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Algal biodiversity of New Zealand wetlands

Distribution patterns and environmental linkages

Cathy Kilroy and Brian Sorrell



Cover: Bealey Spur wetland study area. *Photo: Cathy Kilroy.*

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Distribution patterns and environmental linkages

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Abstract

Algal communities in wetlands have received little attention in New Zealand despite their significant role as primary producers and their increasing use overseas as indicators of wetland condition. This important gap in knowledge of wetland biodiversity was addressed through a series of studies aimed at: 1) quantifying spatial and temporal variability in algal communities in wetlands; 2) exploring linkages between algal communities and local/regional environmental variables; and 3) documenting algal taxa in relatively unimpacted wetlands throughout New Zealand. In a small-scale study in an alpine wetland (a tarn complex) aimed at determining what optimum sampling intensity may be required to represent species diversity and the abundances of common taxa, we found that four to five samples appeared sufficient. In the same wetland, large differences in community composition between sites were associated with differences in alkalinity, water colour (determined as absorbance at 440 nm; gilvin) and pH. However, characteristic community composition was retained over at least two years. In a New Zealand-wide study, relatively unimpacted lowland wetlands had high algal diversity compared to that of other taxonomic groups. Algal communities differed between the North and South Islands, with differences attributable to inter-island differences in water conductivity, pH and dissolved nutrients. Within islands, nutrient, catchment and geological variables explained variation, as well as pH. Based on these and other published studies, we make recommendations for survey protocols to aid future studies of wetland algae. We also discuss the potential for use of specific indicator taxa to support conservation and restoration of New Zealand's wetlands.

Keywords: algae, biodiversity, sampling, wetlands

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1. Introduction

Since the Ramsar Convention was signed in 1971, the significance of wetland ecosystems has been increasingly recognised internationally (Ramsar Convention Secretariat 2006). Many governments worldwide no longer actively encourage wetland destruction by drainage and conversion to agricultural or urban usage (Mitsch & Gosselink 2007), but now value wetlands for their ecological services and often unique biodiversity. Nevertheless, in New Zealand, despite the early signing of the Ramsar Convention in 1976, wetland management has not always been effective (Gerbeaux 2003). The land area under wetlands is currently only about 10% of its historic extent and wetlands continue to be drained and converted (Ausseil et al. 2011; Myers et al. 2013), especially in lowland and coastal areas.

The term 'wetland' covers a wide range of habitat types distinct from rivers and lakes, with the common feature of being 'permanently or intermittently wet areas, shallow water or land/water that support a natural ecosystem of plants and animals that are adapted to living in wet conditions' (definition in the New Zealand Resource Management Act 1991, and see also Sorrell & Gerbeaux 2004). Classification of wetland ecosystems in New Zealand is based on a functional classification using a hierarchical approach, starting at the top with hydrosystem type (e.g. riverine v. palustrine), then wetland class (e.g. bog, fen, swamp, etc., as defined by soil type, water regime, nutrients and pH) and, finally, structural class (whether vegetated or not), with dominant vegetation composition at the lowermost level of the classification, leading to a variety of wetland types (Johnson & Gerbeaux 2004). Different biodiversity values are associated with the various wetland types and their characteristic habitats.

Small organisms, including algae, play fundamental roles in wetland aquatic food webs. Although microscopic, algae can collectively account for a significant component of primary production in wetlands (Goldsborough & Robinson 1996). Algae can form an important resource for herbivorous invertebrates and fish, thus underpinning some wetland food chains (Ewe et al. 2006). Because algal species often have characteristic pH, nutrient availability and light requirements, algae have long been used for bioassessment purposes in streams and rivers (e.g. Kelly & Whitton 1995; Prygiel et al. 1999), and are increasingly being used in wetlands overseas (e.g. Mayer & Galatowitsch 2001; Zheng & Stevenson 2006; Gaiser 2009). General guidelines for their use as indicators are available (e.g. Stevenson et al. 2001). Scope for the use of indicator algal species to provide early warning of changes in condition, including achievement of restoration goals, has also been demonstrated (Lougheed et al. 2007).

