, 3.5.1990.

Typus: Präp. 77A IOK

Slide label: "Lustsee (?) links Stein 3.5.90 Cymbella"

Encyonopsis alpina Krammer & Lange-Bertalot

Locus typicus: Königsee bei Berchtesgaden

Typus: Präp. 5b IOK

Slide label: "Königsee 30.5.88"

Encyonopsis tavorana Krammer

Locus typicus: Wasserbecken an einer Landstraße bei Tavira, Algarve, Portugal, Wandbelag, 753 $\mu\text{S}/\text{cm}$. 7.3.1987.

Typus: Präp. 517B IOK

Slide label: "Wasserbecken an Strasse nah Terra (?), Belg M2118A"

4.4. RESULTS

The *microcephala*-complex occurred at $\geq 1\%$ abundance in 63 of 90 lakes sampled (Figure 4.1) and was often numerically dominant in epilithic communities (median and max density of 17.5% and 84%, respectively). It is infrequent in lotic waters, occurring in $<10\%$ of river samples and only as isolated valves in the majority of these. Ten *Encyonopsis* species and four other forms which cannot be reliably attributed to existing species concepts are described below. These include descriptions of two new species and emended descriptions for three others. Descriptions of all taxa addressed in this study, updated with new observations or data where relevant, are presented in Table 4.1.

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. microcephala</i>	10.0– 23.0(16) *	3.5–4.2	5.4	23– 24(25) (24) [#]	Symmetric–weakly dorsiventral; large or mid–sized valves parallel or weakly convex, or dorsal weakly convex with ventral very weakly convex, straight or rarely concave.	Moderately narrow capitate, subrounded, mostly with dorsal and ventral shoulders present.	1–3 shortened striae at centre, sometimes irregularly positioned, variable between populations, weakly radiate, equidistant at centre and poles.	38–42 Very small circular round.	Krammer, K. (1997b). Intermissio absent. Distal raphe ends weakly ventrally displaced.
<i>Encyonopsis robusta</i> (Hustedt) Bahls 2013 – synonym <i>E. microcephala</i> var. <i>robusta</i> has coarser striae (19–22), lacking dorsiventrality and has a small round central area.									
<i>E. minuta</i>	8.0–17.0 (13)*	2.8–3.5	4.9	24–25	Symmetric–weakly dorsiventral, lanceolate with weakly convex dorsal & ventral sides.	Narrow capitate– subcapitate, subrounded ends lacking clear shoulders. Distal raphe ends strongly ventrally hooked	Almost regular at middle, nearly equidistant at centre and poles.	36–45 Very small mostly circular.	Krammer, K. (1997b). Intermissio present.

Table 4.1 Comparison of the morphology of type material and other comparable *Encyonopsis* taxa. Numbers in parenthesis adjacent to stria density indicate occasional extreme values and represent averages for valve length and width. The number in parenthesis in the comments indicates the number of SEM images measured. Estimates provided for dorsal stria density precedes ventral stria density. Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. minuta</i>	8.7–16.9 (12.4)	2.9–3.8 (3.4)	4.8	(24)26– 30(32) 24– 28(32)	As above.	Variable, typically capitate–subcapitate. With size diminution occasionally subrostrate but always broadly rounded and somewhat protracted.	Parallel to weakly radiate at middle, occasionally with single shortened ventral or dorsal striae near valve centre. Uniform.	~45 Small round or occasional ly oval.	Irish populations. Intermissio present. (n=72)
<i>Encyonopsis</i> sp. 2	9.5–17.2 (13.2)	2.7–3.6 (3.2)	5.2	27–30 24–29	As above.	Protracted with narrowly rostrate ends.	Almost parallel at centre becoming weakly radiate thereafter.	40–50 Areolae round.	Irish populations. Intermissio? (n=25)
<i>E. horticola</i>	11.0– 15.0	3.0–3.5	–	27–28.5 26–27	Moderately dorsiventral, lanceolate to elliptic–lanceolate (never linear lanceolate) with clearly convex dorsal and slightly convex almost straight ventral margin.	Slightly protracted, rostrate–subrostrate. Distal raphe ends strongly ventrally hooked.	Clear shift in orientation near CA dorsally & ventrally with insertion of shortened striae.	~40 Small rounded.	Intermissio absent. Van de Vijver <i>et al.</i> (2009)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. horticola</i>	14.1– 15.8 (15.0)	2.9–3.4 (3.2)	5.1	28–30 26–28	As above.	As above.	As above.	As above but puncta density 45–50.	Irish populations. Intermissio absent. (n=13)
<i>Encyonopsis</i> sp. 3	9.9–13.5 (11.5)	2.5–3.3 (2.8)	4.5	26–30 26–30	As above.	Only very weakly rostrate or non– protracted.	Weakly radiate with equidistant or more radiate shortened striae at centre.	40–50 Rounded to transapica lly oval.	Irish populations. Intermissio absent. (n=12)
<i>E. hibernica</i>	10.1– 13.0 (11.5)	2.6–3.5 (3.1)	4.4	28–31 26–31	Symmetric–weakly dorsiventral, lanceolate with distinctly convex or weakly convex dorsal & ventral margins.	Broadly subrostrate– rostrate without shoulders. Distal raphe ends hooked ventrally with expanded sickle shaped terminal pores.	Usually distinctly radiate at middle, typically with 1–2 shortened dorsal and/or ventral striae, occasionally parallel.	~50 Round– oval & peanut shaped.	Kennedy, Buckley & Allott sp. nov. Intermissio absent. (n=45)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. perborealis</i>	11.0– 20.0	2.5–3.5	5.7	26–28	Slightly dorsiventral, linear lanceolate– elliptical with weakly convex dorsal & ventral margins.	Not to weakly protracted, broadly rounded.	Ventral striae are about the same distance as on the dorsal side.	45–53 Foramina are relatively large round and weakly transapica l.	Krammer, K. (1997b).
<i>E. vandamii</i>	10.0– 19.0	2.9–4.2	4.5	26–30	Slightly dorsiventral, lanceolate with moderately convex dorsal & ventral margins.	Non–protracted, rounded. Weakly subrostrate valves rarely encountered.	Ventral striae are about the same distance as on the dorsal side.	38–42 Transapic ally elongate foramen.	Krammer, K. (1997b).
<i>E. angusta</i>	13–25	3.4–3.6	4.5	32–35	Slightly dorsiventral, linear to linear– lanceolate with very weakly convex dorsal & ventral margin.	Relatively broad capitate, rounded. Dorsal & ventral with clear shoulders.	Only clearly visible in LM with oblique lighting.	~50	Krammer, K. (1997b).

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. alpina</i>	11.0– 20.0	3.0–3.8	5.3	28–32	Slightly dorsiventral, linear to linear– lanceolate with very weakly convex dorsal & ventral margin.	Relatively broad capitate, rounded. Dorsal & ventral of larger forms with clear or	Weakly to clearly radiate, especially at valve centre with shortened striae often observed.	38–42	Krammer, K. (1997b).
	12.7– 17.3 (15.4)	2.7–3.6 (3.2)	5.7	29– 34(35) 28– 33(34)	Smaller valves more lanceolate outline.	appearance of shoulders. Smaller forms without shoulders.		45–55 Usually round to oval or less occasional ly slightly peanut shaped.	Type material re-examined for this study. Intermissio absent. (n=25)
<i>E. alpina</i>	12.4– 17.2 (15.7)	2.8–3.5 (3.2)	5.9	29–35.5 28–35.5	Not or only very slightly dorsiventral – otherwise as above	As above.	As above.	As above.	Irish populations. Intermissio absent. (n=74)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. tavirana</i>	10.0– 18.0	3.4–4.0	4.5	28–30	Slightly dorsiventral, linear to linear– lanceolate with weakly convex dorsal &	Relatively broad capitate, rounded. Dorsal & ventral of larger forms	Dorsally & ventrally equally spaced, sometimes with shortened stria at middle.	38–42	Krammer, K. (1997b)
	13.4– 18.9 (17.4)	3.6–4.2 (3.9)	5.1	29–32 26–30	ventral margin. Smaller valves more lanceolate outline.	with clear or appearance of shoulders. Smaller forms without shoulders.		45–55 Small round– oval to distinctly peanut shaped.	Type material re–examined for this study. Intermissio absent. (n=43)
<i>E. aff. tavirana</i>	9.5–14.4 (12.8)	3.3–3.9 (3.6)	4.3	27–32 26–32	Not or only slightly dorsiventral with linear or slightly convex margins. More clearly convex in smaller valves.	Distinctly broadly capitate with well- developed shoulders.	Typically having only slightly shortened striae at valve centre. More clearly radiate at central area.	As above.	Irish populations. Intermissio absent. (n=32)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>Encyonopsis</i> sp. 1	10.4– 15.8 (13.5)	2.9–3.8 (3.4)	5.3	27–32 (24)26– 32	Not to slightly dorsiventral with slight to weakly convex margins.	Broad capitate to subcapitate, without or with weakly developed shoulders.	Often distinctly radiate at valve centre, with shortened stria often inserted on both sides at or near central area.	Often slightly peanut shaped, but also oval.	Irish populations. Intermissio absent. (n=55)
<i>E. krammeri</i>	11.5– 23.5	2.6–3.8	7.0	(27)28– 30(32)	Slightly dorsiventral, lanceolate, weakly convex dorsal & ventral margin.	Narrowly offset, rounded, without shoulders, occasionally weakly ventrally directed.	Transapical striae weakly radiate and approximately equal dorsally and ventrally.	35–42 Transapic ally extended.	Krammer, K. (1997b).
<i>E. krammeri</i>	9.6–18 (15.6)	2.8–3.3 (3.0)	6.1	32–36 32–35	As above.	As above but also with capitate ends.	As above.	45–50	Population occurring in <i>E.</i> <i>alpina</i> type material. (n=17)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. krammeri</i>	9.9–22.9 (16.3)	2.8–3.6 (3.2)	7.0	30– 35(36) 28– 33(34)	Lanceolate and clearly dorsiventral with convex dorsal and ventral margin.	Generally rostrate, but capitate or less frequently with rounded non-protracted ends, without shoulders.	Weakly radiate and usually distinctly denser towards the poles. Uniform length or occasionally with single shortened stria at centre.	40–50 Transapically extended.	Irish populations. Intermissio absent. (n=50)
<i>E. subminuta</i>	10–25 (16)*	3.4–4.5	5.6	23–26	Symmetric to very weakly dorsiventral, lanceolate with weakly convex dorsal & ventral margin.	Narrow capitate, rounded, dorsal & ventral with or without weak shoulders.	Dorsally & ventrally equally spaced, almost equally spaced at middle and poles.	36–45 Very small mostly circular.	Krammer, K. (1997b).
<i>E. subminuta</i>	14.2– 18.6 (16.6)	3.0–4.1 (3.5)	5.8	25–29 24–28	Mostly symmetric, lanceolate outline with weakly to clearly convex margin.	Predominately clearly capitate to subcapitate or rarely rostrate.	Parallel in appearance at middle, becoming weakly radiate away from valve centre.	45–50 Clearly rounded small pores, or form with peanut shaped puncta.	Irish populations. Intermissio absent. (n=52)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. neerlandica</i>	15–26	3.5–4.5	6.3	25–28 24–26 30 near apices	Weakly (smaller valves) to moderately (larger) dorsiventral, narrow lanceolate (never linear) with convex dorsal and slightly convex ventral margin.	Protracted, acutely rounded, rostrate to subcapitate.	Weakly radiate to moderately radiate near the apices, almost equally spaced throughout.	40–45 Small rounded to transapica lly elongate and slit- like.	Van de Vijver <i>et al.</i> (2012)
<i>E. neerlandica</i>	13.6– 31.6 (19.9)	3.0–4.4 (3.6)	8.3	26–30 25– 29(30)	as above.	As above but also with capitate valves.	As above.	40–45 Round to slightly peanut shaped.	Irish populations. Intermissio absent. (n=50)
<i>E. anacondae</i>	16.6– 24.4	3.2–4.0	–	25–28	Narrowly linear to linear–lanceolate, slightly dorsiventral. Dorsal and ventral margins weakly convex.	Rostrate, deflected weakly to the ventral side.	Weakly radiate.	No SEM.	Bahls (2013).

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

Taxon	Length (µm)	Width (µm)	L:W (max)	Striae (10 µm)	Outline	Poles	Striae	Puncta (10 µm)	Comments
<i>E. krammerioides</i>	19.0– 30.0	3.7–4.3	7.0	22–25	Weakly dorsiventral, narrowly lanceolate with weakly convex margins.	Weakly protracted to subrostrate.	Moderately radial overall.	35–40 Just visible in LM – no SEM data.	Rumrich <i>et al.</i> (2000).
<i>E. delicatissima</i>	11.0– 23.0	2.5–3.0	7.7	29–32 (Hustedt ~35)	More dorsiventral, lanceolate with weakly convex dorsal & ventral margin.	Weakly subrostrate, rounded without shoulders, sometimes weakly ventrally deflected.	Characteristically divergent at valve middle.	–	Krammer, K. (1997b).
<i>E. carraensis</i>	17.2– 22.6 (20)	2.8– 3.5(3.9 +) (3.1)	7.3	26–30 25–29	Weakly dorsiventral, narrow lanceolate with very weakly convex margins, or appearing almost linear ventrally.	Clearly protracted, subcapitate to capitate.	Weakly radiate.	40–50 External areolae openings with diverse shape.	Kennedy, Buckley & Allott sp. nov. Intermissio absent. (n=50)

Table 4.1 (Continued) Symbols as follows (* mode; ! type specimens; # mostly; + initial cell).

The range of values for morphometric variables is compared with boxplots (Figure 4.530) and presented with the species descriptions. A Principal component analysis (PCA) biplot confirms the large overlap within the aggregate, but groups of species based on variation in valve length, width, length/width ratio and stria density are apparent nevertheless (Figure 4.531). Descriptive features of the frustule, including sometimes subtle variation in valve characteristics (e.g. presence or absence of shoulders, extent of dorsiventrality, end shape and stria orientation at central area) enable individual valves in these overlapping groups to be clearly separated in most cases.

Except for a diatom resembling *E. tavirana*, *Encyonopsis* is generally not found in lakes with TP > 30 µg/l-P and is rare below pH 6.5. These findings validate their environmental niche for alkaliphilous and oligosaprobous waters, but with a more oligotraphentic than meso-eutraphentic trophic indicator value compared with Van Dam *et al.* 1994. Differing optima are observed in scatter plots for some individual taxa (Figure 4.532, Table 4.2). Lakes with mixed populations of *Encyonopsis* are associated with several environmental variables and alkalinity and TP gradients are the most important in NMDS ordination plots (Figure 4.533).

4.4.1. TAXONOMIC RESULTS

Diatom morphology

Division bacillariophyta

Class bacillariophyceae Haeckel [emend. Medlin & Kaczmarska 2004]

Subclass bacillariophycidae D.G. Mann in Round *et al.* 1990: 651

Order Cymbellales D.G. Mann in Round *et al.* 1990: 653

Family Cymbellaceae Greville 1833: 263, 409

Genus *Encyonopsis* Krammer 1997a: 11

Encyonopsis carraensis Kennedy, Buckley & Allott sp. nov. (Figures 4.3–4.27)

Type: IRELAND. Co. Mayo: Lough Carra, 53.69138267 N, 9.22379978 W, elev. 18.0 m, coll. B. Kennedy, coll. date 12 August 2014. (Holotype: specimen here designated Finder: Q42 (The England Finder™ Graticule) TCD0018335, illustrated in Figure 4.11; isotype, slide Zu11–19, Alfred Wegener Institute).

LM (Figures 4.3–4.20): Cells solitary. Valves weakly lanceolate to linear-lanceolate and only weakly dorsiventral, with slightly convex margins, occasionally straightened ventrally. Apices are clearly protracted, subcapitate to capitate and rounded, with no indication of shoulders. Valve dimensions (n=51): length 17.2–22.6 µm, width 2.8–3.5 µm, valve L: W ratio 5.5–7.3. Sternum narrow, linear. Central area non-existent or hardly discernible due to only slightly shortened dorsal striae. Raphe filiform, slightly ventrally then dorsally curved with inconspicuous proximal and comma shaped distal raphe fissures. Striae lineate, uniseriate, weakly radiate and almost uniformly spaced throughout the valve length, occasionally slightly coarser and denser at valve centre and apices respectively, ranging from 26–30 dorsally and 25–29 ventrally in 10 µm. Areolae not discernible with LM.

SEM (Figures 4.21–4.27): Striae consist of 5–6 areolae, 40–50 in 10 μm (Figure 4.21).

Very diverse shape and orientation of external areolae openings; predominately apically orientated and slit like, but transapical, rotated, comma, Y-shaped and X-shaped puncta were observed on the same valve and quite variable between populations. Raphe slightly curved to the ventral side, shifting towards dorsal side at valve middle, with slightly enlarged central endings and inconspicuous central pores. Distal raphe fissures deflected ventrally over broadly rounded poles, ending in small pores externally (Figure 4.21). Internal striae with undulating margins. Raised silica struts between oval shaped apically orientated or rotated areolae. Proximal raphe endings not visible below a prominently raised central nodule. Distal raphe endings terminate as well developed helictoglossae.

Etymology: The specific epithet *carraensis* refers to Lough Carra, Ireland's largest marl lake where the species is abundant.

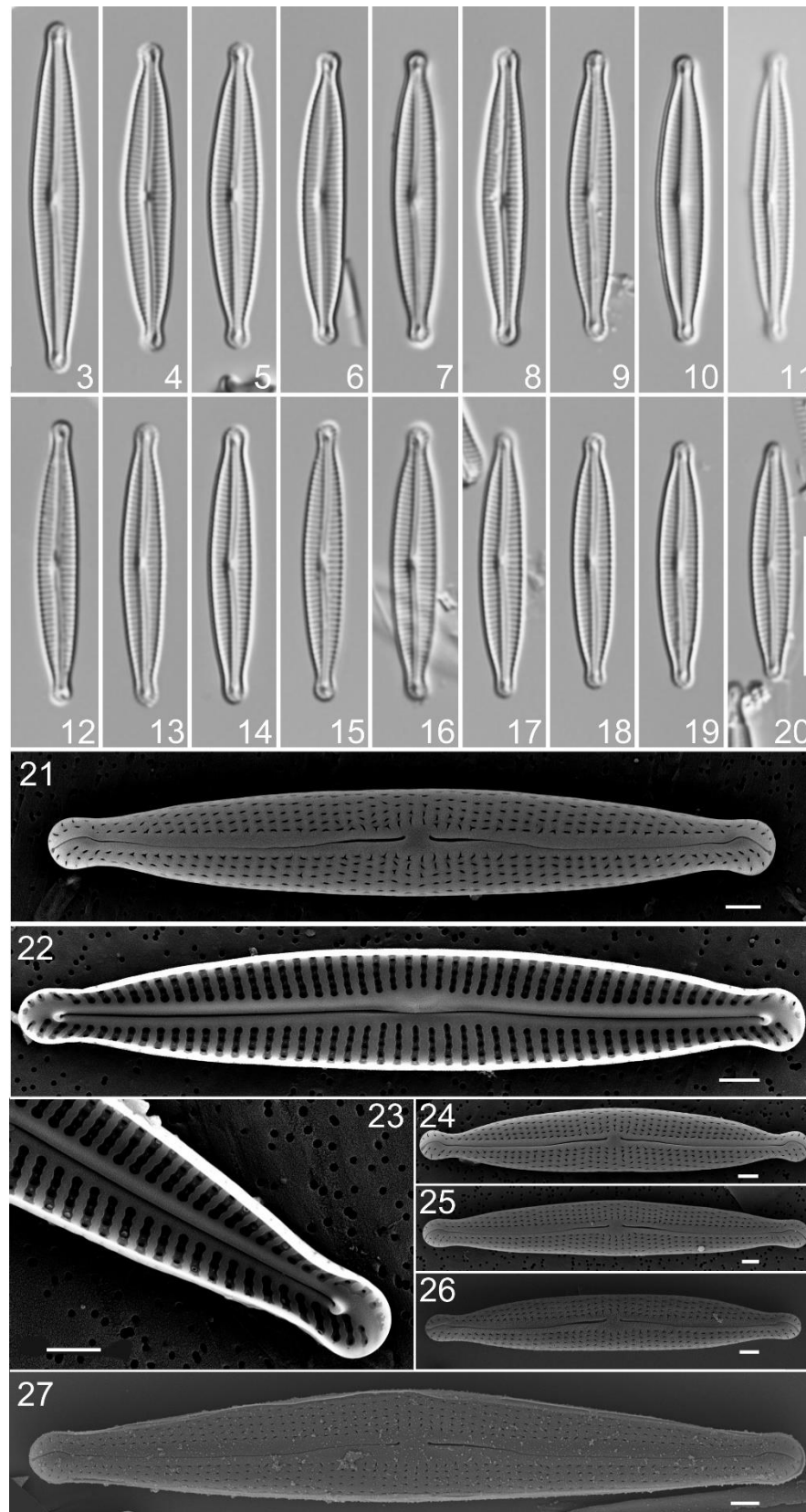
Similar species: The morphological characteristics including high L:W ratio, linear valve outline, distinctly protracted apices and striae density enable differentiation from similar taxa using LM alone (Figures 4.3–4.20). Larger valves of *E. carraensis* are comparable to the narrowest valves of *E. neerlandica*. When both co-occur, the more broadly lanceolate outline and rostrate ends of the latter enables their clear separation. The proximal raphe ends internally are not visible in *E. carraensis* and differing shape of external areolae openings are also discriminating ultrastructural characteristics. The lower L:W ratio, broader lanceolate outline and more obviously capitate apices typically allows a clear separation of *E. subminuta*. There was difficulty differentiating isolated valves of some less clearly protracted and narrow frustules of *E. subminuta* in LM, but this form was very rare.

Taxon	No. lakes	Max. %	pH	Alkalinity (mg/L CaCO ₃)	Conductivity (µS-cm)	True Colour (Hazen)	Total Phosphorus (mg/L-P)	Total Ammonia (mg/L-N)	Total Oxidised Nitrogen (mg/L-N)	Si - Silica (mg/L SiO ₂)
<i>Encyonopsis</i> (ENCYall)	73	83	5.1-8.9 (7.9)	2.5-215 (72)	33-479 (227)	2.5-177 (25)	0.004-0.07 (0.01)	0.005-0.01 (0.018)	0.03-1.33 (0.2)	0.1-6.6 (1.4)
<i>E. alpina</i> (EALP)	21	30	6.8-8.4 (8.2)	8.0-215 (112)	132-479 (262)	2.5-177 (17)	0.005-0.027 (0.008)	0.01-0.06 (0.021)	0.04-0.91 (0.2)	0.3-2.8 (1.3)
<i>E. carrensis</i> (ECAR)	12	39	7.8-8.4 (8.3)	67.0-215 (119)	164-179 (270)	2.5-35 (11)	0.005-0.021 (0.007)	0.01-0.045 (0.018)	0.06-0.91 (0.2)	0.3-2.6 (1.4)
<i>E. hibernica</i> (EHIB)	19	26	7.2-8.5 (7.9)	11-174 (76)	69-351 (214)	12.2-175 (39)	0.007-0.05 (0.016)	0.01-0.1 (0.017)	0.03-1.33 (0.2)	0.6-6.3 (1.8)
<i>E. krammeri</i> (EKRA)	31	43	6.6-8.9 (8.2)	4.0-215 (105)	52-478 (281)	2.5-175 (16)	0.005-0.05 (0.008)	0.01-0.06 (0.021)	0.04-1.33 (0.2)	0.2-6.6 (1.3)
<i>E. minuta</i> (EMIN)	40	17	6.8-8.9 (8.1)	8.0-215 (99)	103-479 (253)	4.2-177 (24)	0.005-0.05 (0.009)	0.01-0.1 (0.018)	0.04-1.33 (0.2)	0.3-6.6 (1.4)
<i>E. neerlandica</i> (ENER)	26	54	5.9-8.9 (7.1)	3.6-109 (10)	41-335 (111)	10.9-177 (51)	0.005-0.027 (0.011)	0.01-0.035 (0.014)	0.1-0.5 (0.1)	0.1-3.1 (0.7)

Table 4.2 The number of lake occurrences, maximum percentage abundance in diatom samples, range and weighted average optima (in parentheses) for physiochemical variable for all common *Encyonopsis* taxa in the study.