Despite the crucial roles and potential use of algae in wetlands, until recently understanding their biodiversity has been relatively neglected in New Zealand. Early work on freshwater algae in New Zealand consisted of identifying taxa from collections from a range of areas, covering various taxonomic groups (Cooper 1994). In relation to wetlands, New Zealand's desmid flora, which is characteristic of wetland habitats, has been comprehensively described in a three-volume taxonomic publication (Croasdale & Flint 1986, 1988; Croasdale et al. 1994). There have also been surveys of the algal flora of the Te Anau mire (Skuja 1976) and, more recently, the diatom flora of some West Coast wetlands (Gerbeaux & Lowe 2000; Beier 2005; Beier & Lange-Bertalot 2007). A survey of diatom communities in small, pristine water bodies in the South Island in 2001 included wetland pools (Vanhoutte et al. 2006), and a study of diatom communities in a range of freshwater habitats concluded that physically stable, pristine habitats, such as bogs, tended to harbour a higher proportion of taxa thought to be endemic to New Zealand (Kilroy et al. 2008). All this information provides a substantial baseline that can be built upon for future assessments of conservation values (e.g. assessments of biodiversity). However, an understanding of algal communities in New Zealand wetlands across environmental and geographic gradients is lacking. Furthermore, there have been no attempts to determine whether or how algal communities are altered following wetland modification, to evaluate algae

as indicators of change, or to assess the value of wetland algal communities in terms of their indigenous or endemic component. Indeed, a synthesis of the biodiversity of various taxonomic groups in major wetlands globally, stated that: 'Periphytic algae have not been assessed at all' (Junk et al. 2006).

This report is a result of a programme initiated with funding from the New Zealand Department of Conservation and supported by the New Zealand Foundation for Research, Science and Technology to address gaps in our knowledge of the diversity, distributions and environmental drivers of aquatic invertebrates and algal communities in wetlands. Studies in this programme since 2004 have improved knowledge of aquatic macroinvertebrates in wetlands (Suren et al. 2008; Suren & Lambert 2010; Suren & Sorrell 2010). Here we present a synthesis of parallel studies on algae in New Zealand lowland wetlands, and also the results of detailed research in a single alpine wetland complex, which were conducted as part of a doctoral study (Kilroy 2007). Together, the studies represent a first attempt to provide information on the distributions and diversity of algae in New Zealand wetlands at a range of scales. The studies were restricted to algal communities associated with substrata (bottom sediments (Benthos) or plant surfaces) in permanent water bodies within wetlands. We did not investigate the effects of variations in water permanence on communities, but acknowledge the importance of addressing this in future studies, because human impacts on wetlands almost always involve hydrological changes.

Although all algal groups were sampled, most attention has been paid to diatom communities. Of all the algae, diatoms are the most speciose, the easiest to identify to species level without necessity for laboratory culture, the most straightforward to preserve, and the most commonly used for wetland assessments overseas (U.S. EPA 2002).

In addition to providing new information about algal patterns (over space and time) and biodiversity values in permanent wetland water bodies in New Zealand, this synthesis aims to contribute to the management of wetlands. In particular, we evaluate the potential for using algae in guiding assessments of the condition of the aquatic component of New Zealand wetlands.

In this report, section 2 describes investigations into spatial and temporal variability of diatom community composition, and relationships to environmental variables, in a single wetland (a tarn in a subalpine fen complex), to inform survey design. Section 3 comprises the results of a survey of algal communities (non-diatom and diatom taxa) in lowland wetlands throughout New Zealand, and preliminary analyses to investigate environmental and geographic patterns. In section 4, recommendations for algal sampling and survey methodology are presented, followed by an evaluation of the potential for use of algae as indicators of wetland condition and a consideration of the implications for conservation.

2. Spatial and temporal variability in benthic algal communities in an alpine wetland

2.1 Introduction

Freshwater algal species composition varies over both space and time at a range of scales. For example, small-scale variability in benthic diatom community composition in streams can be largely explained by local water velocity (Passy 2001), while temporal variability across larger scales can result from differences in nutrient supplies and disturbances (flood frequency) (e.g. Biggs & Smith 2002). Algal community variability over time can result from seasonal changes in temperature and light availability (Biggs 1996). In wetlands, other environmental factors, such as pH and nutrient concentrations, may also vary seasonally (Kilroy et al. 2008). These factors may change in a wetland because of restoration measures or catchment development (e.g. Lundin & Bergquist 1990; Cummins & Farrell 2003). The extent to which algal community composition responds to such seasonal and non-seasonal changes in wetland water quality is unknown.

Suren & Lambert (2010) found that temporal changes in wetland invertebrate communities did not mask differences between wetlands. For algae, it would also be useful to know whether seasonal differences are large enough to influence the design of sampling programmes. In this section, we describe investigations into the variability of diatom communities over space and time using data collected in a subalpine fen wetland in Canterbury. We also investigated the numbers of samples needed to characterise diversity and community composition and the effects of using different types of data (e.g. cell densities, presence/absence data), with a view to assisting with study design of future wetland monitoring programmes.

2.2 Study area

Field sampling was carried out between September 2001 and July 2004, in a subalpine fen wetland area (1030 m a.s.l.) at Bealey Spur, near Arthur's Pass, Canterbury, South Island, New Zealand (Fig. 1). The wetland comprises numerous pools ranging from approximately 4400 m² down to a few square metres, in an area 900 m by 150–200 m. The western side of the wetland is at the foot of a hillslope and presumably receives subsurface flows. The central area is slightly elevated relative to the immediate surrounding area. The eastern part has no obvious stream inputs, but has at least three outflow streams. pH measurements taken across the wetland ranged from pH 5 to 7, which indicates that this is a fen system (Johnson & Gerbeaux 2004). The wetland is on public conservation land and is largely unmodified.

2.3 Small-scale (cm to m) spatial variability

The aims of this study were to investigate the small-scale spatial variability (cm to m) of benthic diatom communities, and to determine the numbers of samples needed to characterise diversity and community composition. The survey was undertaken in the largest pool (approximately 4400 m²) in the wetland area (pool 2 in Fig. 1A). This pool had no obvious surface inputs or outputs. The survey was carried out in about 100 m² of shallow water on the eastern shore (see Fig. 1A), which had a visibly uniform substratum of consolidated algal mat.