Taxon	No. lakes	Max. %	pH	Alkalinity (mg/L CaCO ₃)	Conductivity (µS-cm)	True Colour (Hazen)	Total Phosphorus (mg/L-P)	Total Ammonia (mg/L-N)	Total Oxidised Nitrogen (mg/L-N)	Si - Silica (mg/L SiO ₂)
<i>E. subminuta</i> (ESUB)	30	25	6.8-8.9 (8.2)	4.0-215 (115)	103-479 (276)	2.5-176 (16)	0.005-0.025 (0.008)	0.01-0.1 (0.019)	0.04-0.91 (0.2)	0.3-3.6 (1.4)
<i>E. aff. tavorana</i> (ETAV-IE)	13	47	7.5-8.9 (8.1)	34.0-209 (151)	103-424 (318)	17.6-176 (42)	0.005-0.07 (0.032)	0.005-0.1 (0.036)	0.1-1.05 (0.3)	0.3-3.1 (1.8)
<i>Encyonopsis</i> sp. 1 (ESP1)	43	71	5.1-8.9 (7.9)	2.5-209 (65)	36-424 (208)	5.6-177 (32)	0.005-0.05 (0.009)	0.005-0.05 (0.016)	0.03-1.05 (0.2)	0.2-3.6 (1.5)
<i>Encyonopsis</i> sp. 2 (ESP2)	25	6.3	6.9-8.9 (8.2)	21.5-215 (119)	103-479 (274)	2.5-176 (15)	0.005-0.05 (0.009)	0.01-0.05 (0.021)	0.05-0.9 (0.2)	0.3-3.6 (1.5)
<i>Encyonopsis</i> (ENCYunid)	30	2.9	6.2-8.9 (7.9)	4.0-215 (78)	33-479 (229)	5.8-100 (24)	0.004-0.03 (0.008)	0.01-0.1 (0.02)	0.09-0.91 (0.2)	0.2-3.6 (1.6)

Table 4.2. (Continued) The number of lake occurrences, maximum percentage abundance in diatom samples, range and weighted average optima (in parentheses) for physiochemical variable for all common *Encyonopsis* taxa in the study.



Figures 4.3–4.27 *Encyonopsis carraensis* sp. nov. Figs 3–20. LM. Fig. 11. Holotype specimen. Figs 3–20. Valves showing morphological range and size variation in populations. Figs 21–27. SEM. Fig. 21. External view. Figs 22–23. Internal view. Figs 24–27. Variation in valve outline. Scale bar in Fig. 20 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .

Ultrastructurally, both taxa differed in puncta shape. Valve length of *E. carraensis* has a similar range to *E. krammeri*, but a combination of moderate dorsi-ventral outline and more distinctly arched dorsal margin enables a clear differentiation. Some populations of *E. krammeri* with distinctly capitate poles can be distinguished by clear dorsiventral outline and higher stria density. Puncta shape, orientation and form of the distal raphe fissures also differ significantly in SEM. The L:W ratio and outline of *E. krammerioides* Lange-Bertalot & Rumrich in Rumrich *et al.* (2000: 117) are comparable, but narrower width, higher stria density and clearly protracted ends of *E. carraensis* are differentiating. Conspecificity with *E. anaconda* Bahls (2013) can also be ruled out based on rostrate ends and broader lanceolate valve outline equivalent to *E. neerlandica* of this taxon, but type material is unavailable for comparison of ultrastructural differences (L. Bahls *pers comm.*).

Distribution & Ecology: *Encyonopsis carraensis* was found in 12 lakes reaching a maximum relative abundance of 39% in lower Lough Corrib, Co. Galway. It occurs in oligotrophic calcareous lakes (Figure 4.28) where the upper littoral is dominated by a marl precipitating community of *Schizothrix* Kützing ex M.Gomont (1892: 2002) and *Dichothrix* G.Zanardini ex É.Bornet & C.Flahault (1886: 373).



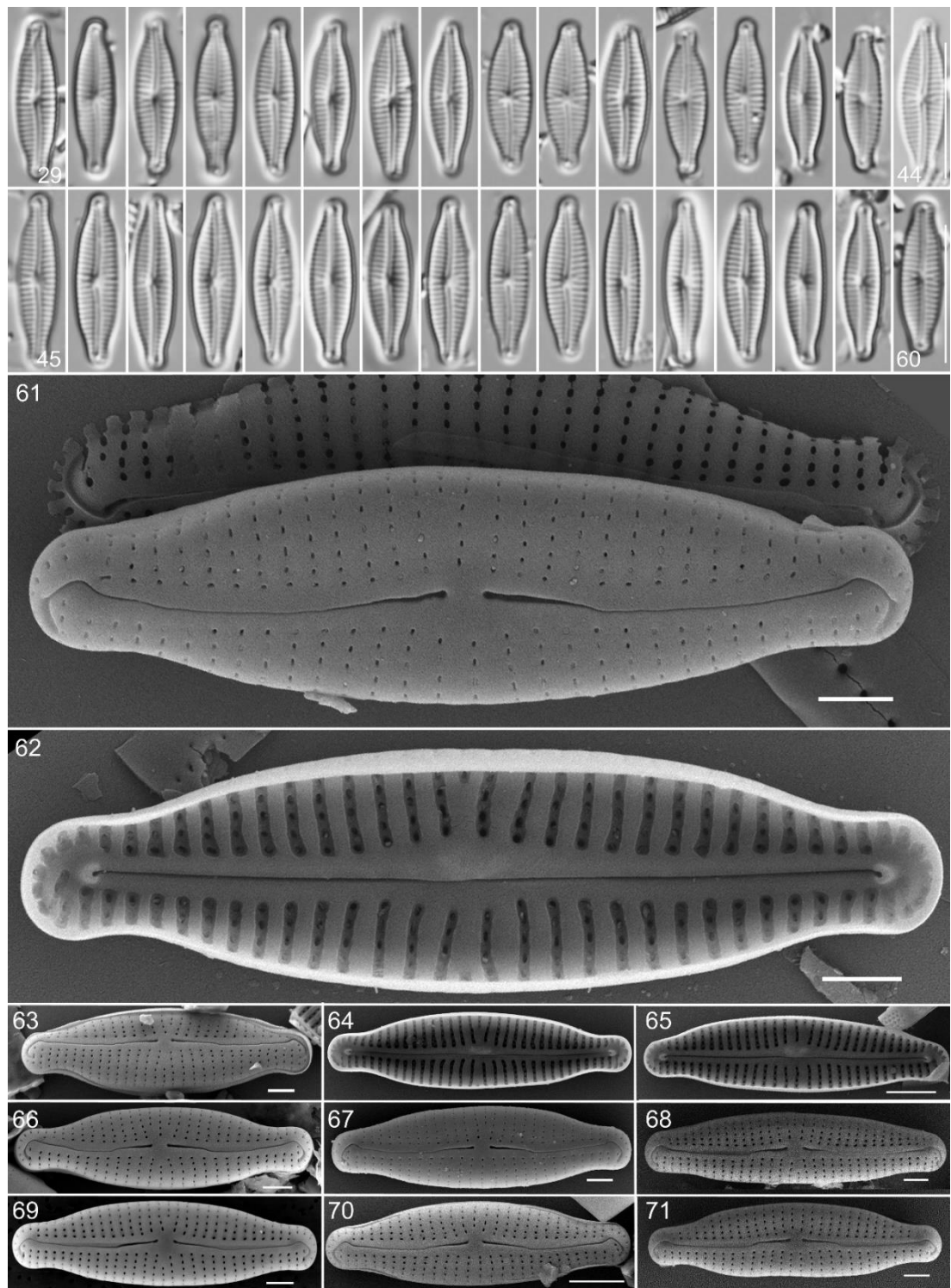
Figure 4.28 Lough Carra, Co. Mayo. Shoreline at Pleasure Island (a) the type locality of *Encyonopsis carraensis* with an extensive deposit of submerged (b) and exposed (c) marl precipitating cyanobacteria over boulders.

Encyonopsis hibernica Kennedy, Buckley & Allott sp. nov. (Figures 4.29–4.71)

Type: IRELAND. Co. Clare: Lough Inchicronan, 52.92222687N, 8.9026442W, elev. 26.0 m, coll. Dr. Ruth Little, coll. date 16 April 2012. (Holotype: specimen here designated Finder: N47 (The England Finder™ Graticule) TCD0018336, illustrated in Figure 4.44; isotype, slide Zu11–20, Alfred Wegener Institute).

LM (Figures 4.29–4.60): Cells solitary. Valves lanceolate and weakly dorsiventral, with convex margins and without clear shoulders. Apices clearly protracted, with broadly rounded subcapitate poles. Valve dimensions (n=45): length 10.1– 13.0 µm, width 2.6– 3.5 µm, valve L:W ratio 3.3–4.4. Sternum narrow, linear. Central area small to hardly discernible due to only slightly shortened dorsal or ventral striae. Raphe filiform, ventrally then dorsally curved with inconspicuous proximal and strongly deflected distal raphe fissures. Striae lineate, uniseriate, weakly radiate throughout the valve length or sometimes parallel, more commonly with shortened, radiate or deflected stria at central area, ranging from 28–31 dorsally and 26–31 ventrally in 10 µm. Areolae not discernible with LM.

SEM (Figures 4.61–4.71): Stria consist of 5–7 areolae, 46–60 in 10 μm (Figure 4.61). External and internal areolae openings are round, oval or slightly peanut shaped. Raphe slightly curved to the ventral side, shifting towards dorsal side at valve middle, with slightly enlarged central endings and inconspicuous central pores. Distal raphe endings strongly deflected ventrally over broadly rounded poles, terminating in expanded fissures externally (Figure 4.61). Internal striae with straightened margins. Proximal raphe endings not visible below a prominently raised central nodule. Distal raphe endings terminate as well developed helictoglossae.



Figures 4.29–4.71 *Encyonopsis hibernica* sp. nov. Figs 29–60. LM. Fig. 44. Holotype specimen. Figs 29–60. Valves showing morphological range and size variation in several populations. Figs 61–71. SEM. Fig. 61. External view. Fig. 62. Internal view. Figs 63–71. Variation in valve outline. Scale bar in Fig. 60 = 10 μm for all LM figures except holotype specimen. Scale bar in all SEM micrographs = 1 μm and = 2 μm in Figs 65–70.

Etymology: The specific epithet *hibernica* refers to Ireland where the species is found.

Similar species: The morphological characteristics including valve dimension, broadly rounded subcapitate apices and stria orientation at the central area allow differentiation from similar taxa using LM alone (Figures 4.29–4.60). *E. hibernica* has a comparable outline to less clearly protracted, smaller valves of *E. minuta*. Although stria density overlap, the latter typically has coarser parallel stria, only occasionally having a single shortened or offset stria at the central area. Absence of an intermissio, differing shaped terminal fissures and puncta are also discriminating ultrastructural characteristics. The valve dimensions and striation pattern at the central area of *E. hibernica* are often comparable to *E. horticola*. However, valve apices in the latter are rostrate to subrostrate and never capitate to subcapitate. Striae may be weakly radiate to sub-parallel near the central area in *E. hibernica* but always have a clear change in direction in *E. horticola*. The shape of their terminal raphe fissure also differs. The linear-lanceolate outline and lower stria density of *E. bobmarshallensis* are clearly differentiating, while *E. perborealis* Krammer (1997: 109) also has a more linear outline, stria density in the lower range of *E. hibernica*, less distinctly or non-protracted poles, weakly radiate striae and different shaped puncta.

Distribution & Ecology: *Encyonopsis hibernica* is found in samples from 19 lakes reaching a maximum relative abundance of 26% in Lough Inchicronan, Co. Clare. It favours mesotrophic lakes with elevated nutrients and substrate at the type location has macroscopic colonies of *Chaetophora* F.Schrank (1783: 19) with lesser *Dichothrix* G.Zanardini ex É.Bornet & C.Flahault with low levels of calcification.

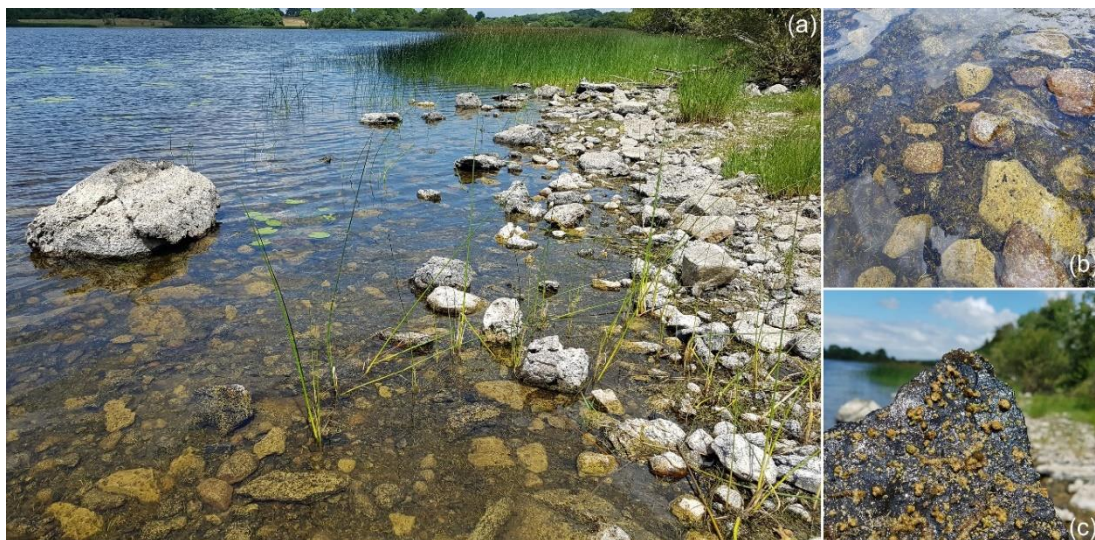
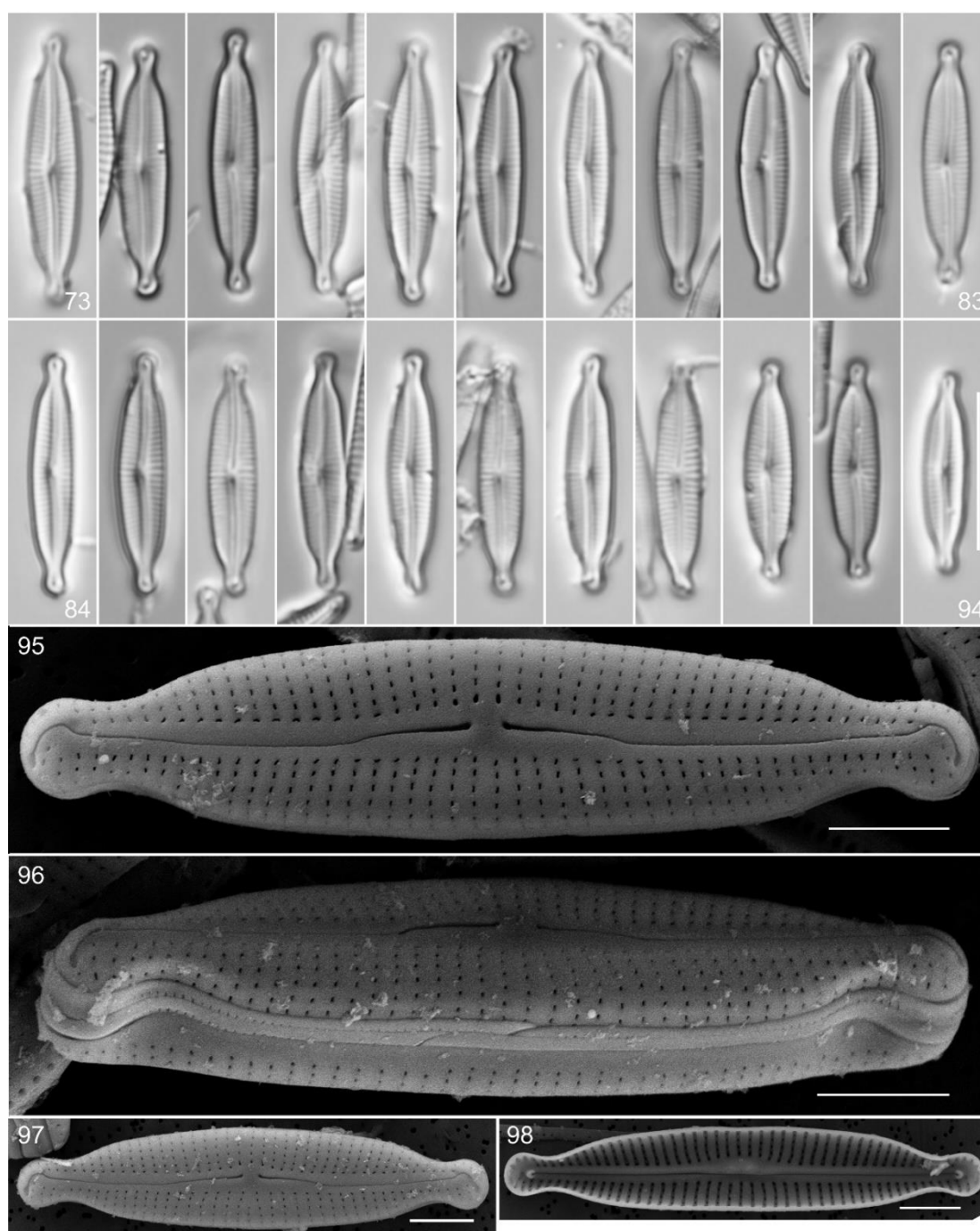


Figure 4.72 Lough Inchicronan, Co. Clare (a) the type locality of *Encyonopsis hibernica* showing the shallow littoral (b) and closeup of epilithic habitat (c) with associated macroscopic colonies of *Chaetophora* F.Schrank and *Dichothrix* G.Zanardini ex É.Bornet & C.Flahault, also with some minor calcification.

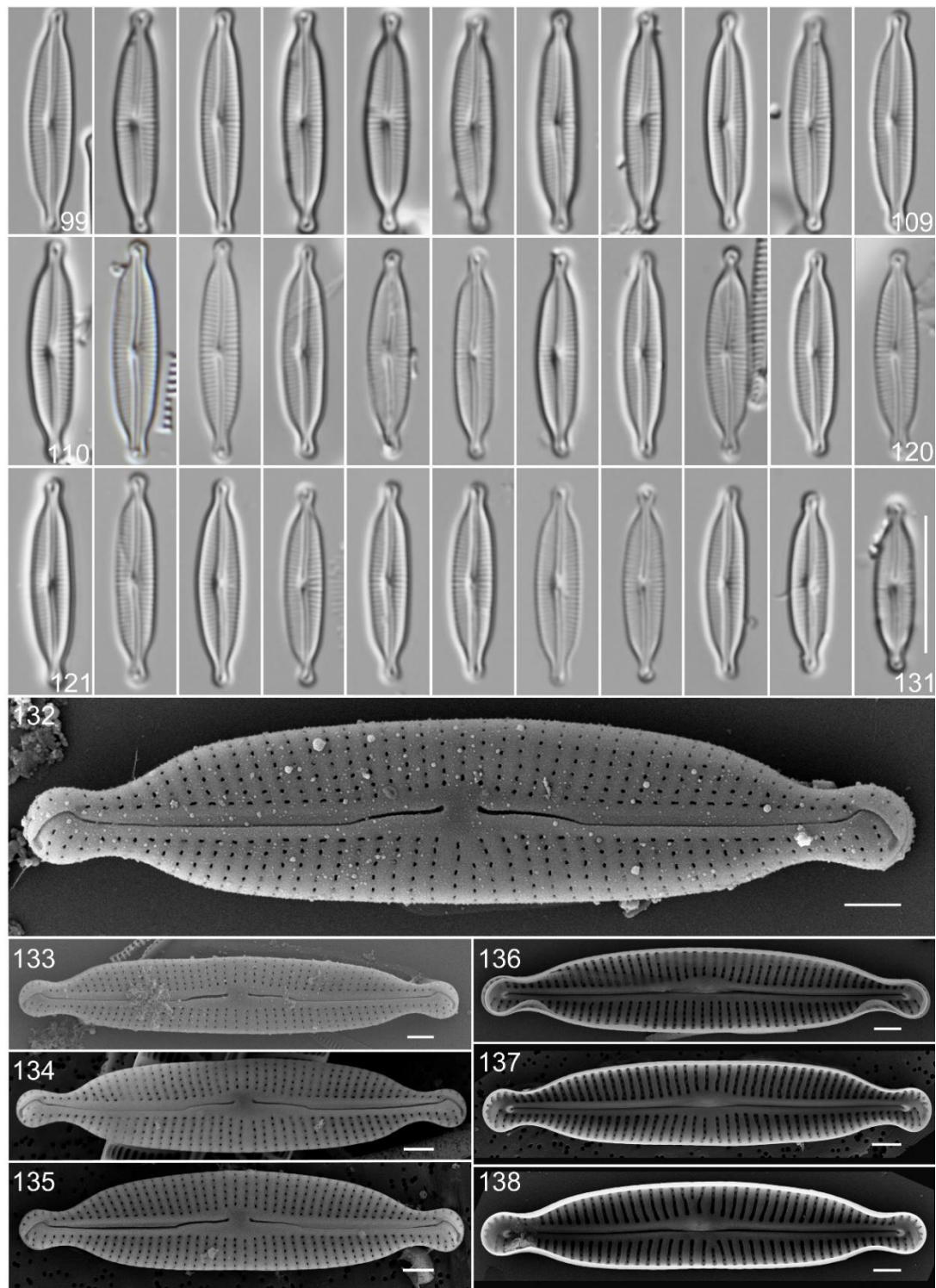
Encyonopsis alpina Krammer & Lange-Bertalot (Figures 4.73–4.98 and Figures 4.99–4.138)

Description: Valves not or only slightly dorsiventral with weakly convex dorsal and weakly convex to straight ventral margin. Clear shoulders may be visible or absent. Length 11–20 μm ; width 2.7–3.8 μm ; maximum L:W ratio 5.9. Apices clearly protracted and capitate. Axial area narrow and central area only slightly differentiated dorsally. Striae weakly to clearly radiate, only occasionally with a somewhat shortened stria at valve centre. Striae uniform or occasionally appearing somewhat more coarsely spaced at valve centre (Figures 4.73 & 4.95). Average stria density only slightly higher dorsally (32.5 in 10 μm) than ventrally (31 in 10 μm). Raphe filiform, curved to the dorsal side, terminating in inconspicuous pores proximally and having small round to oval shaped pores distally. Puncta small rounded to slightly peanut shaped. Irish populations are morphologically equivalent to the type population, with the most

significant difference from the species diagnosis relating to a higher range in stria (max 35.5 in 10 μm) and areolae density reported here.



Figures 4.73–4.98 *Encyonopsis alpina* Krammer & Lange-Bertalot Typus: Prap. 5b IOK. Individuals from type material Typus: Prap. 5b IOK. Figs 73–94. LM. Figs 73–94. Valves showing morphological variability and variation in size in type population. Figs 95–98. SEM. Fig. 95. External view. Fig. 96. External view of tilted frustule illustrating the arrangement of the intact cingulum, composed of two bands, with copulae tapered near the middle and having a single visible row of pores. Figs 98. Internal view. Scale bar in Fig. 94 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .



Figures 4.99–4.138 *Encyonopsis alpina* Krammer & Lange-Bertalot. Individuals from Ireland. Figs 99–131. LM. Figs 99–131. Valves showing morphological and variability in size for populations from Irish lakes. Figs 132–138. SEM. Figs 132–135. External view. Figs 136–138. Internal view. Scale bar in Fig. 131 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .

Ecology: Occurring in some alpine oligotrophic lakes with high oxygen concentration.

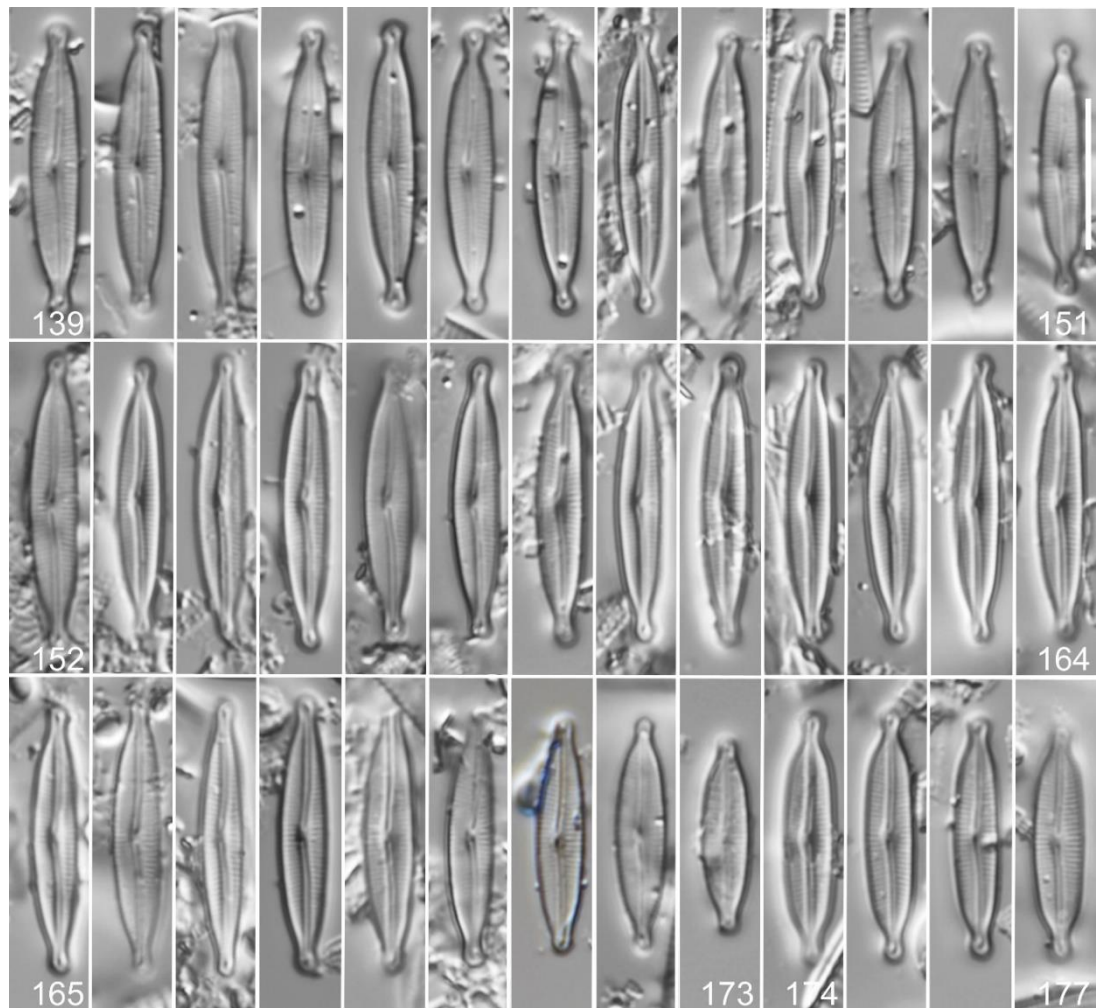
E. alpina is a cosmopolitan taxon and occurs in well buffered, moderate to high alkalinity lakes (n=21) reaching a maximum relative abundance of 30%. A total phosphorus optimum close to 10 µg/l-P suggests a sensitivity score S=1 for lake ecological assessment (Figure 4.532).

Encyonopsis angusta Krammer & Lange-Bertalot (Figures 4.139–4.151)

Description: In the diagnosis valves were described as weakly dorsiventral, linear to linear elliptical in outline with very weakly convex dorsal and ventral sides. Poles relatively broadly rounded capitate, with clear shoulders. Axial area is narrow and central area absent. Length 13–25 µm; width 3.4–3.6 µm; maximum L:W ratio 4.5, but when remeasured from original plates was determined to be ≥ 5.0 . The combination of characters enabling identification were proposed as; outline with distinct shoulders, relatively broad capitate ends, very elongate, having approximately 33 delicate transapical striae in 10 µm.

Several *Encyonopsis* taxa are present on the type slide and valves illustrated in Figures 4.139–4.151 most closely follow the illustrations of *E. angusta* provided by Krammer (1997b). The selected valves have a lower minimum valve width (2.9 µm) and greater maximum L:W ratio (6.2) but are otherwise comparable to the diagnosis. Another diatom more with an affinity to *E. krammeri* is well represented on the slide (Figures 4.152–4.173). These valves are clearly dorsiventral and have slightly less protracted, narrower rostrate to subcapitate poles. However, they probably represent a continuation of the same diatom described above (see plates of *E. krammeri* below and discussion). *E. alpina* (Figures 4.174–4.177) was also found on the slide, while *E. subminuta* and *E. minuta* are less frequent and are not illustrated.

Ecology: Reported to be frequent in suitable oligotrophic alpine and pre-alpine lake habitats in waters with high oxygen concentration. Not found with certainty in Ireland, although valves with a similar morphology are occasionally encountered but are considered to represent a capitate form of *E. krammeri*.



Figures 4.139–4.177 *Encyonopsis* Typus: Prap. 77A IOK. Figs 139–177. LM. Figs 139–151. *E. angusta* Krammer & Lange-Bertalot. Figs 152–173. *Encyonopsis krammeri* Reichardt. Figs 174–177. *E. alpina* Krammer & Lange-Bertalot. Scale bar in Fig. 151 = 10 µm for all LM figures.

Encyonopsis tavirana Krammer (Figures 4.178–4.200)

Description: Valves weakly dorsiventral, linear to linear-lanceolate in outline with very weakly convex dorsal and ventral sides, smaller forms are more lanceolate. Poles are relatively broadly rounded capitate, dorsally and ventrally with clear shoulders or indication of shoulders in larger valves, smaller forms without shoulders. Axial area very narrow and central area absent. Length 10–18.9 μm ; width 3.4–4.2 μm ; maximum L:W ratio 5.1. Average stria density slightly higher dorsally (30.5 in 10 μm) than ventrally (29 in 10 μm) but have a higher range than given with the diagnosis when re-measured (29–32 in 10 μm dorsally and 26–30 in 10 μm ventrally). Raphe filiform, curved more ventrally with proximal ends bent to dorsal side. Areolae are more distinctly bent or peanut-shaped relative to comparable taxa. A combination of characters can be used to separate the more robust valves of *E. tavirana* from others including, a linear lanceolate outline with robust valves having very well-developed shoulders, a greater maximum valve width, clearly capitate broadly rounded poles, and approximately 30 transapical striae or less in 10 μm .

Populations of a diatom closely resembling *E. tavirana* from Irish lakes are comparable in morphology, including broadly capitate valves and poles, distinct shoulders and relatively fine striae (Figures 4.201–4.245). But average valve width and maximum L: W ratio (4.3 versus 5.1) are lower relative to the type population. This species is likely to have been frequently confused with *E. microcephala* in the past, given their similar robust outline and equivalent valve width. However, ultrastructural characteristics clearly differ from the type population of *E. microcephala* illustrated in Krammer 1997b. Distal raphe endings are more gently deflected over the apices and terminate

in a small rounded pore in the latter taxon. Internally the clearly constricted alveoli and lower stria density (23–25 in 10 μm) are also differentiating.

Ecology: Originally found in the Portuguese Algarve and abundant in wells, mountainous regions and oligotrophic waters with high oxygen content. Irish populations with an affinity to *E. tavorana* occur in 13 lakes with a maximum relative abundance of 47%. It occurs in more eutrophic lakes and has a higher nutrient optimum relative to the type population or compared to others in the *microcephala*-complex, suggesting a diatom sensitivity score $S=3$ for lake ecological assessment (Figures 4.532–4.533).

***Encyonopsis* sp. 1** (Figures 4.246–4.287)

Description: Valves weakly dorsiventral, linear to linear-lanceolate in outline with weakly convex to sublinear dorsal and ventral sides, smaller forms are more lanceolate. Poles are rounded capitate to subcapitate, with or without clear shoulders. Axial area very narrow and central area absent. Length 10.4–15.8 μm ; width 2.9–3.8 μm ; maximum L:W ratio 5.3. Average stria density only slightly higher dorsally (30 in 10 μm) than ventrally (29 in 10 μm) with shortened or bent striae often found at valve centre. Raphe filiform, curved more ventrally with proximal ends bent to dorsal side. Areolae are small oval to peanut shaped.

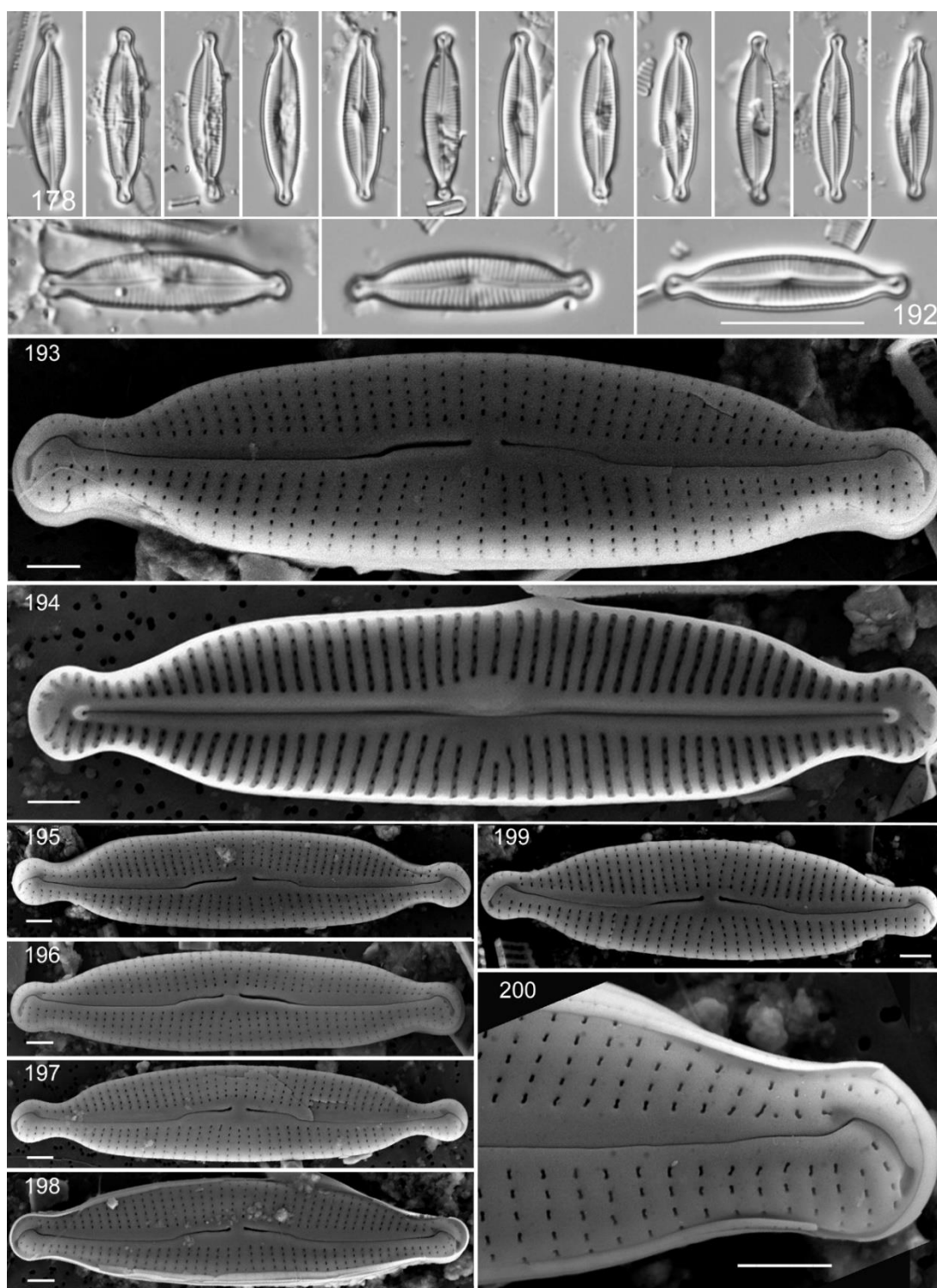
This form has an equivalent ultrastructure to *E. aff. tavorana* with a slightly expanded terminal fissure, comparable shaped puncta and dorsal stria density. It lacks the very robust outline and distinctive shoulders and has a higher maximum L:W ratio ($L/W=5.3$ versus 4.3). These features usually allow for a clear separation from that species in LM. Average valve width in the type population of *E. tavorana* is higher than this form, but

maximum L:W ratio is equivalent. This diatom also closely resembles *E. alpina* in LM but slightly coarser, angled orientation of stria at central area and a lower average L:W ratio is usually sufficient to separate most populations of these diatoms.

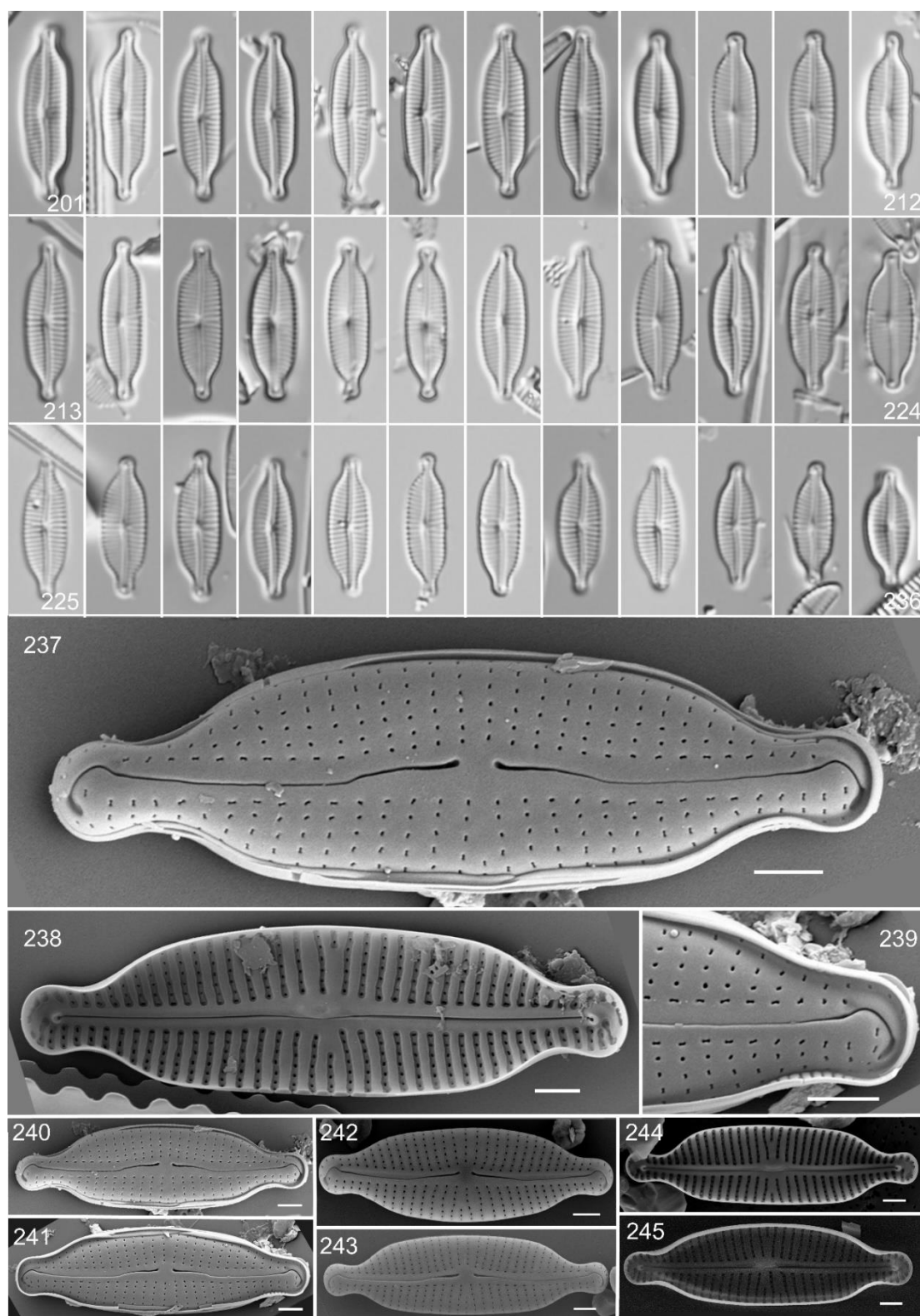
Ecology: This form is most likely to have been misidentified as *E. alpina* in the past. Individual frustules are occasionally difficult to differentiate and both frequently co-occur. It is a widespread diatom found in 43 lakes and reaches a high relative abundance in epilithic habitat (max.= 71%). It has a relatively broad tolerance across the alkalinity gradient but a low optimum for TP, which is equivalent to populations of *E. tavirana* and *E. alpina* but significantly different ($p < 0.01$) from *E. aff. tavirana*.

Encyonopsis minuta Krammer & Reichardt (Figures 4.288–4.352)

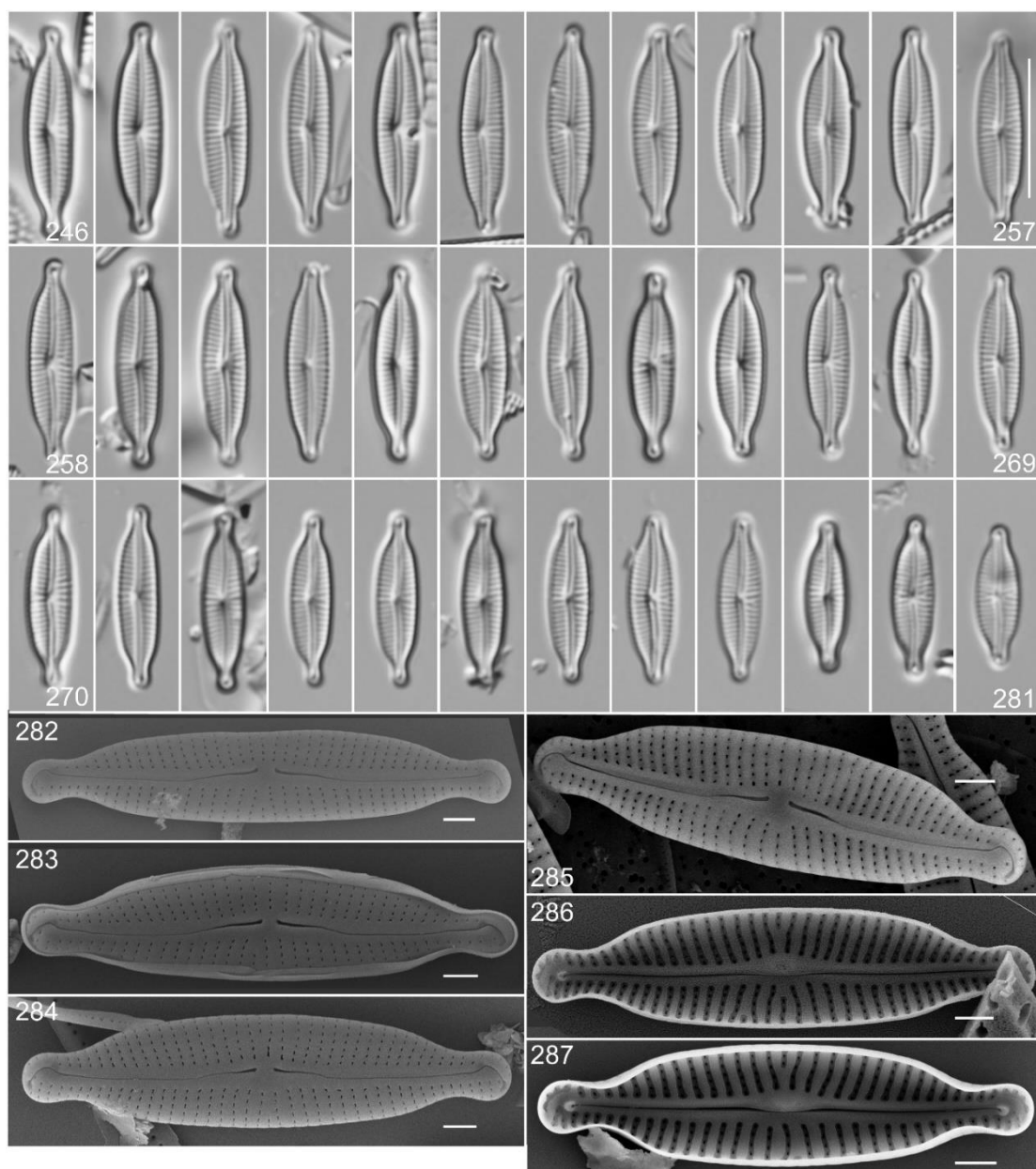
Description: Outline symmetric to weakly dorsiventral, lanceolate with weakly convex dorsal and ventral sides. Poles are capitate to subcapitate, always broadly rounded and at least slightly protracted even in diminutive valves. Typically lacking clear shoulders with a gently sloping outline. Axial area is very narrow and central area absent. Length 8.0– 17.0 μm ; width 2.8–3.8 μm ; maximum L:W ratio 4.9. Striae parallel to weakly radiate and rarely with only a slightly shortened single stria at centre in smaller valves. Striae appearing quite uniform and easily visible over valve length towards poles. Maximum stria density in Irish populations higher than reported for type description (~30–32 in 10 μm). Raphe filiform, dorsally deflected at centre, with a distinctive short section recurved back towards centre on ventral side and lacking obvious expanded terminal raphe endings. Areolae always small and rounded.