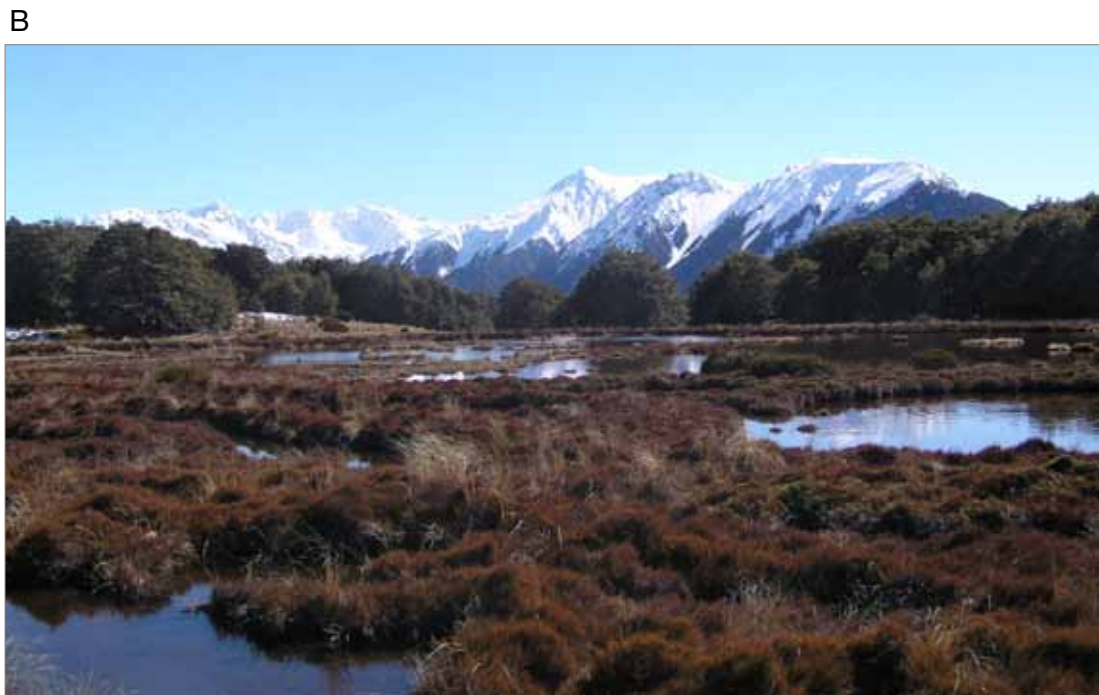
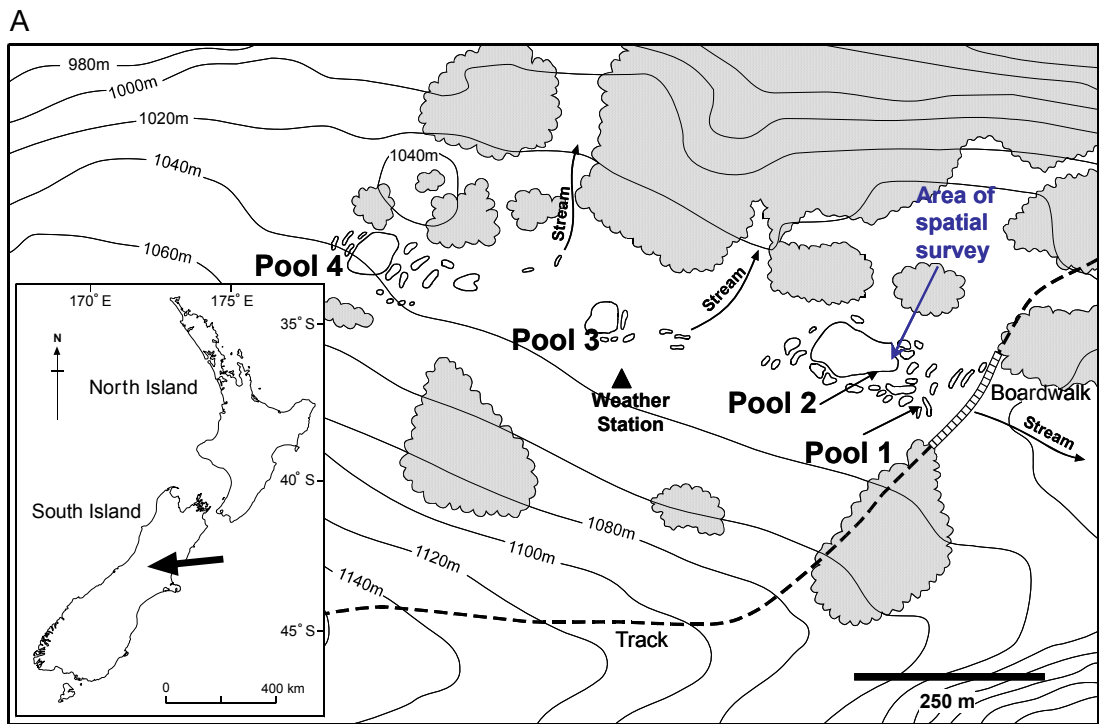


Figure 1. Bealey Spur wetland study area. A. Map showing locations of the spatial study (section 2.3) and the four pools sampled over time (section 2.4). Note that not all of the small pools in the wetland are shown. The shaded areas are beech forest. B. Bealey Spur wetland, looking northwest from pool 1.