Figures 4.178–4.200 *Encyonopsis tavorana* Krammer Typus: Prap. 517B IOK. Figs 178–192. LM. Figs 178–192. Valves showing range of morphological variability and variation in type population. Figs 193–200. SEM. Fig. 193. External view. Fig. 194. Internal view. Figs 195–199. SEM showing variation in outline and apices in type population. Fig. 200. Close up of valve at pole showing form of distal raphe ending and shape of areolae. Scale bar in Fig. 189 = 10 μm for LM figs 178–189 and in Fig. 192 = 10 μm for LM figs 190–192. Scale bar in all SEM micrographs = 1 μm .



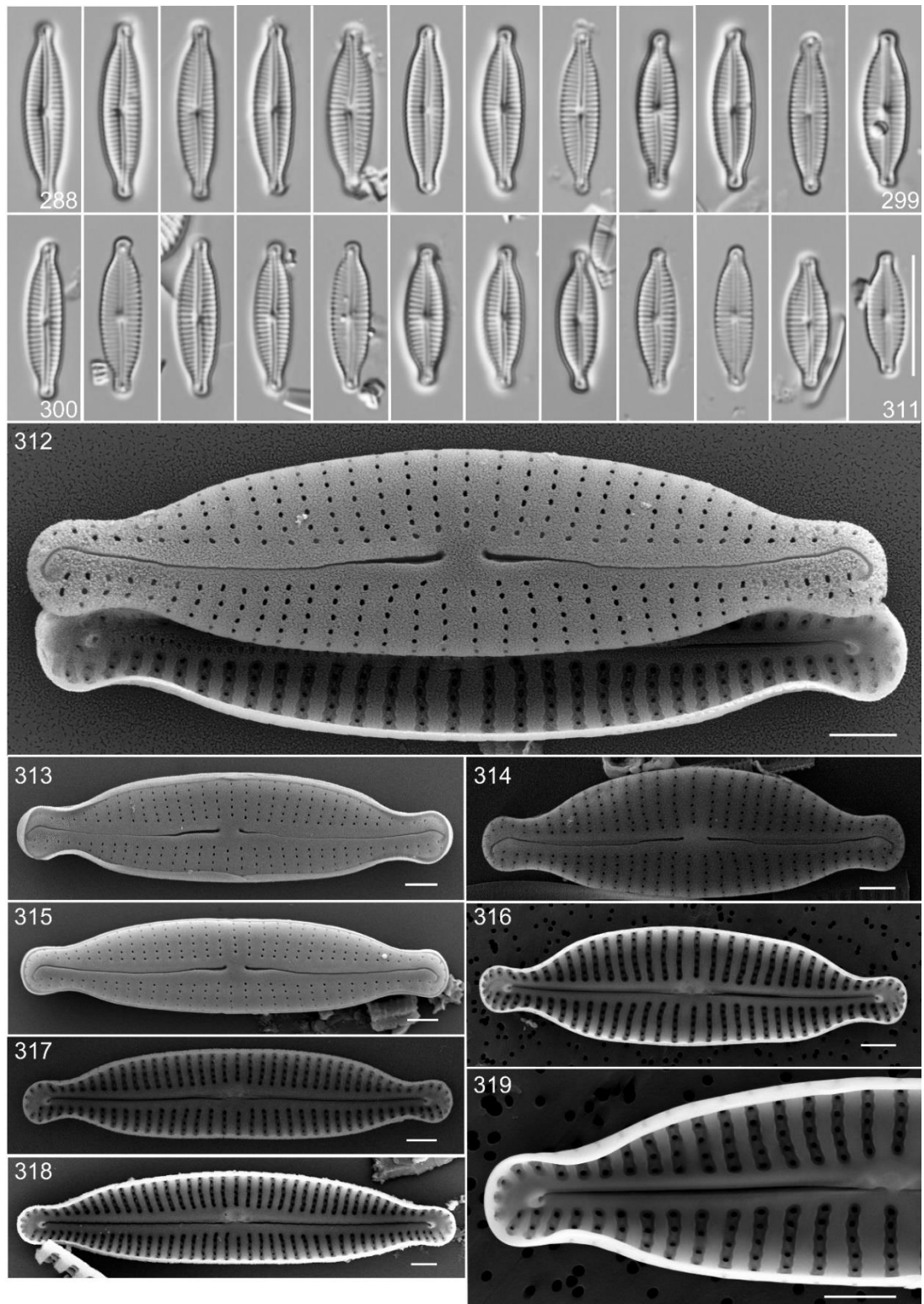
Figures 4.201–4.245 *Encyonopsis* aff. *tavrana*. Figs 201–236. LM. Figs 201–236. Valves showing morphological variability for a size diminution series. Figs 237–245. SEM. Figs 237 & 239–243. External view. Figs 238 & 244–245. Internal view. Scale bar in Fig. 236 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.



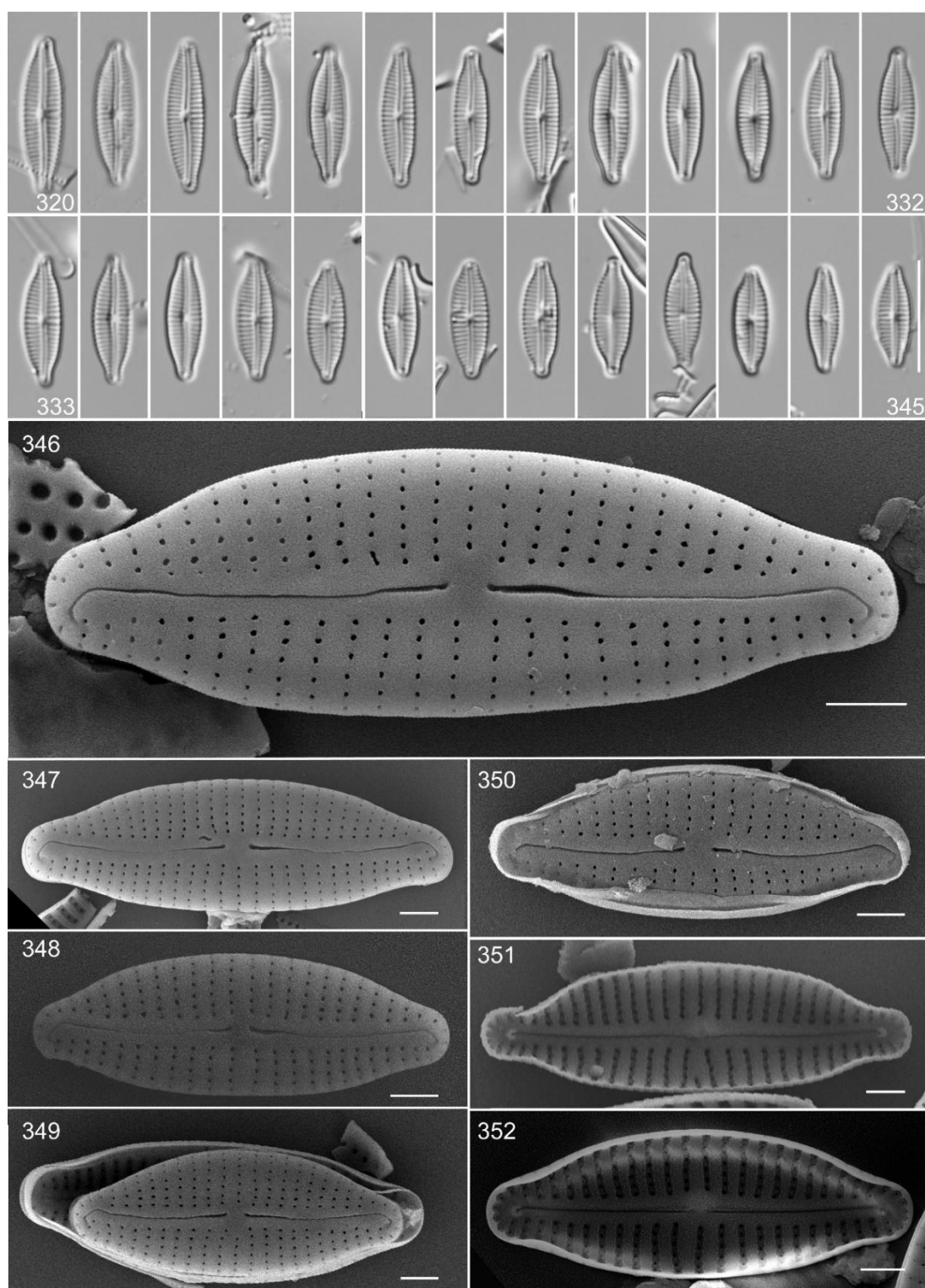
Figures 4.246–4.287 *Encyonopsis* sp. 1 Figs 246–287. LM. Figs 246–281. Valves showing morphological range and size variation in populations. Figs 282–287. SEM. Figs 282–285. External view. Figs 286–287. Internal view. Scale bar in Fig. 257 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.

Easily confused with *E. microcephala* in past studies but populations converge and may be difficult to separate in LM. The size reduction series (Figures 4.320–4.352) are less clearly protracted and have a higher maximum stria density but retain the distinctive ultrastructure of more clearly capitate frustules. The general size, outline and broadly rounded poles in combination with relatively coarse sub-parallel striae are often sufficiently clear to permit identification in most cases.

Ecology: Described as cosmopolitan and well distributed in suitable habitats in temperate to boreal regions. Well distributed in this dataset occurring in 40 lakes, reaching a maximum relative abundance of 16.5%. Density is greatest at the upper end of the alkalinity gradient and a total phosphorus optimum near 10 µg/l-P suggests a diatom sensitivity score of S=1 for lake ecological assessment.



Figures 4.288–4.319 *Encyonopsis minuta* Krammer & Reichardt Figs 288–319. LM. Figs 288–311. Valves showing morphological range and size variation in populations with clearly capitate apices. Figs 312–319. SEM. Figs 312–315. External view. Figs 316–318. Internal view. Fig. 319. Internal view of raphe fissure with clear intermissio visible. Scale bar in Fig. 311 = 10 µm for all LM figures. Scale bar in all SEM micrographs = 1 µm.



Figures 4.320–4.352 *Encyonopsis minuta* Krammer & Reichardt Figs 320–345. LM. Figs 320–345. Illustrating morphology of populations with a size reduction series. Figs 346–352. SEM. Figs 346–350. External view with valves maintaining bluntly rounded poles. Figs 351–352. Internal view showing distinctive intermissio at central area. Scale bar in Fig. 345 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .

***Encyonopsis* sp. 2** (Figures 4.353–4.396)

Description: Outline symmetric to weakly dorsiventral, lanceolate with weakly convex dorsal and ventral sides. Poles are narrow rostrate. Vales with gently sloping outline lacking shoulders. Axial area is very narrow and central area absent. Length 9.5–17.2 μm ; width 2.7–3.6 μm ; maximum L:W ratio 5.2. Striae parallel at valve centre becoming weakly radiate and visible over the length of the valve. Stria density 27–30 dorsally and 24–29 ventrally in 10 μm . Raphe filiform, dorsally deflected at centre, with a distinctive short section recurved back towards centre on ventral side and lacking obvious expanded terminal raphe endings. Areolae always small and round.

This form closely resembles *E. minuta* with smaller valves converging in morphology and occasionally were not possible to differentiate in LM and SEM. Larger valves are more linear lanceolate in outline and have narrow rostrate ends compared to the broadly rounded apices of *E. minuta*. Valves lack a very clear separation of proximal raphe endings that are always more clearly visible internally in the latter. All remaining characters are equivalent and its relationship with *E. minuta* requires further investigation. Ultrastructurally, it has an affinity with *E. eifelana* Krammer (1997: 102) but lacks the more obviously truncate ends and lower striae density of this taxon.

Ecology: Found in 25 lakes but usually at low densities (<1–3%) with a maximum relative abundance of 6.3% in L. Lene, a calcareous lake in the midlands. Its ecology is equivalent to *E. minuta* and both diatoms co-occur.

Encyonopsis horticola Van de Vijver & Compère (Figures 4.397–4.404)

Description: Valves are moderately dorsiventral, lanceolate to elliptic-lanceolate but never linear lanceolate. Clearly convex dorsal margin and slightly convex ventral margin. Apices are slightly protracted, broadly rounded, rostrate to subrostrate but

never capitate to subcapitate. Valve dimension almost equivalent to type population from a limited number of valves examined. Length 11–15.8 μm ; width 2.9–3.5 μm ; maximum L:W ratio 5.1. Axial area is very narrow and central area almost absent. Striae are clearly radiate with a clear shift in orientation and with shortened dorsal and ventral stria at valve centre. Stria density 27–30 dorsally and 26–28 ventrally in 10 μm . All other characteristics equivalent to that given with type diagnosis. A slight gap in internal proximal raphe endings illustrated in Van de Vijver & Compère (Fig. 43) is concealed under a silica flap in Irish population.

Ecology: Few documented records for this taxon exist in natural waterbodies in Europe since it was first described from a small pool in the national botanic garden of Belgium. A single population at low relative abundance (3.3%) occurs in L. Gur, Co. Limerick. This lake is mesotrophic to eutrophic (mean TP=0.03 mg/l-P; max. chlorophylla=24 $\mu\text{g/l}$) and at poor ecological status when last classified for the WFD.

***Encyonopsis* sp. 3** (Figures 4.405–4.416)

Description: Valves are moderately dorsiventral, lanceolate to elliptic-lanceolate. Clearly convex dorsal margin and slightly convex ventral margin. Apices are rounded nonprotracted or appearing only very slightly protracted with careful focusing. Length 9.9–13.5 μm ; width 2.5–3.3 μm ; maximum L:W ratio 4.5. Striae (26–30 in 10 μm) are weakly radiate and usually shortened both dorsally and ventrally at central area. Raphe filiform, clearly deflected dorsally at proximal and distal ends. Proximal endings slightly dilated and terminating in an inconspicuous pore. Distal raphe fissures are ventrally curved then acutely hooked towards ventral margin. Puncta are oval shaped and slightly larger in diameter towards axial area. This form may represent a size

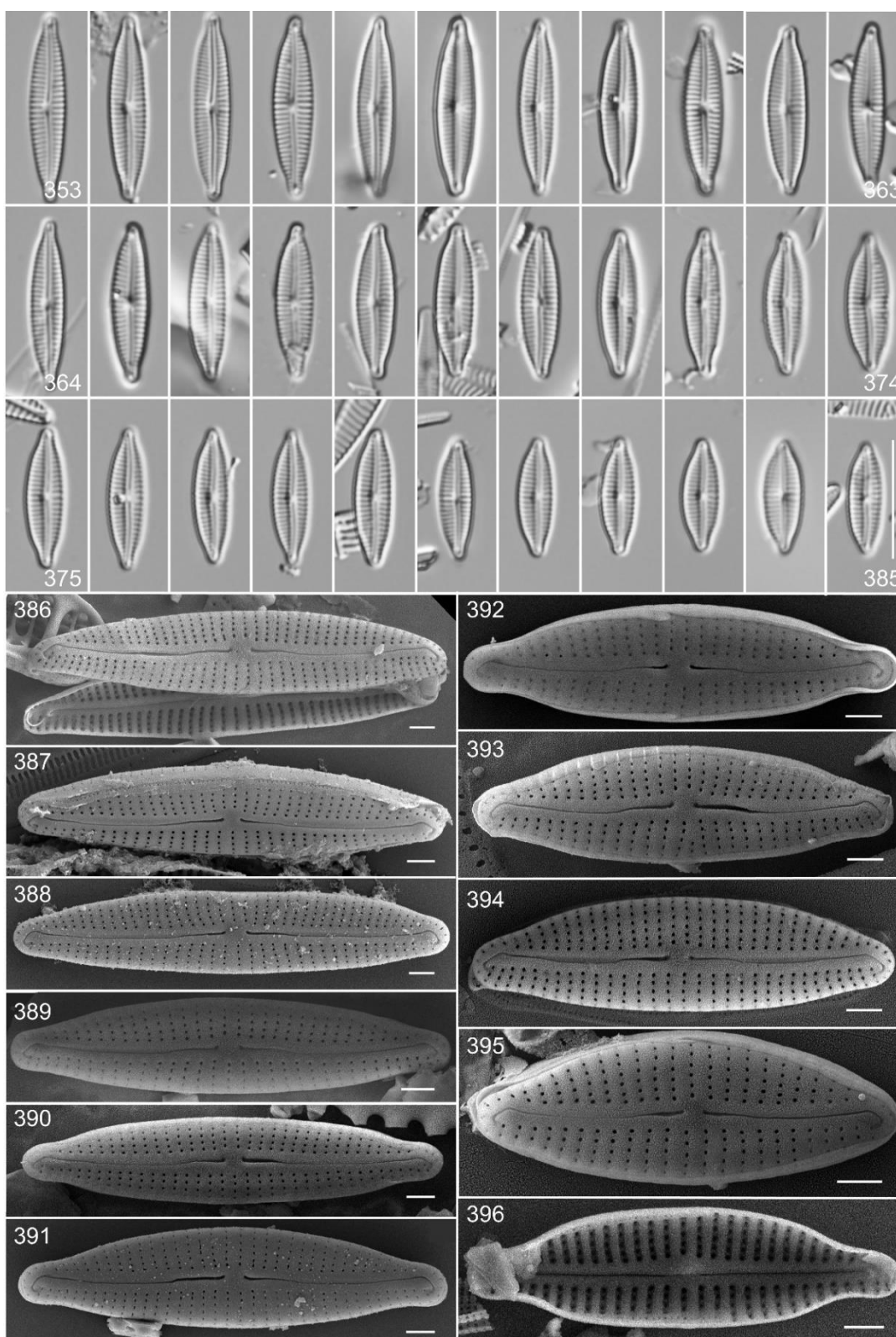
reduction of *E. horticola* but also has an affinity with *E. perborealis* and *E. vandamii*, although its morphology does not fit any of these taxa exactly.

Ecology: A rare form occurring in only 4 lakes and always at low relative abundance (1–6.5%). Environmental data from these lakes suggest an optimum at mesotrophic conditions.

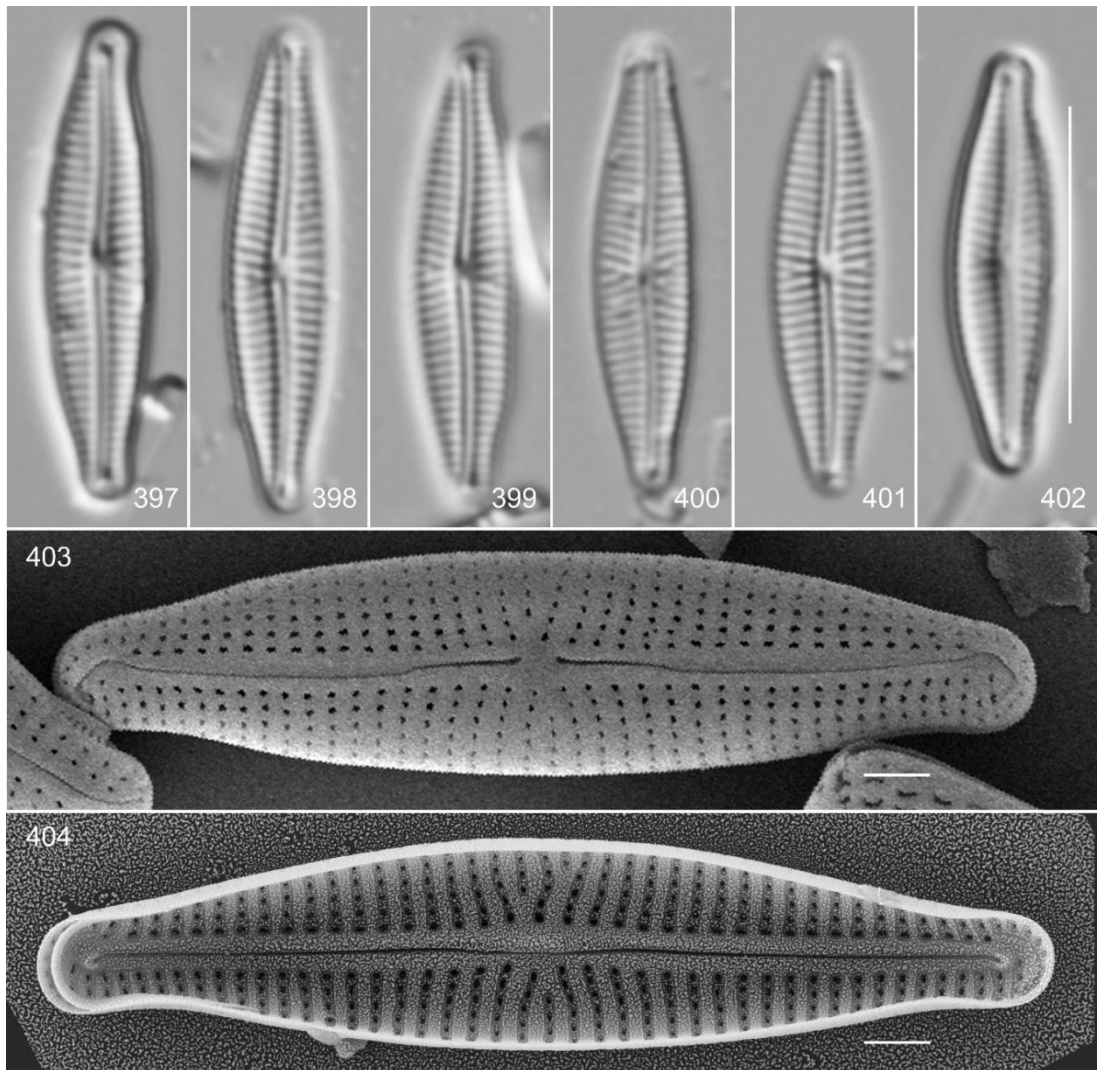
Encyonopsis krammeri Reichardt (Figures 4.417–4.480)

Description: Two different forms with differing apices are documented here. Valves are moderately dorsiventral and narrow-lanceolate in outline in the most widespread form. Dorsal margin is more clearly curved relative to the weakly convex ventral side, without shoulders. Poles are rostrate and may be weakly deflected ventrally, but variable in populations. Axial area very narrow and central area absent to weakly developed. Length 9.6–23.5 μm ; width 2.6–3.8 μm ; maximum L:W ratio 7.0. A more clearly capitate form with a near lanceolate outline (Figures 4.461–4.472) was found with type material of *E. alpina* and also occurs in calcareous lakes in Ireland. It shares the same ultrastructural combination of features as *E. krammeri*, including identical raphe design, a characteristic thin plate-like silica thickening visible internally between the helictoglossa and apices (Figure 4.442; 4.479–4.480) and transapically elongate linear shaped puncta. Striae density is higher (32–36 in 10 μm) in these populations than originally published for *E. krammeri*.

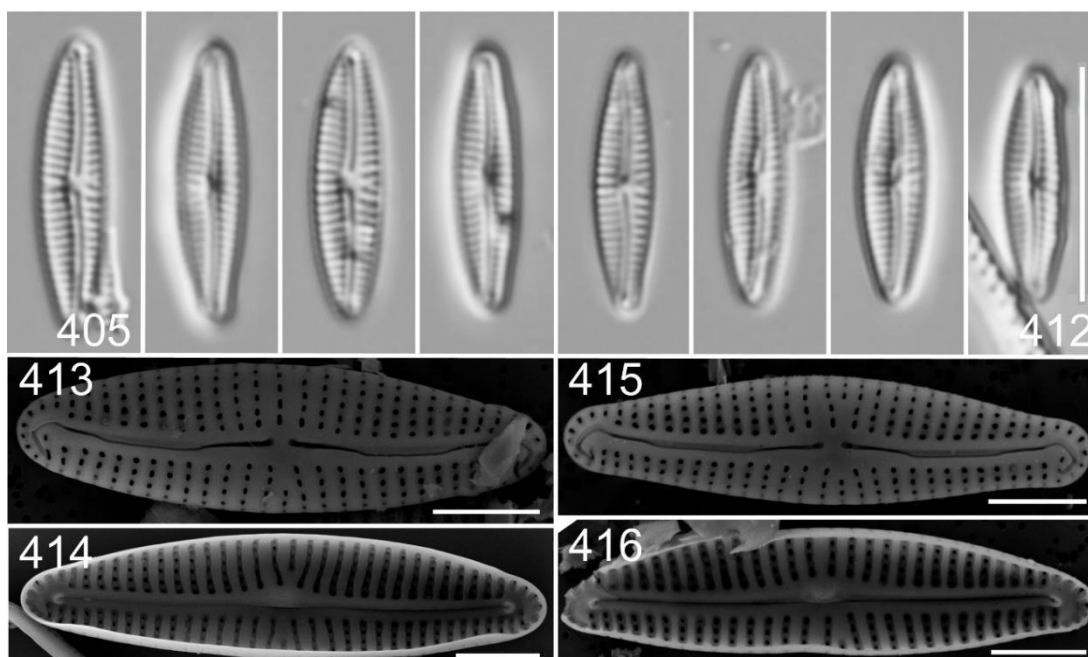
Ecology: A cosmopolitan and widespread diatom found in 31 lakes and reaching a maximum relative abundance of 43%. It occurs at higher densities in calcareous lakes in oligotrophic to mesotrophic conditions (Figure 4.532).



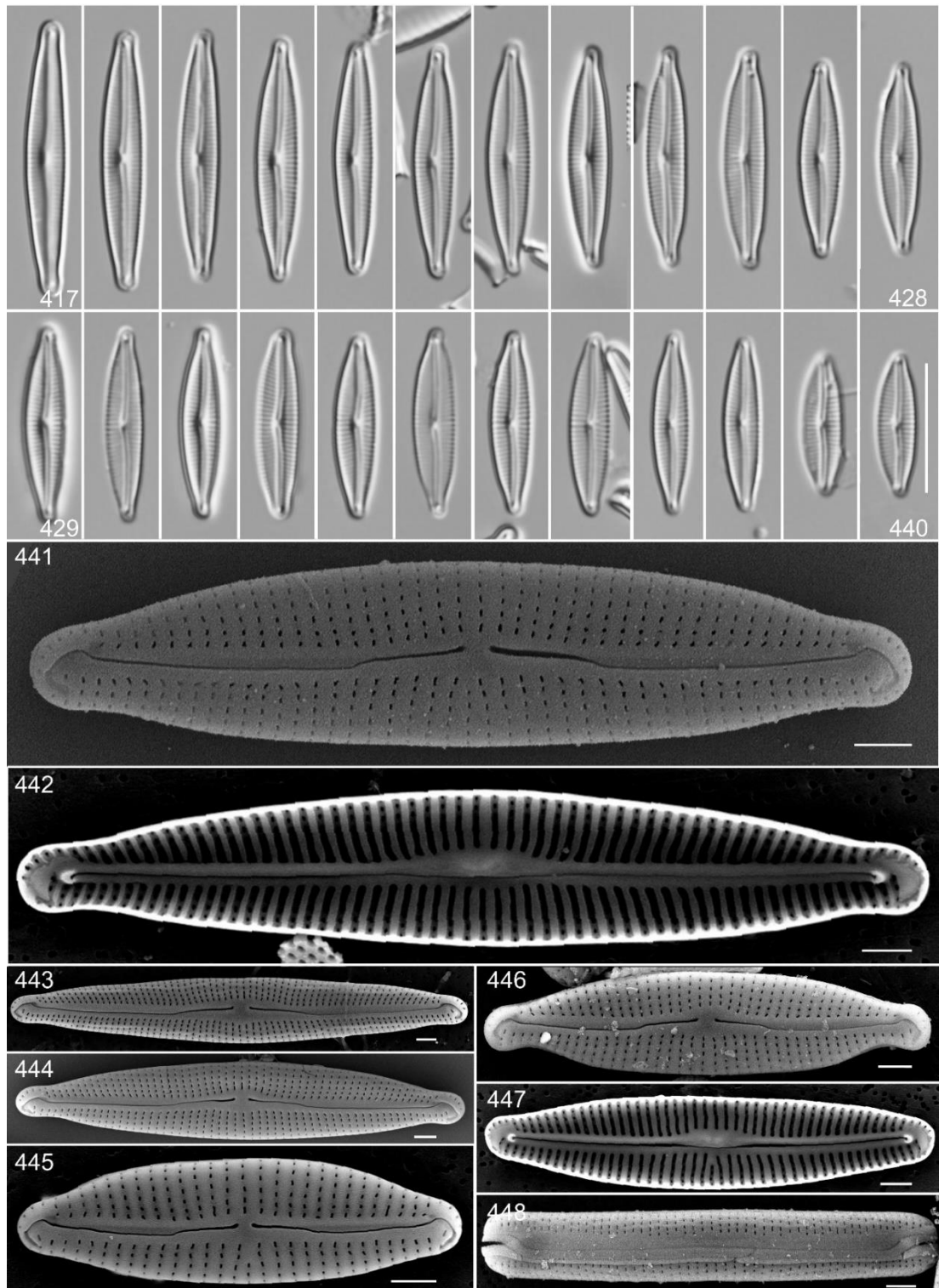
Figures 4.353–4.396 *Encyonopsis* sp. 2 aff. *minuta* Figs 353–385. LM. Figs 353–385. Valves showing morphological range and size reduction in populations. Figs 386–396. SEM. Figs 386–395. External valve view with narrow rostrate apices. Fig 396. Internal view lacking a clear intermission at central area. Scale bar in Fig. 385 = 10 μ m for all LM figures. Scale bar in all SEM micrographs = 1 μ m.



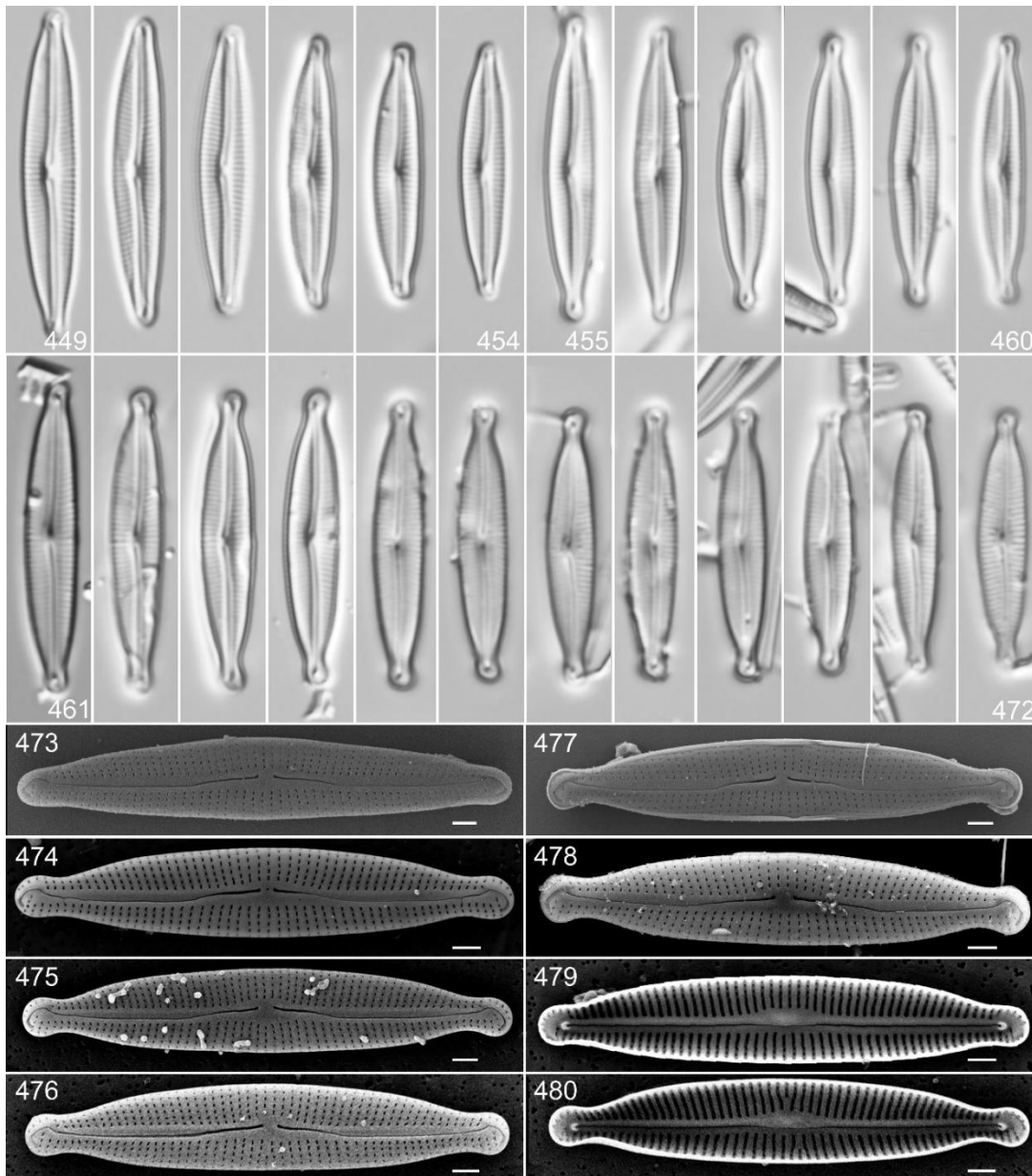
Figures 4.397–4.404 *Encyonopsis horticola* Van de Vijver & Compère Figs 397–402. LM. Figs 397–402. Valves showing morphological range of a single population in L. Gur, Co. Limerick. Figs 403–404. SEM. Fig 403. External view. Fig. 404. Internal view. Scale bar in Fig. 402 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .



Figures 4.405–4.416 *Encyonopsis* sp. 3 Figs 405–412. LM. Figs 405–412. Valves showing morphological range and size variation. Figs 413–416. SEM. Figs 413 & 415. External view. Figs 414 & 416. Internal view. Scale bar in Fig. 412 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 2 μm .

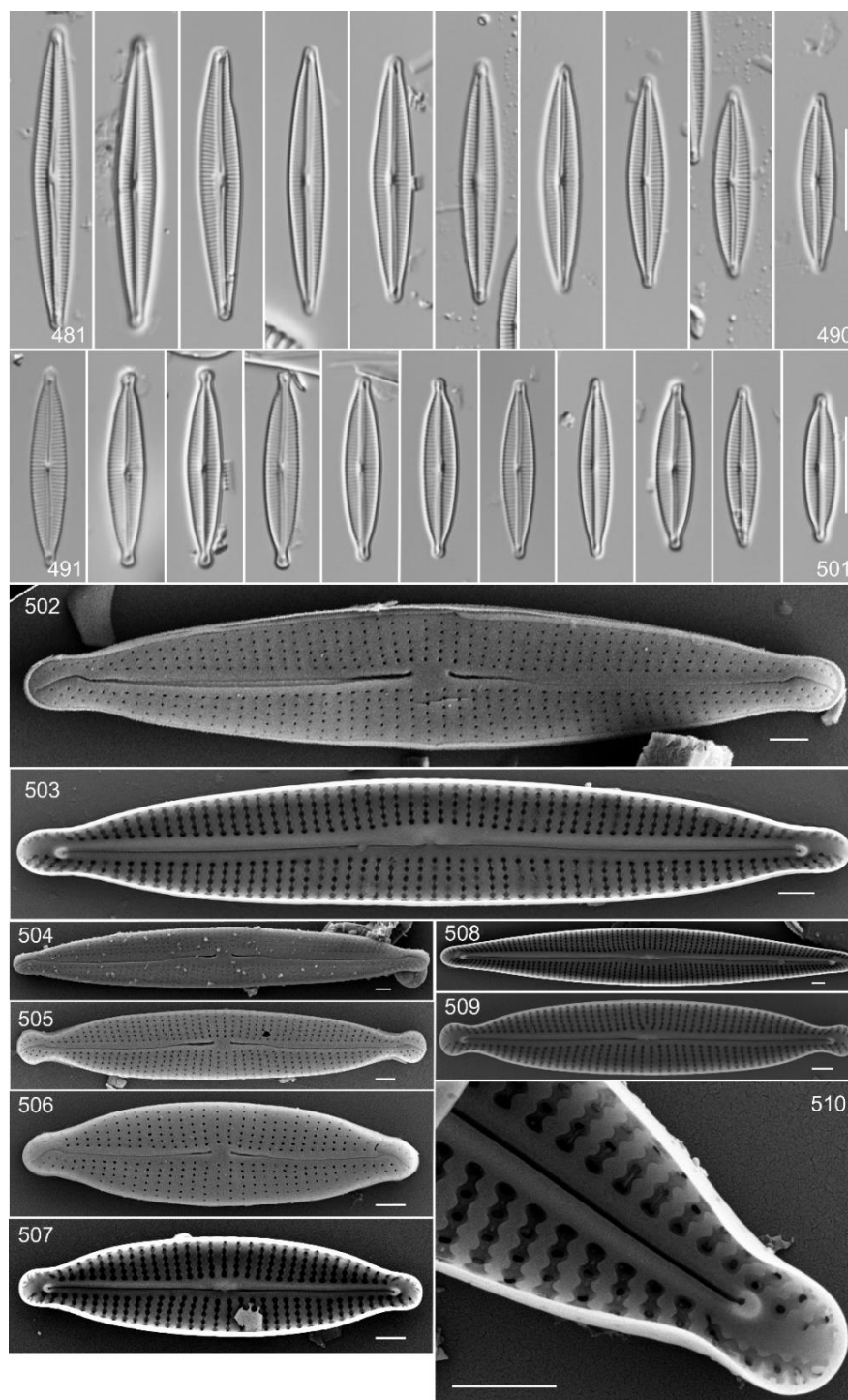


Figures 4.417–4.448 *Encyonopsis krammeri* Reichardt. Figs 417–448. LM. Figs 417–440. Valves showing morphological range and size reduction series in Irish lakes. Figs 441–448. SEM. Figs 441 & 443–446. External view. Figs 442 & 447. Internal view. Fig. 448 Girdle view. Scale bar in Fig. 440 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .



Figures 4.449–4.480 *Encyonopsis krammeri* Reichardt. Figs 449–472. LM. Figs 449–454. Valves illustrating the dorsiventral form and narrow rostrate poles for the most commonly encountered form. Figs 455–460. A population with more protracted subcapitate poles. Figs 461–472. A more lanceolate form corresponding to *E. angusta* Krammer & Lange-Bertalot with more clearly capitate rounded ends. Figs 473–480. SEM. Figs 473–478. External view of frustules with differing outline and apices but with equivalent ultrastructural morphology. Figs 479–480. Internal view with thickened silica between helictoglossa and end of apices. Scale bar in Fig. 472 = 10 μm for all LM figures. Scale bar in all SEM micrographs = 1 μm .

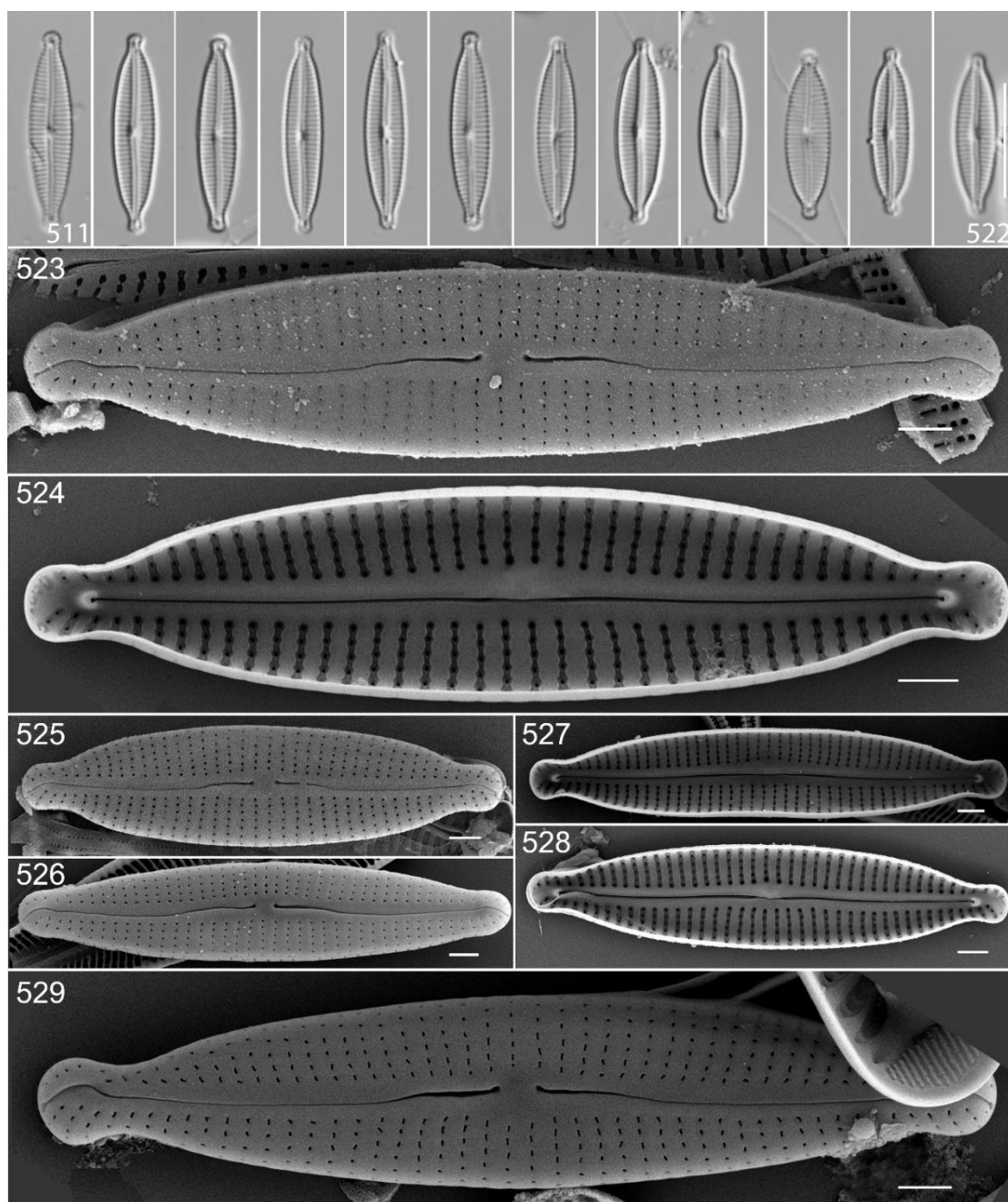
Encyonopsis neerlandica Van de Vijver, Verweij, Van der Wal & Mertens (Figures 4.481–4.510)



Figures 4.481–4.510 *Encyonopsis neerlandica* Van de Vijver, Verweij, Van der Wal & Mertens. Figs 481–501. LM. Figs 481–501. Valves showing morphological range and size reduction series in populations from Irish lakes. Figs 502–510. SEM. Figs 502 & 504–506. External view showing variation in size and shape of apices. Figs 507–510. Internal view. Figs 507–509. Variation in valve outline and shape of apices. Fig. 510. Detail of valve apex and helictoglossa. Scale bar in Fig. 490 = 10 μ m for LM figures 481–490. Scale bar in Fig. 501 = 10 μ m for LM figures 491–501. Scale bar in all SEM micrographs = 1 μ m.

Description: Characterised by relatively large valves for a diatom in the *microcephala*-complex, lanceolate to broadly lanceolate in outline with convex margins and rostrate to subcapitate apices. Length 13.6–31.6 μm ; width 3.0– 4.5 μm ; maximum L:W ratio 8.3. Mostly comparable to the type description but populations in Irish lakes also have distinctly capitate rounded apices (Figures 4.492–4.494, 4.505, 4.509). Larger valves and initial cells may have non-protracted narrowly rounded ends. Striae are parallel or weakly radiate at centre, becoming increasingly radiate towards the poles. Stria density 25–30 dorsally and 24–29 ventrally in 10 μm . Stria density in Irish populations exceed that reported for type material and valves have a higher maximum L:W ratio. Axial area narrow and central area absent, rarely with a single slightly shortened stria at valve centre. Ultrastructural characters are equivalent to that given with the original species description.

Ecology: *E. neerlandica* is widespread in peatland catchments in the West of Ireland, occurring in 26 lakes and reaching a maximum relative abundance of 54%. Its ecology is equivalent to that reported by Van de Vijver *et al.* (2012) and is found in low to moderate alkalinity, near-neutral humic lakes. It is also found in a number of samples from Cronaniv Burn (max. 9.5%), a river in a near-pristine peatland catchment overlying siliceous geology in Co. Donegal and less frequently in the Bundorragha River, a lake outflow in Co. Mayo. Although found in near-pristine reference lakes, it has a maximum abundance at mesotrophic condition, suggesting a sensitivity value of S=2 for lake ecological assessment (Figure 4.532).

***Encyonopsis subminuta* Krammer & Reichardt (Figures 4.511–4.529)**

Figures 4.511–4.529 *Encyonopsis subminuta* agg. Figs 511–522. Valves showing morphological range and size variation in populations from Irish lakes. Figs 523–529. SEM. Figs 523–527. External and internal view of *Encyonopsis subminuta* Krammer & Reichardt. Fig. 528–529. Internal and external of *E. aff. subminuta*. Scale bar in Fig. 522 = 10 μm for LM figures. Scale bar in all SEM micrographs = 1 μm .

Description: Valves are symmetric or appear only very slightly dorsiventral, lanceolate with weakly convex dorsal and ventral sides. Valve apices are clearly protracted capitate to subcapitate, rarely rostrate and without clear shoulders. Axial area is narrow linear and central area absent. Length 10–25 μm ; width 3.0–4.5 μm ; maximum L:W ratio 5.8. Striae parallel to weakly radiate at centre, weakly radiate towards apices and uniform in appearance. The upper range of stria density in Irish populations is slightly higher than reported elsewhere (Table 4.1) and a form with distinct peanutshaped puncta (Figures 4.528–4.529) was also well represented and co-occurs with nominate valves (Figures 4.523–4.527). Raphe is filiform, proximal ends curve dorsally with slightly dilated endings. Distal fissure deflected ventrally, terminating in a small pore on the valve face.