2.3.1 Methods

Samples were collected from a floating platform to minimise substratum disturbance. Sampling was at 19 points on a 2-m grid, starting about 0.2 m out from the pool margin. Distances on gridlines perpendicular to the pool edge (y gridlines) were measured, and distances on parallel (x) gridlines were guided by the observer controlling the platform from the bank. There were only three points at the 0.2-m distance because of a discontinuity in water depth. At each sampling point, a Petri dish 50 mm in diameter and 10 mm deep was inverted and gently pushed into the substratum. A sheet of rigid plastic was then slid across underneath the dish, to enclose a shallow

'core' of surface substratum. Previous sampling had established that most of the live diatoms were in the surface layer to about 8 mm deep. Three subsamples were then taken (about 1.5 cm apart) from each 50-mm core by pushing the cut-off end of a 10-mm diameter syringe to the bottom of the Petri dish and withdrawing the resulting small plug. Samples were preserved in 2% glutaraldehyde within 3 h of collection. At each sampling point, water depth was measured and the consistency of the substratum noted. Because of wind mixing of the water, and the absence of surface flows into the pool, it was assumed that the overlying water was more or less uniform in chemical composition. Absence of consistent gradients in water chemistry has been confirmed for much larger wetland water bodies (e.g. Weillhofer & Pan 2006). Field measurements of pH and conductivity at multiple points on the pool confirmed narrow ranges of pH (6.4–6.8) and conductivity (10–12 $\mu\text{S}/\text{cm}$).

In the laboratory, each sample was homogenised for 10 s, then made up to a known volume with distilled water. Two aliquots of 0.5 mL were pipetted into the chamber of an inverted microscope (Leica Diavert). Counts of the live diatoms in each sample were made across two to three transects of the chamber, viewed at a magnification of 400 \times . Up to 600 live cells, defined as cells containing chloroplasts, were counted per sample. Counts of 400 to 600 are generally recommended as adequate to represent the diversity in most diatom communities (e.g. Pan et al. 1996; and see discussion in U.S. EPA 2002). A trial in the present study also showed that calculated abundances for most species stabilised with counts of 300 to 400 cells. Diatom identifications were confirmed from permanent slides of cleaned diatom frustules, prepared using standard methods (Round et al. 1990) and examined at 1000 \times . Identifications were made using a wide range of taxonomic texts (refer to Kilroy 2007 for the complete list of references). Mean biovolume for each species was estimated by measuring the dimensions of up to 30 individual cells from randomly selected samples, then converting these to volumes based on the shape of the cell (cylinder, ellipsoid, etc.).

2.3.2 Data analyses

All cell counts were normalised to numbers per mm^2 and the data were converted to biovolumes. Biovolumes can be important because they can better reflect the dominance of different species in a community than cell counts. For example, a cell of *Neidium iridis* is >250 times larger than a cell of *Kobayasiella* sp. Percentage numbers and biovolumes were also calculated. Finally, the data were reduced to a matrix of presence (1) or absence (0). Thus, there were five datasets available for analysis.

We used non-parametric Analysis of Similarity (ANOSIM routine, PRIMER v. 6) to investigate the similarity of communities along the x and y axes of the sampling grid, and within and between gridlines. For each of the five datasets, we ran two nested ANOSIMs: samples at each point nested within gridlines parallel to the pool edge (x); and samples nested within gridlines perpendicular to the pool edge (y). Spatial changes in densities of individual taxa were checked using non-parametric Kruskal-Wallis tests. Because the substratum consistency at three of the sampling points was obviously less consolidated than the area in general, we also checked to see whether this affected community composition. Cell densities and biovolumes were log-transformed prior to analysis to downweight the effect of abundant taxa, as recommended by Clarke & Warwick (2001).

We estimated the numbers of samples required to adequately account for the abundance of each taxon, and for overall diversity (as species richness), using an adaptation of a formula discussed by Biggs & Kilroy (2000, p. 18):

$$\begin{aligned} & \text{Approximate number of samples required to estimate the population mean within 20\% of the} \\ & \text{mean, with a probability of 5\% (N)} \\ & = (120 * \text{variance of available samples}) / (\text{mean of available samples})^2 \end{aligned}$$

The formula was adjusted iteratively if $N < 7$.¹ All count data were log-transformed prior to the calculation, so that the data met the requirements of homoscedasticity and normality of distribution. The number of samples needed to account for taxon richness was also estimated, on untransformed data.