Ecology: A cosmopolitan species in well buffered alkaline lakes with low nutrient concentration (Figure 4.532). It occurs in 30 lakes with a maximum relative abundance of 25%.

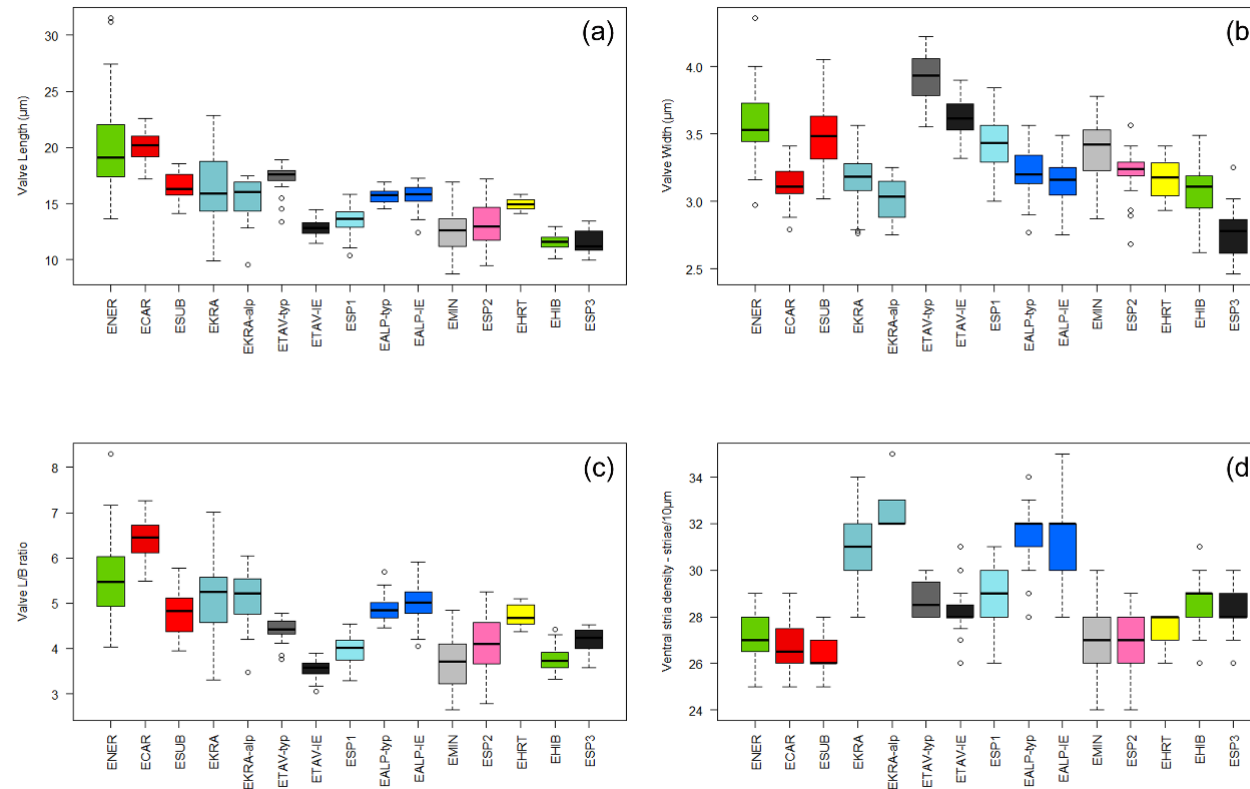


Figure 4.530 Box plots of the distribution of valve characters measured with SEM for *Encyonopsis*. Only diatom frustules having data for all measured characters is plotted. Boxes represent the 25–75% quartiles (excluding outliers) with the median shown as a horizontal bold line. Whiskers represent the upper and lower data points less than 1.5 times the box height and outliers are individual points outside the whiskers. A. Boxplot comparing the distribution of valve length data. B. Comparison of valve width. C. Comparison of valve length to breadth ratio. D. Comparison of ventral stria density. All data are measured using populations of *Encyonopsis* from Ireland unless otherwise stated. *E. neerlandica* (ENER), *E. carraensis* (ECAR), *E. subminuta* (ESUB), *E. krammeri* (EKRA), *E. krammeri* capitate form in *E. alpina* type material (EKRA-alp), *E. tavirana* type material (ETAV-typ), *E. aff. tavirana* Irish populations (ETAV-IE), *Encyonopsis* sp. 1 (ESP1), *E. alpina* type material (EALP-typ), *E. alpina* Irish populations (EALP-IE), *E. minuta* (EMIN), *Encyonopsis* sp. 2 (ESP2), *E. horticola* (EHRT), *E. hibernica* (EHIB), *Encyonopsis* sp. 3 (ESP3).

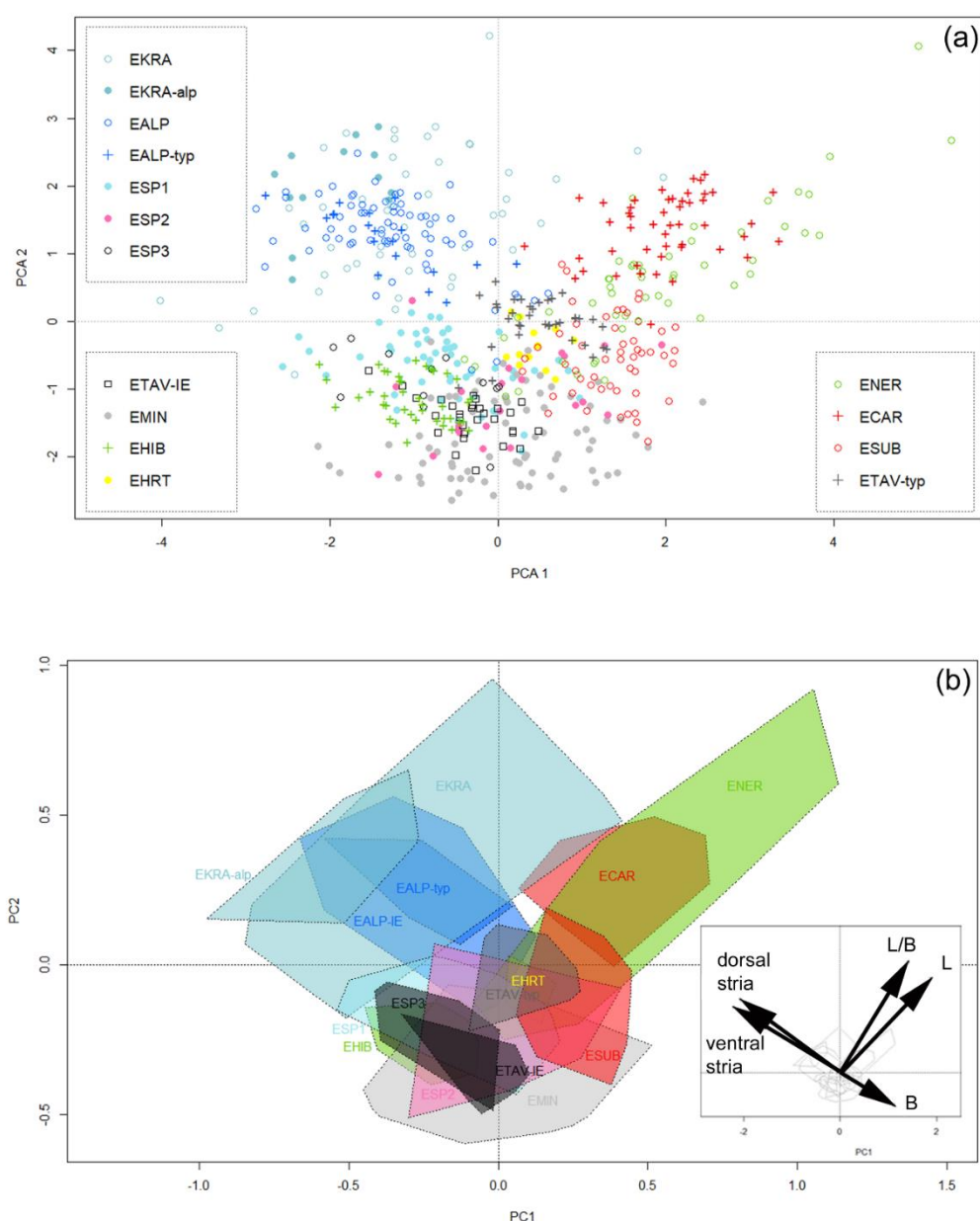


Figure 4.531 Comparison of valve morphometry for *Encyonopsis* taxa. A. PCA plot showing positions of *Encyonopsis* taxa in relation to first and second principal component axes. B. Data fitted to convex hulls with the morphological variables measured in SEM displayed as arrows (inset). Taxon codes are equivalent to Figure 4.530.

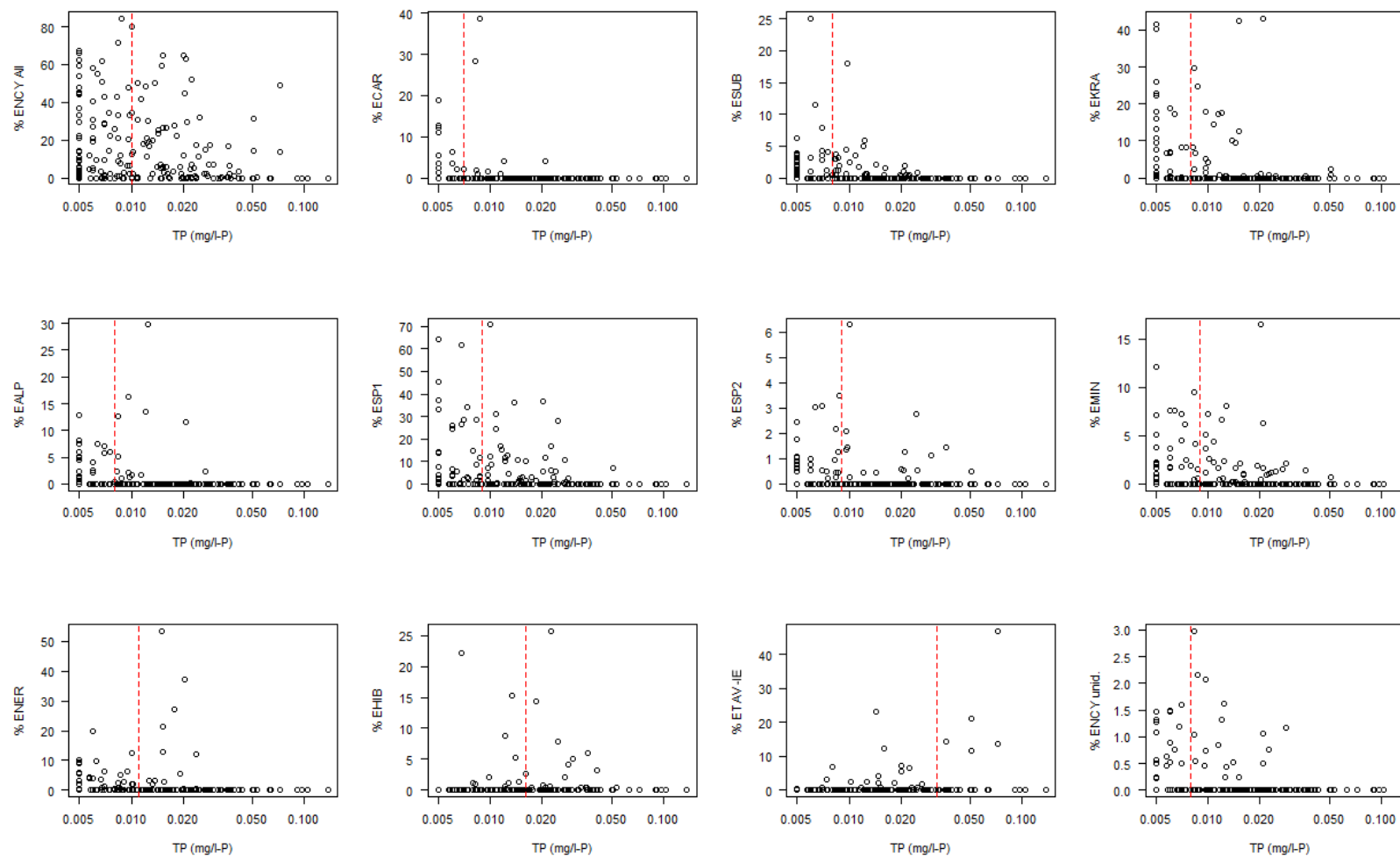


Figure 4.532 Scatter plots of the distribution of *Encyonopsis* taxa in relation to A. Total phosphorus (TP) and B. Alkalinity. Vertical lines indicate the location of optima derived from weighted averages of species occurrences for TP (red) and alkalinity (blue). Taxon codes correspond to Fig. 530, with All (ENCY All) and Unidentified (ENCY unid.) *Encyonopsis* categories illustrated for comparison.

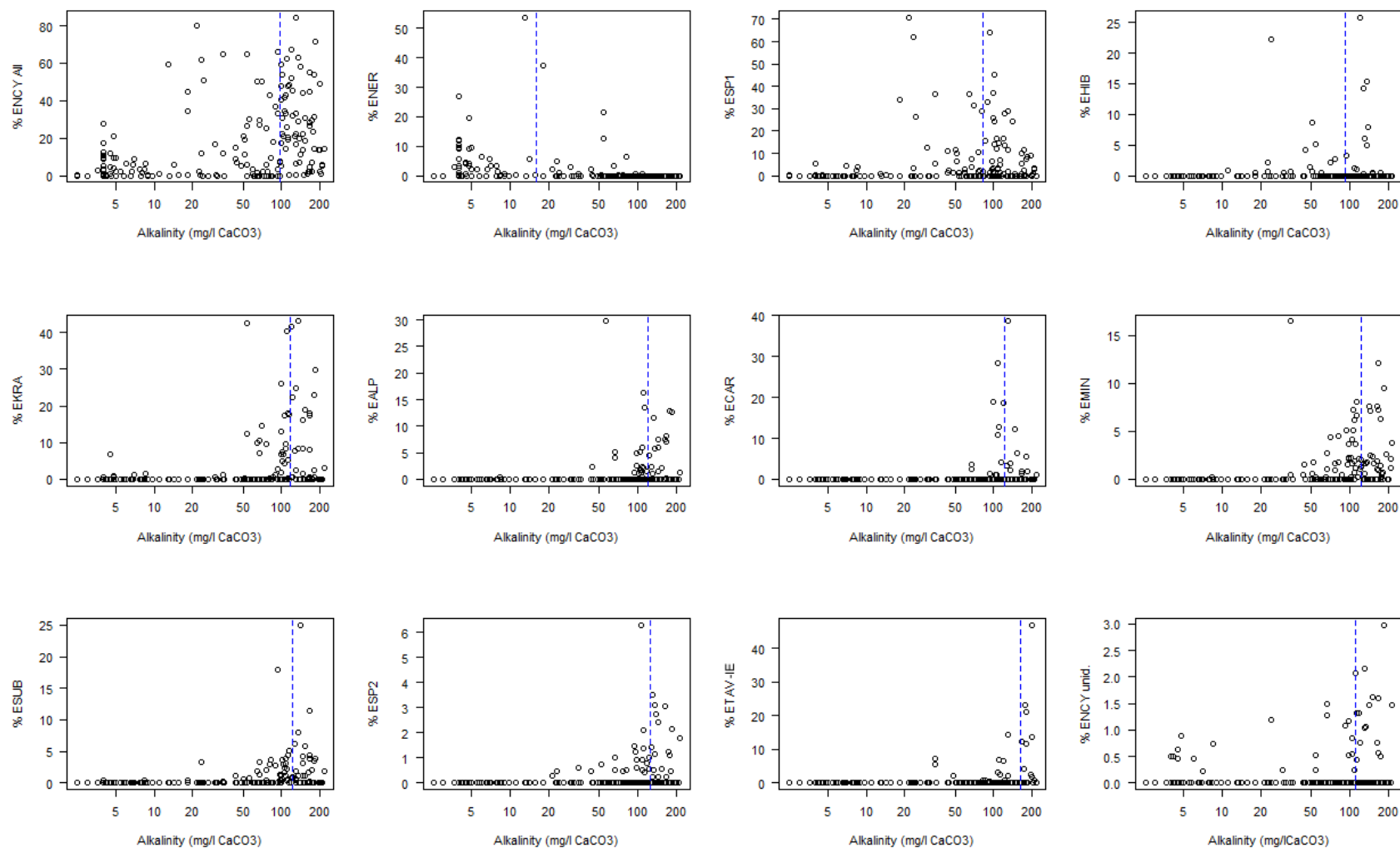


Figure 4.532 (Continued) Scatter plots of the distribution of *Encyonopsis* taxa in relation to A. Total phosphorus (TP) and B. Alkalinity. Vertical lines indicate the location of optima derived from weighted averages of species occurrences for TP (red) and alkalinity (blue). Taxon codes correspond to Figure 4.530, with All (ENCY All) and Unidentified (ENCY und.) *Encyonopsis* categories illustrated for comparison.

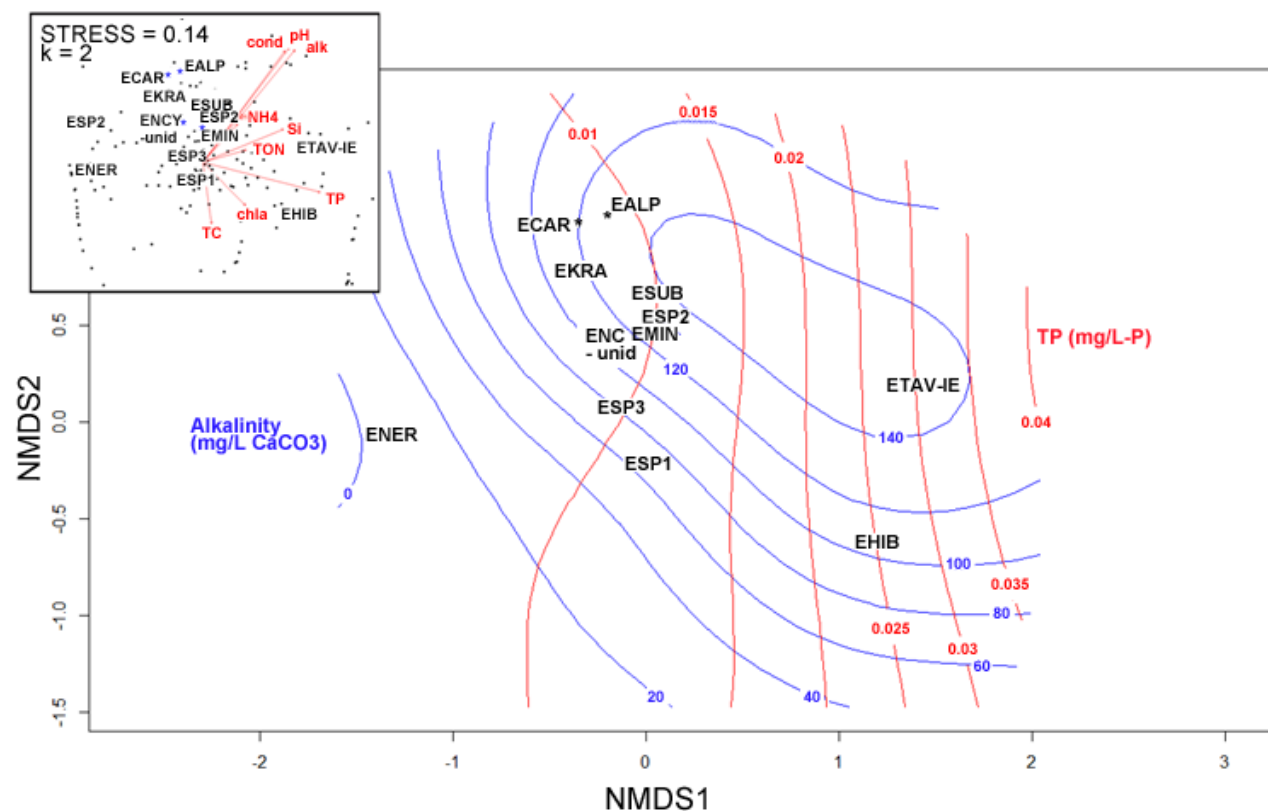


Figure 4.533 NMDS biplot of Bray-Curtis dissimilarity matrix of untransformed relative abundance of *Encyonopsis* in lake periphyton samples. Inset is a plot of lakes with significant environmental factors ($p < 0.05$) fitted *a posteriori*, including true colour (TC), chlorophyll a (chla), total phosphorus (TP), total oxidised nitrogen (TON), silica (Si), total ammonia (NH₄), conductivity (cond), alkalinity (alk) and pH. Main plot shows the location of *Encyonopsis* species added as weighted averages of site scores with surface contour plots for TP (red curves) and Alkalinity (blue curves) gradients fitted *a posteriori*.

4.5. DISCUSSION

The former *Cymbella microcephala*-complex was a convenient and temporary name for an aggregate of diatoms that were difficult to classify because of the close similarity of its constituent taxa. Krammer (1997a, b) examined this group more critically and designated further species in a new genus *Encyonopsis*. Investigations outside oligotrophic alpine habitats of the original study since then has revealed many new taxa, suggesting their potentially greater significance for bioassessment (Bahls 2013, Graeff & Kociolek 2013, Van de Vijver *et al.* 2009, 2012).

Two new species, *E. carraensis* and *E. hibernica* from Irish lakes are described here. Both have a morphology typical of diatoms in the *E. microcephala*-complex; relatively small sized valves, moderately dorsiventral to linearlanceolate cymbelloid outline, weakly radiate striae with density in the range 24–36 in 10µm and puncta not discernible in LM. In common with previous assessments (Gottschalk & Kahlert 2012) their small frustules lack immediately obvious discontinuities in critical characters that could be used to convincingly set them apart. But a detailed analysis with SEM confirm that they are not conspecific with established species concepts. In LM, *E. carraensis* is most like *E. neerlandica* or *E. subminuta*, but a combination of broadly protracted ends with narrow linear-lanceolate outline and high L:W ratio allows them to be clearly separated. Interestingly, *E. carraensis* presents a greater diversity of external areolae openings than other *Encyonopsis* and like *E. palmeti*, they are often also predominately apically rotated. *E. hibernica* is equivalent in size and has a similar stria density to several other very closely related taxa, yet clear differences in morphology are also apparent. It is most likely to be confused with

diminutive valves of *E. minuta* and is occasionally mistakenly used to illustrate this species. Ultrastructural differences include proximal raphe ends concealed under a silica flap internally, and distal raphe endings and puncta shape are also differentiating. In LM a combination of broadly protracted ends, stria density and shortened offset striae at valve centre is usually sufficient to separate it from others. Both diatoms probably have a more widespread distribution outside Ireland (e.g. see undescribed forms in a Swedish lake study in Gottschalk & Kahlert, 2012).

Further evaluation of *E. angusta*, *E. alpina* and *E. tavirana* using type material is outlined. The ultrastructure of the latter taxa is illustrated for the first time, as SEM was not provided when they were originally described. Their overlap in stria density is greater than previously reported and confirmed in Irish populations of *E. alpina*. This is perhaps unsurprising, as Krammer apparently gave an average deviation in range for the character. He suggested that a narrower range of values was easily obtained from measurements of fewer individuals; a pragmatic attempt to help distinguish otherwise closely related taxa (Krammer 1997b, page 88). For small sized diatoms this approach can add confusion when populations from new areas are being assessed. Stria density is a primary character used to differentiate taxa in the *E. microcephala*-complex, in combination with other features that can be equally challenging to resolve in LM. There was some uncertainty around what the diatom *E. angusta* represented as a result. Relative to the cosmopolitan *E. alpina*, reports of this species are infrequent and additional documented references were not found. Unfortunately, only LM images from the type slide of *E. angusta* are possible as the diatom sample could not be located for investigation with SEM (Bánk Beszteri *pers. comm.*). The diatoms we select have a narrower minimum valve

width and greater maximum L:W ratio than originally described. The L:W in the original plates when remeasured and is also greater than given with the diagnosis (≥ 5.0). There is a possibility that *E. angusta* is merely a capitate form of *E. krammeri*, with a higher stria density and lanceolate valve outline relative to populations typically encountered. This form occurs on the type slide of *E. alpina* and in the periphyton of some calcareous lakes in Ireland. These valves have ultrastructural features characteristic of *E. krammeri* including linear puncta and an equivalent raphe structure. In fact, an identical valve was inadvertently selected to illustrate both species (see Tafel 144 Fig. 12 and Tafel 193 Fig. 2 in Krammer 1997b). This may be a legitimate error in labelling as noted elsewhere in the monograph (e.g. *E. subminuta* incorrectly labelled as *E. minuta* in Tafel 151 Fig. 1–7 and Tafel 150 Fig. 23). Alternatively, it provides support for a closer resemblance of these diatoms than their diagnosis suggested. Satisfactory characters to differentiate *E. angusta* and *E. krammeri* are now less certain and we propose that they are conspecific, with *E. krammeri* having priority. Maximum stria density and shape of the valve ends should therefore be emended for *E. krammeri*. The reference to narrower valves of *E. angusta* refer to its comparison with *E. tavirana*, as its range is encompassed entirely within that of *E. alpina*. Based on our findings, the range of stria density for *E. alpina* should also be revised upwards and is therefore no longer a clear differentiating character for these species either. The reported puncta density was a diagnostic character (approximately 50 versus 38–42 in *E. alpina*). However, we could not replicate these findings and determined puncta density to be nearly equivalent for most of the similar sized diatoms examined in the study. Puncta density is difficult to determine accurately in such small valves, given

curvature in the striae and frustule. Estimated densities are found to vary considerably and are dependent on the distance measured and the location where it is done on the valve. In this case it is a character that can only be determined accurately with SEM. Unusually, no SEM images were provided with the original publication for either diatom, as was done elsewhere for other new taxa. If the type material cannot be located for further ultrastructural investigation, a search of diatoms in L. Lustsee may help reaffirm the validity of *E. angusta*.

Several species documented here are first records for Ireland. We report two potential morphodemes which have an affinity with *E. tavirana*, but which differ to an extent from the formal diagnoses given for this species. The robust outline of what we designate as *E. aff. tavirana* is conspicuous, has similar stria density and ultrastructure, and differs principally in valve dimension. It is prevalent in moderately-eutrophic lakes with a high optimum for total phosphorus and ammonia (Table 4.2) contrasting with the oligotrophic preference of *E. tavirana* in Portugal. It was suggested during review that this diatom would be identified as *E. microcephala* by some diatomists. Its ecological niche provides support for the interpretation but differences in morphology are apparent, nevertheless. A finer stria density and weakly constricted internal alveoli are differentiating, while the form of the terminal raphe endings and greater tendency for peanut shaped puncta contrast with the valves of *E. microcephala* illustrated by Krammer (1997b). It is surprising that a greater number of published images of the ultrastructure of *E. microcephala* are not available, given its apparent widespread distribution. Investigation of further populations with SEM would help to determine the full extent of morphological variability and the possibility of conspecificity with other

diatoms. Examination of epipelagic samples in Irish lakes would help to validate the reports of *E. microcephala* for the paleolimnological studies where it has been reported (Chen *et al.* 2008, O'Dwyer & Taylor 2010). *Encyonopsis* sp. 1 is morphologically very similar to *E. aff. tavirana* and its narrower valves and more weakly developed shoulders potentially represent a size reduction of this diatom. These divergent forms may potentially also be explained by cryptic diversity as suggested by Graeff & Kociolek for some North American *Encyonopsis* (Graeff & Kociolek, 2013). It may also be analogous to the case of *Nitzschia palea* (Kützing, W.Smith 1856: 89) and the narrower variety var. *debilis* (Kützing, Grunow 1880: 96), long established indicators of highly polluted and oligotrophic situations, respectively (Trobajo *et al.* 2009). In this case combined morphological, genetic and mating studies of the *N. palea* agg. by Trobajo *et al.* (2009) determined that traditional separation of these infraspecific forms based on morphology was not supported, yet molecular data also indicated a more complex situation than could be explained solely by phenotypic variability. As useful and abundant indicator taxa in Irish lakes, both taxa require molecular characterisation to help establish their relationships.

Populations of *E. neerlandica* and *E. krammeri* with capitate apices document a greater range of phenotypic variability than described previously for these species. End shape of the poles is a variable character in many diatoms including *Encyonopsis* (e.g. see *E. minuta*) but molecular data would allow for the possibility of clades within these populations to be investigated. A form of *E. subminuta* with distinctive peanut-shaped areolae contrasts with *E. subminuta sensu stricto*, which retains the distinctive small rounded puncta illustrated with its diagnosis. Both

these forms regularly co-occur in samples and can only be identified with SEM. Krammer noted that taxa with small rounded areolae were conservative for puncta shape and this is confirmed for numerous valves of *E. minuta* examined in this study. If this is the case, then cryptic diversity may represent a viable explanation for the latter form. Although their ecological niche is rarely overlapping, it closely resembled capitate valves of *E. neerlandica* in LM. A relatively small number of valves are examined for the latter, but a greater valve width, more constricted internal alveoli and clear intermissio are differentiating. These criteria represent very subtle differences in morphology between some established taxa. The trend in the *microcephala*-complex is for an increasing number of species concepts differing in only one or two critical characters (e.g. *E. cilaosensis* Le Cohu & Gassiole and *Encyonopsis* sp. 1 and 2 in this study). The complex may provide a further interesting opportunity to explore the relative significance of evolutionary divergence, phenotypic plasticity and biogeographical patterns of distribution for the group (Cox 2014).

We provide a documented record for the rare *E. horticola* from a natural lake and confirm the poor ecological status classification in Lough Gur where it occurs. However, not all taxa in the complex have diverging indicator value. *Encyonopsis* sp. 2 has an equivalent niche to that of the closely related *E. minuta*. Morphologically, it differs only by distinctively narrow rostrate apices and possibly by a less clear separation of internal proximal raphe endings in SEM. That it could represent further morphological expression of the genotype of *E. minuta* cannot be exclusively ruled out at this time. But in LM their valves maintain consistently different end shape relative to the broadly rounded poles in the size reduction

series of *E. minuta* and it is found in many independent lake samples. However, in SEM, identification is not always so clear when populations converge. From a water quality monitoring perspective, the ecology of closely related forms may be similar, so a redundancy in assessment has been suggested (Rimet & Bouchez 2012, Kelly & Ector 2012). For example, Kelly *et al.* (2014) found that the niche of two similar *Nitzschia* species which had been merged up to that point, was explained more by local geology and habitat, rather than by anthropogenic pressures. Kennedy & Allott (2017) determined that a greater taxonomic resolution of *Brachysira* Kützing (1836) provided limited additional value for differentiating trophic status in Irish lakes. Potapova & Hamilton (2007) however, found that infraspecific forms of *Achnantheidium* (Kützing, Czarnecki 1994: 157) differed significantly in their ecological characteristics, and recommended them as indicators for water quality assessment. In a case study of the utility of species complexes for trophic assessment, Pouličková *et al.* (2017) reported statistically significant relationships with TP for some (pseudo) cryptic species, where aggregates of these taxa were non-significant.

The TP optimum of a merged *Encyonopsis* aggregate is equivalent to that generally reported for the group (~10 µg/l TP), yet its distribution spans the oligotrophic-eutrophic gradient (Figure 4.532). Generalist taxa in species complexes can dominate modern datasets and conceal the potential indicator value of oligotraphentic or eutrapphentic species when niches are estimated empirically. Lakes in this study correlate well with a TP gradient when ordered based only on the dissimilarity of their *Encyonopsis* populations (Figure 4.533). Some applied metrics currently classify reference assemblages of e.g. *E. carraensis* or *E. alpina* as

equivalent to lakes of lower quality where e.g. *E. aff. tavirana* or *E. hibernica* are abundant. We conclude that taxonomic sufficiency will need to be evaluated on a taxon by taxon basis. For some complexes the highest level of resolution that can be reliably achieved will be optimal, whereas merging of others will result in no loss of information when used in that limited context.

Like other species complexes (e.g. *Achnantheidium minutissimum* agg.) routine identification of small *Encyonopsis* valves is challenging in LM. Nevertheless, although there were problems clearly identifying individuals in mixed populations, unidentified forms never exceeded 3% relative abundance in this study. The extent of unassigned valves is too low therefore to effect environmental indices based on weighted averages. Given differences in autecology, a greater differentiation of small *Encyonopsis* will help to reduce the potential for misclassification of ecological status in lakes.

4.6. ACKNOWLEDGEMENTS

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CHAPTER 5

Conclusions and Recommendations

5.1. OVERVIEW

My study aimed to explore epilithic diatom distributional patterns in the periphyton of a limnologically diverse range of lakes in Ireland. My major objective was to identify the primary environmental factors structuring littoral assemblages and to evaluate the value of epilithic diatoms for lake ecological classification. I explored diatom diversity in reference and impacted lakes to determine how assemblages responded to pervasive environmental stressors in the landscape. A large scale study replicated in spring and summer was used to address deficits in understanding of the implication of seasonality for diatom assessments. A further focus was to provide a contemporary taxonomic analysis for two prevalent diatom indicator groups. These outputs can be considered in terms of exploring the extent of unknown algal diversity in Irish freshwaters, while assessing the environmental indicator value of taxonomic complexes. This chapter summarises core outputs before integrating the findings in a broader context while incorporating recommendations where further research and development would be beneficial.

The original study aims, and specific hypotheses have been fully satisfied by providing the first comprehensive evaluation of epilithic diatoms as indicators of ecological status in Irish lakes. Six core groups of littoral diatom assemblages were identified that can now be used to effectively describe and communicate the major

patterns in epilithic assemblages here. The Lake Trophic Diatom Index was validated as a relevant measure of phytobenthos response to lake total phosphorus condition. In contrast to studies in other geographic regions, Irish lakes were found to have comparable reference expected-values across the lake types, notwithstanding that diatom composition differed amongst the lake groups. Contrary to the findings in some similar studies, physical disturbance in exposed littoral habitats did not prohibit the success of diatoms from a lake monitoring perspective. My findings indicated that seasonal variability was not a major factor and that sampling in either spring or summer is unlikely to present a major bias for monitoring programs. I recommend sampling in both seasons however, to ensure confidence in classifications is maintained within acceptable limits.

While diatom diversity in a limnologically diverse range of rivers and lake types was better documented, intensive morphological and ultrastructural investigations allowed me to assess the taxonomy and ecology of two widespread algal genera, *Brachysira* and *Encyonopsis*. As such, my study represents the only recent contemporary taxonomic investigation of freshwater algae in Ireland. New species were described and significant differences in autecology of taxa that are routinely aggregated for environmental assessments was established. These findings will provide a basis for a better understanding of diatom biogeographical patterns while also helping to inform new diatom metrics and environmental reconstructions in future. For several morphotaxa I established taxonomic findings of importance for diatom classifications. Recent independent publications have since confirmed the validity of my original taxonomic outputs by diagnosing new

taxa for some of the documented morphotypes, while I am contributing to a review of *Brachysira* in European waters based on my thesis outputs.

The broad conclusions of the two-separate works, namely the established benefit of higher resolved diatom taxonomy and ecology, versus use of diatoms for ecological assessment in the context of a successful application of an index developed in another region, may appear contradictory. However, this question can only be fully resolved empirically after all of the common morphotypes in the Irish diatom flora have been investigated. The benefit of increased taxonomic resolution may differ amongst different lake types and in different monitoring contexts as I established for *Brachysira* and *Encyonopsis*. But diatom taxonomic resolution might also be considered in the context of as yet other unmeasured components of lake phytobenthos which have a large potential efficacy for use in environmental monitoring.

5.2. MAIN CONCLUSIONS

1. 210 samples from 91 lake waterbodies were assessed, with 86 lakes sampled in both spring and summer seasons. New diatom records for Ireland have resulted but many identified taxa represented a mix of species and species complexes and significant taxonomic challenges remain to be resolved in many cases. *Acanthidium minutissimum*, *Encyonopsis* and *Brachysira* accounted for one in every two diatoms given that oligotrophic to mesotrophic lakes dominated the dataset.
2. Periphyton and diatom populations were compositionally diverse but could be characterised by a simplified typology of three lake alkalinity types, or

six key groups of diatom assemblages that integrated the effects of both natural and anthropogenic gradients. Discrete classifications are artificial in the sense of the observed overlap and gradual turnover of taxa in response to continuous gradients in lake environmental condition. Nevertheless, the approach provided a useful option to summarise broad distributional patterns in the diatom composition across the network of lakes.

3. Diatom variation across the candidate reference network was associated with the primary alkalinity-pH gradient but correlated with true colour and was strongly negatively associated with total phosphorus. Distinctive assemblages of alkalibiontic *Encyonopsis* dominated high status marl lakes with *Mastogloia*, *Achnantheidium minutissimum*, *Cymbella affinis* and other indicators of oligotrophic calcifying waterbodies. *Achnantheidium minutissimum* had a higher prevalence at intermediate alkalinities with a concomitant shift from alkalibiontic *Encyonopsis* to *Encyonopsis neerlandica*. As alkalinity further reduced, *Achnantheidium* became subordinate to *Brachysira*, *Eunotia* and *Frustulia* and a single dystrophic reference lake was characterised by high abundance of acidophilous *Tabellaria flocculosa*, *Eunotia mucophila* and an absence of *Achnantheidium*.
4. Indicator taxa were prevalent in all of the identified groups but as expected there were few significant indicator diatoms in cluster-1 given the high prevalence of the generalist *Achnantheidium minutissimum* and cluster-4 which was intermediate in nutrient condition between the calcareous oligotrophic and eutrophic higher alkalinity waterbodies.

5. Lake morphometry had low significance for littoral diatom assemblages given comparatively low amounts of the total variation explained by the physical variables, but the significant effect of exposure via average wind velocity remained to be more fully validated.
6. Littoral diatom assemblages were strongly coupled to regional landcover through its influence on water chemistry and provided a useful option to classify lake phytobenthos response to total phosphorus enrichment.
7. When diatom samples from reference and non-reference oligotrophic lakes were evaluated with the Lake Trophic Diatom Index (LTDI) there was no difference in metric scores between the lake alkalinity types. The similarity of metric-total phosphorus relationships amongst three alkalinity types and the subsequent distribution of Ecological Quality Ratios (EQR) indicated that lake alkalinity type did not have a major influence on the expected reference value of the LTDI in Irish lakes.
8. Notwithstanding the high prevalence of diatom-complexes, transferability of the Lake Trophic Diatom Index (LTDI) was successfully validated for Irish lakes with a significant relationship observed in all lake alkalinity types. The strength of the relationship was weakest in low alkalinity lakes as reported in other regions. Nevertheless, diatom assemblages in more weakly-acidic low alkalinity lakes also responded to nutrient enrichment.
9. Seasonal differences in diatom composition were minor and significant indicator taxa were rarely abundant. The total explained variance and the primary environmental gradients identified in the spring and summer data were also broadly equivalent. Lake specific differences were apparent with

responses to natural and trophic gradients. In contrast to lotic habitats, seasonality did not have a major influence on diatom assessments in temperate lakes. The clearest seasonal pattern that emerged was an increasing variability in LTDI scores at higher metric values. The factors driving lake specific variation were not identified but agree with previous findings. The consequence for monitoring programs was that sampling in both seasons was recommended in order to improve confidence in classifications.

10. Epilithic diatoms have been identified to be less sensitive than lake macrophytes in lake classifications. It was suggested that this was owing to the effective positioning of diatom status class boundaries and the lack of inclusion of a functional metric such as periphyton biomass in the diatom based approach.
11. Diatom samples from 212 rivers and 95 lakes were investigated to establish the extent of diversity of *Brachysira* and *Encyonopsis* in Irish waters. Original type material for three *Encyonopsis* was also re-evaluated and *E. angusta* was proposed as a synonym of *E. krammeri*.
12. *Brachysira* and *Encyonopsis* populations comprised of a combination of species and new species. Dominant morphotypes were characterised with detailed ultrastructural studies but these require supporting molecular data and additional checks of type material before they can be formally diagnosed. Four new species were reported and results indicated that diversity in these taxa is high. If generalized to other algal groups it suggested that freshwater algal flora is poorly described in Ireland.

13. The extent to which resolved species complexes enhanced environmental indicator value was taxon specific but was demonstrated for some diatoms. This new information can be incorporated in future metric revisions to reduce sampling variability, but further studies are required for a greater range of diatoms.

5.3. DISCUSSION

5.3.1. DIATOM-ENVIRONMENT RELATIONSHIP

Despite mixed statements about the potential of epilithic diatoms to accurately reflect the trophic condition of lakes, I found littoral assemblages to be significantly related to total phosphorus in the three lake alkalinity types that were investigated (Bennion *et al.*, 2014; DeNicola *et al.*, 2004; Kelly *et al.*, 2014; Riato and Leira, 2020; Rimet *et al.*, 2015; Schneider *et al.*, 2020).

A fundamental tenet of the WFD is a requirement to assign waterbodies to distinctive types, after which common concepts of reference condition can be evaluated in an intercalibration of member state classification systems (Pardo *et al.*, 2012). Lake typologies are established using abiotic criteria to characterize the natural communities expected in different environments and help improve the precision of classification results. The national lake typology in Ireland incorporates mean depth (< or > 4m), lake area (< or > 50 ha), alkalinity (< 20, 20-100, > 100 mg l⁻¹ CaCO₃) and altitude (for lakes > 300 m), but the relevance of this system for lake phytobenthos was not considered when the typology was validated (Free *et al.*, 2006). There was only a single lake above 300 metres altitude in my data, so

an altitude effect was not tested. Nevertheless, altitude and climate are known to correlate with distinctive diatom assemblages (Gottschalk and Kahlert, 2012), but also reflect atmospheric deposition and climate forcing that has been identified to significantly influence upland waterbodies (Battarbee *et al.*, 2014; Clarke *et al.*, 2005; Karst-Riddoch *et al.*, 2005). Further monitoring will be required to address this issue and the case to include diatom monitoring in established chemical monitoring programs in the Irish uplands is strong. The results of variance partitioning indicated that lake physical attributes were not significant for epilithic diatoms in Irish lakes. Significant typological variables have been found to differ amongst trophic levels in other lake studies so divergent responses to abiotic criteria are not unexpected and lake depth also had a limited relevance for littoral diatoms in UK lakes (Bennion *et al.*, 2014; Catalan *et al.*, 2009). There was a more natural grouping of lakes and diatom assemblages using the simplified alkalinity typology adopted from European investigations and this is suggested as a workable approach from a lake diatom perspective in Ireland (Kelly *et al.*, 2014). Nevertheless, the chosen arbitrary alkalinity thresholds may have lesser biological relevance for diatom assemblages here. This cannot be established without a much greater number of reference and high status waterbodies sampled across a fuller gradient of alkalinity, dissolved organic carbon, and ideally supported with palaeoecological data.

While the total explained variation in diatom-environment multivariate ordinations was moderately low, it was in line with values reported in similar studies. I did not fully explore all possibilities to explain patterns of residual variation in seasonal diatom metric scores. Many of these lakes have since been resampled in three-yearly cycles for WFD monitoring, with twelve larger waterbodies incorporating a

greater number of within-lake sampling stations ($n = 2-6$). A more in-depth analysis of this time series would have greater precision for identifying casual factors associated with different levels of variation on a lake to lake basis. More effective recording of shoreline habitat features, meteorological data, the extent of grazing pressure and algal biomass would help to capture a greater number of processes and interactions to improve the explanatory power in future studies (Evtimova and Donohue, 2016; Kahlert *et al.*, 2002; Rimet *et al.*, 2015).

Nevertheless, from an applied monitoring perspective the performance of the Lake Trophic Diatom Index (LTDI) was sufficiently robust to capture diatom assemblage response to increasing enrichment by phosphorus. This represents an improvement on a previous study where diatom metrics were found to be poor indicators of trophic state (DeNicola *et al.*, 2004). The cross-regional transferability of diatom indices is not guaranteed because of potential differences in species realized niches, species pools and/or ecosystems, local adaptation or taxonomic identifications (Pajunen *et al.*, 2020). However, my findings confirm a broader applicability of the LTDI (Fidlerová and Hlúbíková, 2016). It confirmed the metric was responsive to the phosphorus gradient primarily, as was intended when it was developed. The major difference in findings relative to international studies was the consistent relationship between the LTDI and total phosphorus across all three lake alkalinity types (Bennion *et al.*, 2014; Schönfelder *et al.*, 2002). The implication of these findings is that member state reference concepts in comparable regions are more influential for lake classifications than potential biogeographical or habitat factors. While the WFD intercalibration process has successfully bridged these gaps to an extent, differences in member state monitoring systems and nutrient

standards are still apparent (Kelly *et al.*, 2014; Poikane *et al.*, 2019). The findings were also unexpected in light of prediction models that established a range in total phosphorus levels related to geology and landcover in reference lakes (Vighi and Chiaudani, 1985; Vinogradoff and Oliver, 2015). The upper range of diatom-inferred total phosphorus in Irish lakes also broadly agrees with the limits reported in these models (Leira *et al.*, 2006; Taylor *et al.*, 2006). While there are no naturally eutrophic Irish lakes, the extent of oligotrophic to mesotrophic (and dystrophic) lake conditions is less certain (Taylor *et al.*, 2006). My results suggested that there were no type specific differences amongst LTDI values, but there is a possibility that the metric is not sufficiently sensitive to enable subtle differences to be identified. Precision of the metric might be investigated by determining if a greater number of diatom sensitivity classes would improve performance while at the same time reconciling trophic optima for a greater number of resolved species-complexes.

The overall LTDI model is fit for purpose in terms of providing an empirically stable range of EQR scores and set of status class boundaries that meet WFD classification objectives. Nevertheless, there are areas that will require further improvement. The metric has good comparability with lake classifications in high and good status lakes but provides less stringent classifications with further declines in lake quality. While there is no formal requirement, or necessarily an ecological rationale either, for concordance amongst the different biological groups, it has the practical consequences of making metrics with the lowest stringency redundant. The lower sensitivity of the diatom metric will need to be addressed in future developments and the good moderate boundary position will need to be reviewed as a priority. While this status class boundary has the greatest ecological underpinning of all the

diatom status classes, there is good evidence that significant deterioration in lake ecology including in the condition of lake phytobenthos has occurred before declines are captured in the diatom index (Doddy *et al.*, 2019; Free *et al.*, 2006; Free *et al.*, 2016).