Table 1. Summary of abundances and frequencies of diatom taxa identified from 57 samples collected from 19 sampling points on a 2-m grid in a wetland pool, with the number of samples required to produce reliable estimates of mean abundance for the common species. Taxa named with a letter (e.g. sp. A) refer to species morphotypes recognised by Kilroy (2007), but not formally described.

TAXON	MEAN DENSITY (CELLS PER mm ²)	PRESENCE IN SAMPLES (%)	PRESENCE AT SAMPLING POINTS (%)	ESTIMATE OF NUMBER OF SAMPLES
<i>Kobayasiella</i> sp. A	5423	100	100	2
<i>Eunophora</i> cf. <i>oberonica</i>	767	100	100	3
<i>Brachysira brebissonii</i>	434	100	100	2
<i>Encyonema neogracile</i>	761	98	100	4
<i>Kobayasiella parasubtilissima</i>	639	98	100	5
<i>Frustulia krammeri</i>	272	95	100	10
<i>Tabellaria flocculosa</i>	162	93	100	10
<i>Frustulia magaliesmontana</i>	150	90	100	4
<i>Tabellaria</i> sp.	61	79	100	35
<i>Stenopterobia</i> sp. A	33	67	95	50
<i>Pinnularia</i> cf. <i>macilenta</i>	78	66	100	50
<i>Frustulia</i> sp. A	67	55	95	>100
<i>Eunotia bilunaris</i> var. <i>mucophila</i>	27	50	84	97
<i>Brachysira microcephala</i>	37	48	79	>100
<i>Achnanthydium minutissimum</i>	41	43	74	>100
<i>Eunotia</i> sp. A [small]	17	38	89	
<i>Encyonopsis</i> sp. A	16	36	79	
<i>Neidium iridis</i>	14	33	74	
<i>Eunophora berggrenii</i>	11	29	58	
<i>Pinnularia biceps</i>	11	29	63	
<i>Brachysira</i> sp. C	12	22	63	
<i>Stenopterobia curvula</i>	6	17	47	
<i>Frustulia</i> cf. <i>cassieae</i>	10	16	47	
<i>Eunotia</i> sp. B [large]	5	14	42	
<i>Brachysira wygaschii</i>	4	14	37	
<i>Pinnularia</i> sp. A	5	12	26	
<i>Diatoma hiemale</i>	8	5	16	
<i>Navicula</i> sp. A	1	2	11	

¹ The factor of 120 arises because: (1) the denominator in Biggs & Kilroy (2000) is the square of the half-width of the confidence interval around the mean, i.e. $(0.2 * \text{mean})^2$, which is the same as $25/(\text{mean})^2$; (2) we combine this with an approximation of the square of the critical value for the t-distribution of 4.8 (taking the critical value as approx. 2.2, applicable to approx. 9–15 samples, the critical value was included in the original formula), and $25 * 4.8 = 120$. This formula underestimates the number of samples required where $N < 7$, because the critical value for the t-distribution changes more rapidly at low N. In this case, N was recalculated, with the aim of matching the final estimated N as closely as possible with the N equivalent to the factor shown in the following table.

N	RECALCULATED USING FACTOR OF:
7	140
6	150
5	165
4	190
<3	250

2.3.3 Results

Water depth at the sampling points was 33–41 cm (mean 37.7 ± 2.44). Since the depth difference was small, it was considered to be ecologically insignificant and was not considered further.

Twenty-eight diatom taxa were identified from the 57 samples. Three taxa were present in all samples (*Kobayasiella* sp. A, *Eunophora* cf. *oberonica*, *Brachysira brebissonii*) and nine were present at all 19 sampling points (Table 1). Examples are illustrated in Fig. 2. Seven taxa were