The lack of thresholds of change in continuous EQR scales has also been identified previously as a problem in such classification approaches (Donohue *et al.*, 2009; Poikane *et al.*, 2016). The empirical nature of existing status class boundaries that should be more ecologically focussed and an absence of functional attributes and metrics to broaden the conceptual basis to assess the health and condition of lake phytobenthos are all areas that should be investigated and further developed. While filamentous algal occurrences are incorporated with lake macrophyte monitoring in Ireland, there is greater environmental indicator value in other benthic communities that could be incorporated in an algal multimetric with diatoms (Charles *et al.*, 2020; DeNicola and Kelly, 2014; Doddy *et al.*, 2019; Schneider *et al.*, 2019). However, there is also complementarity in some of this information and there is little benefit to substituting one laboratory-based approach with an alternative one (Kelly *et al.*, 2008). Field based observational metrics could be progressed as there was good evidence during diatom sampling that benthic cyanobacteria, diatom biomass and other algae can also provide additional sources of data for environmental assessments. Organic phosphorus is the dominant natural form of phosphorus expected in Irish lakes and the replacement of organic-P algal specialists in response to nutrient enrichment is arguably a more significant threshold change that should be the target of metrics. The widespread prevalence of heterocystous cyanobacteria and mat forming

members of the Oscillatoriales all provide useful opportunities to do this. There is no evidence that diatoms are not also sufficiently sensitive to capture the same response, with taxa such as *Didymosphenia* and possibly *Cymbella/Encyonema* and a wider range of diatoms, but it is not yet explicitly defined in metrics.

It is unlikely that diatoms would not feature in any future classification system given their significant responses to relevant pressure gradients demonstrated in this study. One approach to improving ecological linkages based on structural changes in diatom composition suggested incorporating Red List species which have been demonstrated to be capable of discriminating between reference and non-reference sites (Cantonati *et al.*, 2020). The approach is already used elsewhere (Schaumburg *et al.*, 2004). Cantonati *et al.*, (2020) also referred to investigating the possibility of making greater use of diversity within genera, i.e. species complexes. Diatom metrics based on greater resilience of multi-species aggregates in biofilms versus dominance by a single r-strategist species in the same genus has significant appeal. My diatom taxonomy investigations outlined below will allow this hypothesis to be tested and new proposals for status class boundaries might therefore be identified. Diatom guilds and teratological forms were not intensively applied in my study but their environmental indicator value is also a focus of much diatom based research (Berthon *et al.*, 2011; Falasco *et al.*, 2009; Marcel *et al.*, 2017). Teratological forms were excessive in one low alkalinity lake Lough Lettercraffroe but were typically only 1.0%-2.0% in other lakes. The simplification of taxonomy promised by guilds also appeared to have less relevance with motile taxa prevalent across the trophic gradient as found in Swedish lake study (Gottschalk and Kahlert, 2012).

There is also potential to improve the environmental relevance of the stressor gradient used to assess the performance of metrics which has been based just on total phosphorus to date. Lake dissolved organic carbon and pH have received more limited attention in terms of lake phytobenthos monitoring so far. Given the complexity of existing and future climate projections this has become increasingly relevant (Monteith *et al.*, 2007; Ockenden *et al.*, 2017). The pH and dissolved organic carbon gradient was associated with a sequence of *Achnanthydium-Brachysira-Eunotia* amongst the low alkalinity lake network. Periphyton communities may have a role in helping to disentangle fluxes in natural and anthropogenic organic carbon with increased nutrient pressure that could result from future projected scenarios (Vinogradoff and Oliver, 2015). However, this will require existing sampling programs to be spatially and statistically optimised to maximise the opportunity to detect subtle trends.

5.3.2. DIATOM TAXONOMIC RESOLUTION AND INDICATOR VALUE

The premise of all biological monitoring is simple and in the case of diatom assessment requires only that taxonomic identifications be consistently done for taxa whose environmental niches have been robustly established. That species boundaries have been drawn too widely for traditional indicator groups like in former taxonomic aggregates such as *Brachysira exilis* (Kützinger) Round & D.G.Mann and *Cymbella microcephala* Grunow in Van Heurck is not in question (Mann, 1999). With the exception of some very characteristic diatoms, more detailed investigations have thus far led to an almost endless increase in species, although the opposite outcome has resulted in some cases like *Gomphonema*

Ehrenberg that has been found to be over described (Abarca *et al.*, 2020; Mann, 2010; Mann and Droop, 1996). Several studies have confirmed that refining taxonomic resolution improves indicator values and potentially the precision of assessments (Potapova and Hamilton, 2007; Pouličková *et al.*, 2017). Nevertheless, a potential indicator redundancy within species complexes suggests that their full implication for applied monitoring remains to be more fully validated (Jüttner *et al.*, 2013; Kelly and Ector, 2012; Kelly *et al.*, 2015).

Taxonomic research on freshwater algae in Ireland is traditionally limited, despite the fact that these studies are justified (Jüttner *et al.*, 2013; Lange-Bertalot, 1980; Metzeltin, 1995; Urbánková *et al.*, 2015; Van de Vijver *et al.*, 2008; Vis *et al.*, 2020; Williamson, 1991). My study provides the first contemporary examination of diversity and environmental niches in two taxonomically problematic diatom groups that are widely distributed in Ireland and in other global freshwaters. The objective was twofold – to improve diatom classifications, and to identify if increased taxonomic resolution was useful for environmental assessments.

The study had a focus on only a relatively small number of species and the dominant *Achnantheidium minutissimum*-complex was assessed in less detail in this thesis. Widespread diatoms in aggregates of *Cymbella affinis* Kützing, *C. helvetica* Kützing, *Fragilaria capucina* agg., *Gomphonema pumilum* agg., and *G. parvulum* (Kützing) Kützing have been provisionally assessed and no new diversity is reported. Nevertheless, many of these are new records for the Irish flora based on a preliminary check list (Guiry, 2019). However, taxonomic progress in all of the dominant aggregates of species will be required if biogeographical patterns are to

be elucidated and new metric concepts are to be validated. Rare species have a role here too but have not been targeted for critical examination in most cases. Additional morphological analysis incorporating SEM studies was part completed for several diatoms that could not be identified in standard floras or in the literature searches completed to date. Potentially new species will be progressed for publication after they have been more fully diagnosed (Appendix J, Figure J.1). An unknown *Delicatophycus* M.J.Wynne with a low abundance was encountered in marl lakes. A greater number of new species have been described in this genus in the last decade but it remains relatively species poor with only 27 species documented since it was erected in 2003 (M.D. Guiry, 2020). In the same lake type a problematic *Amphora* Ehrenberg ex Kützing with intermediate ultrastructural characteristics could not be identified and was not represented in a recent revision of the genus (Levkov, 2009). It co-occurs with *A. indistincta* Levkov and both diatoms potentially have indicator values that merit separation from *A. pediculus* (Kützing) Grunow. *Achnantheidium minutissimum* (Kützing) Czarnecki diversity is the most ubiquitous diatom in Ireland. But its diversity is very complex although there has been greater progress in resolving its taxonomy and would allow Irish populations to be more critically examined. However, there are limitations to what can be achieved in light microscopy (Pinseel *et al.*, 2017; Potapova and Hamilton, 2007). A small naviculoid diatom that could not be assigned to a particular genus and temporarily assigned to the nomenclaturally artificial genus *Naviculadicta* Lange-Bertalot was investigated in SEM (Wetzel *et al.*, 2015). It possibly represents a new *Nupela* Vyverman and Compere. The generitype was described from shallow mountain lakes with a peaty bottom, low conductivity and moderately low pH

(Vyverman and Compère, 1991). The genus is relatively uncommon in Irish waters but occurred in several dystrophic lakes with elevated total phosphorus and has a more widespread distribution (Rondón and Catalan, 2017).

The genus *Brachysira* dominates periphyton communities in rivers and lakes although it is also distributed across a wider range of oligotrophic habitats (Werum, 2004). My review will help to improve the precision of future assessments because the range and overlap of morphological characteristics provided in original diagnosis has been expanded in many cases (Kennedy and Allott, 2017). I discovered two new species, *B. praegeri* B.Kennedy & Allott and *B. conamarae* B.Kennedy & Allott. The latter can be definitively identified only in SEM and is an example of former cryptic diversity in the group around *B. procera* Lange-Bertalot & Gerd Moser and *B. microcephala*. The possibility that it merely represented infraspecific variation in one of these diatoms was raised during peer review, given limited critical characters in its description. Similar to *B. garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot, an absence of silica ribs bordering the raphe was identified as a significant character. Variability in this character however has been identified in populations of e.g. *B. brebissonii* R.Ross in Hartley, R.Ross & D.M.Williams, although not in populations examined from Ireland. The combination of characters enabled it to be validly published but it would be beneficial to further validate the hypothesis with supporting molecular data. Endemic species are a characteristic of the genus *Brachysira* (Lange-Bertalot and Moser, 1994). I have observed a population resembling *B. conamarae* in Loch Osgaig in the Highland region in Scotland, but *B. praegeri* has not yet been reported to occur elsewhere and it is more distinctive. It is less easy to validate the

existence of restricted biogeography's with certainty, yet both are confined to near-pristine oligotrophic waterbodies in western Ireland. They merit consideration in the context of conservation and incorporation in a protected Red List (Denys, 2000). The diatom *Frustulia curvata* Kulichová & Urbánková has recently been describe from a single lake, Loughavaul Lower in Connemara, Co. Galway (Urbánková *et al.*, 2015). Despite extensive sampling across European habitats it has not been found in any other region outside the type location and is reported to be rare. The occurrence of a further two rare populations of *F. curvata* in my data, in Lough Beaghcauneen in Connemara and Lough Barra, Co. Donegal, awaits confirmation with SEM given the close resemblance of this diatom to *F. crassinervia* (Brebissoni ex W.Smith) Lange-Bertalot & Krammer. Incorporating Irish samples however, Veselá *et al.* (2012) established molecular diversity and geography to be closely linked in a pan European study of *Frustulia* Rabenhorst. Distinctive patterns in the occurrence of *Frustulia* between western Ireland and the Aquitaine region in France that were absent elsewhere have a significant biogeographical relevance. It demonstrates that studies of diatoms like *Frustulia* and *Brachysira* also have a more fundamental significance in addition to use in water quality assessments. Detailed studies are required to provide a full understanding of diatom geographic distributions that is linked to both environment and biogeography (Urbánková *et al.*, 2016). As already established in a recent study of the Desmidiáles (John *et al.*, 2011), my thesis confirmed previously unknown algal diversity in near reference lakes in the western periphery in Ireland, but this algal diversity remains to be further explored. Without a greater focus to address the "missing-species pool" then it can be reliably stated that the freshwater algal flora will remain only partially

known (De Clerck *et al.*, 2013). This is not unexpected in terms of the accepted globally undocumented protist diversity and in light of continuous new diatom findings even in some of the most intensively studied regions in the world (Van De Vijver *et al.*, 2020). However, when considered that the correct specific epithet for even dominant populations of *Brachysira* and *Encyonopsis* could not be determined with certainty is remarkable.

The ultrastructure of a diatom with a close affinity to *B. intermedia* (Østrup) Lange-Bertalot was documented. SEM images of *B. intermedia* confirm it as a new species but type material for the nominate population will be more comprehensively investigated to include SEM analysis before it is published (Rondón and Catalan, 2017). The morphotype is more abundant than *B. intermedia* and is widely distributed elsewhere (Lange-Bertalot *et al.*, 2017). I concluded that heterogeneous groups of morpho-species in *B. microcephala* (Grunow) Compère (*B. neoexilis* Lange-Bertalot in Lange-Bertalot & Gerd Moser) required levels of molecular variation within their populations to be established before new species could be diagnosed. However, further diatoms with a close affinity to these *Brachysira* morphotypes have been formally described and will need to be considered in future analyses (Bulatov, 2020; Mora *et al.*, 2017). Many of the morphotypes are challenging to identify in light microscopy and further cryptic diversity is highly likely as established for model species including *Sellaphora* Mereschkowsky and *Pinnularia* Ehrenberg (Pinseel *et al.*, 2019; Vanormelingen *et al.*, 2013).

All of the reported *Brachysira* were clearly oligotraphentic with total phosphorus optima below the 10 $\mu\text{g}^{-\text{L}}$ -P detection limit. Differences in environmental optima

between these taxa are not significant therefore for environmental assessments based on traditional weighted averaging metric approaches (Zelinka and Marvan, 1961). There are some exceptions to this general conclusion. *Brachysira vitrea* (Grunow) Ross in Hartley, *B. neglectissima* Lange-Bertalot and *B. liliana* Lange-Bertalot in Lange-Bertalot & Moser are predominately alkaliphilous relative to analogous populations of *B. microcephala* and *B. garrensis* that occur in circumneutral to acidophilous waters (Van Dam *et al.*, 1994). Like occurred in *Encyonopsis* the better separation of taxa provides more realistic multivariate dispersions in community analysis, or if diatoms are needed to validate alkalinity-based lake typologies. A further exception is *B. garrensis* which had a lower pH optimum and a particularly high relative abundance in two heavily modified coastal peatland lakes impounded for water abstraction. The role of pH, dissolved organic carbon and coastal influences on water chemistry and how they affect the distribution of this diatom will need to be further explored. However, changes in its relative abundance relative to declines in *B. microcephala* might have an application where pH or dissolved organic carbon flux is significant. The taxonomic identity of *B. procera* could not be established with certainty and a recommendation was to assign a diatom sensitivity score equivalent to *B. microcephala* for ecological status assessments.

The taxonomic challenge within the former *Cymbella microcephala* complex was even more problematic but there was greater value demonstrated for trophic assessments after these were resolved in my study. Given uncertainties in the morphology of *Encyonopsis alpina* Krammer & Lange-Bertalot, *Encyonopsis angusta* Krammer & Lange-Bertalot and *Encyonopsis tavirana* Krammer and a lack

of good quality SEM images, I re-investigated their ultrastructure using original type material. Unfortunately, only the type slide of *E. angusta* was available so SEM observation was not possible. Nevertheless, on the basis of nomenclatural priority I proposed a synonymy with *Encyonopsis krammeri* Reichardt. Populations of *E. krammeri* in Irish marl lakes closely resembled those in the type slide of *E. angusta*. My ultrastructural analysis also provides greater certainty in the species concepts for *E. alpina* and *E. tavirana* and all of the other dominant small celled *Encyonopsis* in Irish waters. Records attributed to some of these diatoms in the past will need to be revisited as a consequence of the greater range of phenotypic variability that was documented. Two diatoms that closely resembled *E. alpina* and *E. tavirana* were widespread in freshwater samples and should be prioritised for further investigations given differences in their environmental indicator values. Ironically, the apparently cosmopolitan *Encyonopsis microcephala* (Grunow) Krammer was not encountered but the recently described *Encyonopsis neerlandica* Van de Vijver, Verweij, Van der Wal & Mertens was widespread in oligotrophic to mesotrophic lakes. I discovered two new diatom species, *Encyonopsis carraensis* B.Kennedy, Y.Buckley & Allot and *Encyonopsis hibernica* B.Kennedy, Y.Bukley & Allott and established differences in their autecology that is beneficial for monitoring lake water quality (Kennedy *et al.*, 2019).

Perhaps linked to the prominence of the widely invasive and ecologically problematic diatom *Didymosphenia geminata* (Lyngbye) Mart.Schmidt in A.W.F.Schmidt, speculation on the invasive potential of other taxa with rapidly expanding ranges in Europe has arisen (Bothwell *et al.*, 2009; Bothwell *et al.*, 2014; Pérès *et al.*, 2012; Rimet *et al.*, 2010). One such diatom that might be considered in

this context is *Encyonopsis horticola* Van de Vijver & Compère. *E. horticola* was first described from a small enclosed pond in the national botanic garden of Belgium (Van de Vijver *et al.*, 2009). Physicochemical data was unavailable but macrophyte and diatom communities were indicative of mesotrophic to meso-eutrophic conditions and therefore considered to be less typical of the *C. microcephala* complex. I confirmed a single population from meso-eutrophic Lough Gur and more recently confirmed it for the first time in the UK in the ecologically comparable mesotrophic lake Kenfig Pool, Wales. Outside of the type location, this is a rare diatom in Europe but isolated reports from France and the Iberian peninsula are equivalent to the Irish and Welsh observations (Novais, 2011). It occurred in Portugal in Odeleite Reservoir where it also had a low abundance in nutrient enriched waters. An increased prevalence of this diatom in further anthropogenically influenced lakes might offer evidence for the hypothesis of an expanding range, but it is its increased tolerance to enriched waters that is also relevant. Given differences in trophic optima established with weighted averaging, *E. horticola*, *E. hibernica* and *E. cf. tavirana* should be separated from other oligotraphentic diatoms in the former *C. microcephala*-complex to maximise the precision of diatom metrics.

5.4. RECOMMENDATIONS

While it incorporated a larger number of lake types and condition than investigated previously it is recognised that this study provided only a provisional look at diatom ecology in Irish lakes. There were important gaps in environmental gradients that

could be filled with more detailed studies. Some lake conditions are lower in frequency across the Irish landscape as a consequence of natural and anthropogenic factors but targeting smaller waterbodies to improve representation of high-status moderate alkalinity lakes would be beneficial. Combinations of the identified clusters provides a template to organise more focussed research.

An upland lake study should be prioritised. Little is known about these waterbodies and a proportion are impacted by depositional processes or are prone to projected climate effects as has already been demonstrated internationally. Another interesting question relates to the extent that epilithic assemblages in high alkalinity limestone lakes, that are also calcifying, relate to marl lake littoral assemblages with more extensive crusts of *Schizothrix fasciculata* Gomont ex Gomont. There are gradual transitions in this habitat type in larger western lakes that would facilitate within lake studies. These might be combined with nutrient diffusing substrata to allow hypotheses over differences in phosphorus limitation in the littoral to be tested. Linkages between the decline in marl crust integrity related to phosphorus and dissolved organic carbon loading and how it is reflected in diatom metrics needs to be more clearly established.

The diatom flora in habitats of high conservation value have also been infrequently investigated in Ireland. These include bogs, springs, turloughs, estuaries and saltmarshes where diatoms are functionally important. Petrifying springs have even higher rates of dispersal limitation with localised environmental selection pressures and offer a particularly exciting prospect for study. It would allow the sub-

disciplines of diatom taxonomy, ecology and biogeography to be combined and examined in the context of the investigations of spring habitats in continental Europe.

The next stages of development for lake phytobenthos assessment methods should be an integrated study to incorporate the precision of diatom-based monitoring with the flexibility of macroalgal assessments to identify the most parsimonious algal groups to include in a periphyton multimetric.

Morphometric methods such as sliding landmarks could be used in follow up taxonomic studies to resolve some of the taxonomic challenges that have arisen. But molecular characterisation would add greater value in terms of populating DNA reference libraries to provide the basis for future metabarcoding applications (Rivera et al., 2018). A national diatom flora to update the seminal work of Niels Foged will also be pursued (Foged, 1977).

Regression methods should be used to test for statistical significance of environmental optima and to explore potential dependencies in environmental gradients, lakes and diatom species interactions.

APPENDIX J

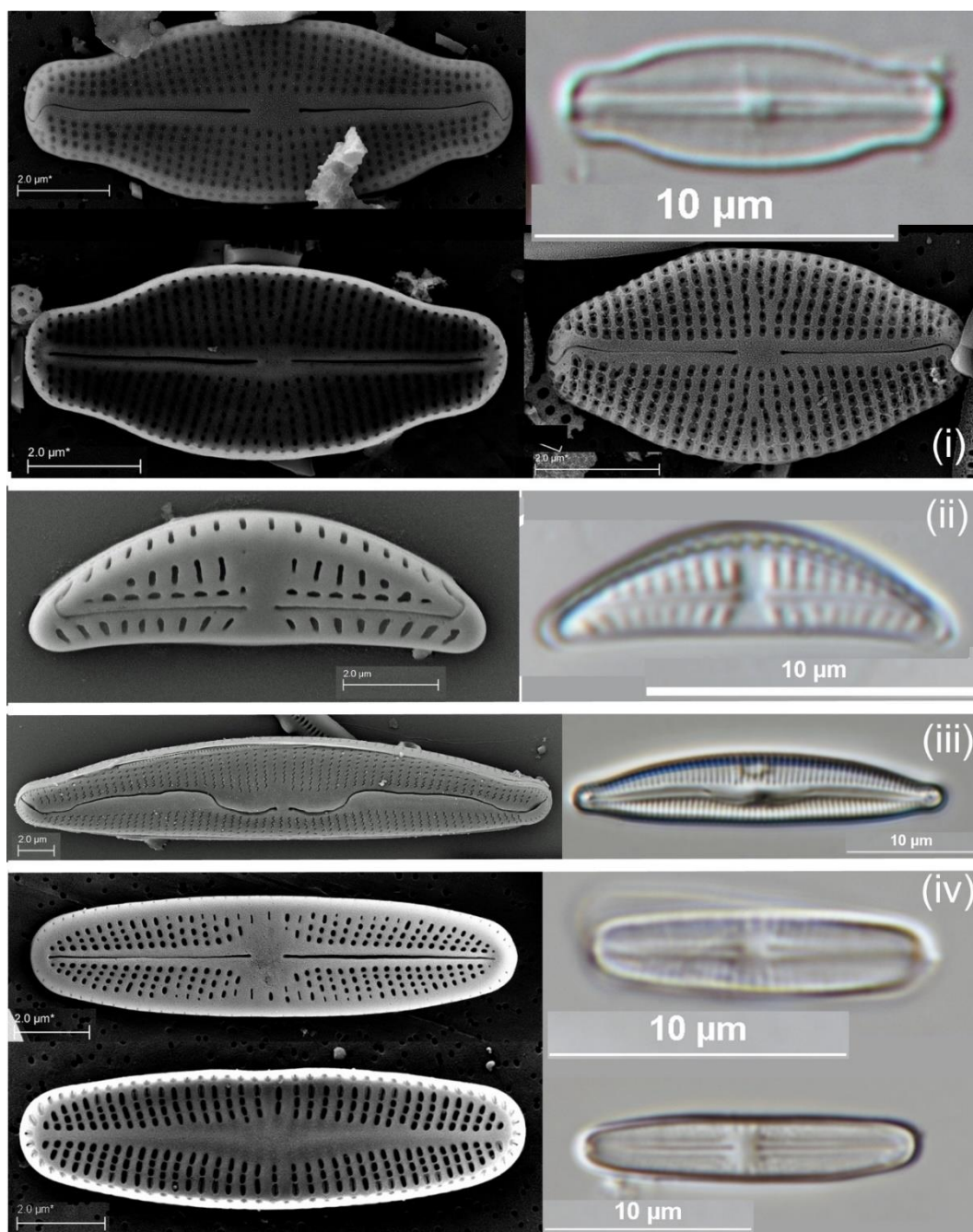


Figure J.1 Light and scanning electron microscope images of unidentified diatoms from the survey of Irish lake epilithon sample (i-iv). *Naviculadicta* or *Nupela* sp. (i). *Amphora* sp. (ii). *Delicatophycus* sp. (iii). *Achnanthidium* sp. (iv).

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