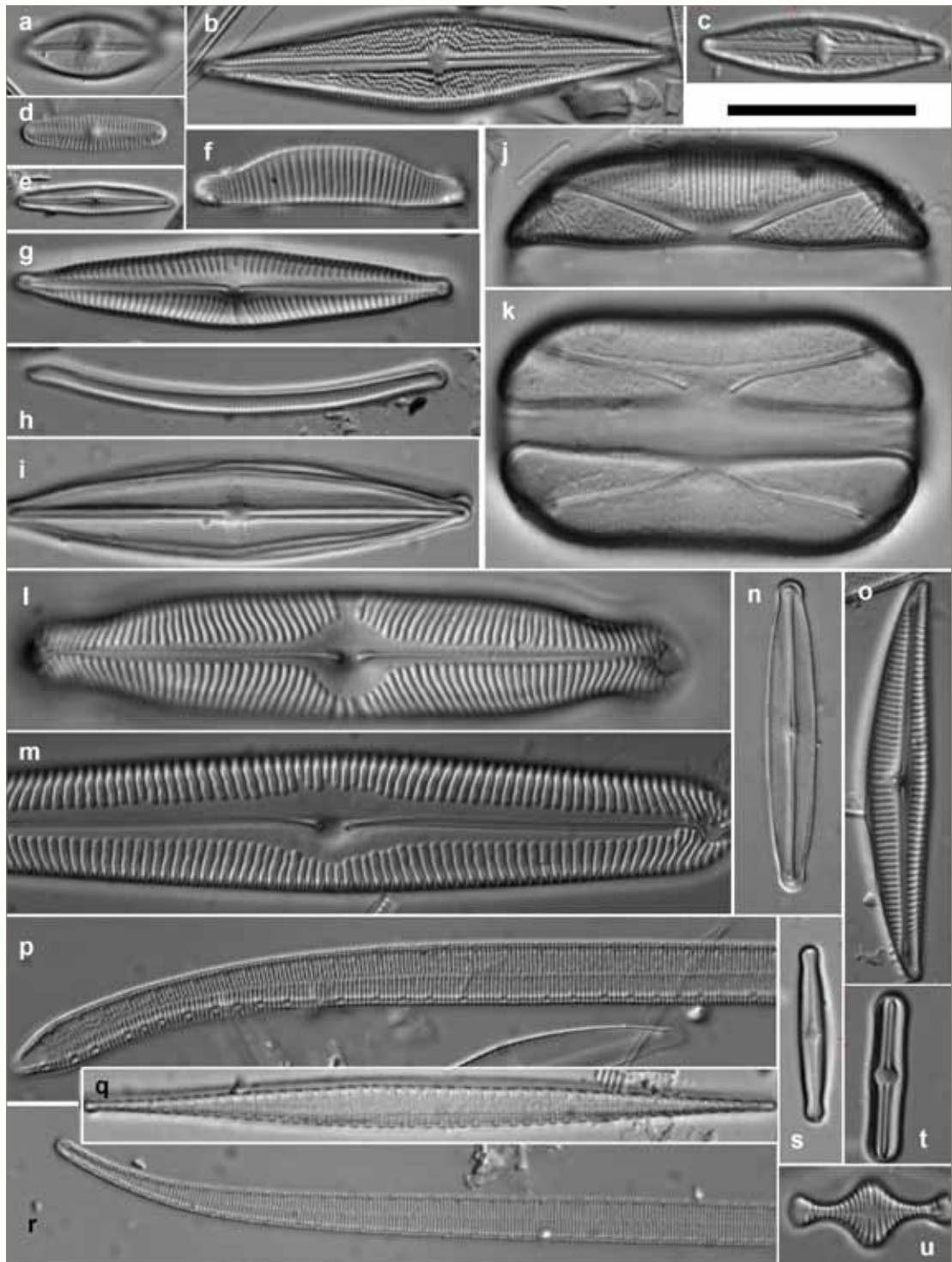


Figure 2. Diatoms in Bealey Spur wetland. (a) *Achnanthes altaica*; (b) *Brachysira wygaschii*; (c) *B. brebissonii*; (d) *Chamaepinnularia* sp. A; (e) *Encyonopsis* sp. B; (f) *Eunotia* cf. *implicata*; (g) *Encyonopsis* sp. A; (h) *Eunotia bilunaris* v. *mucophila*; (i) *Frustulia* sp. A; (j) *Eunophora berggrenii*; (k) *Eunophora* cf. *oberonica*; (l) *Pinnularia biceps*; (m) *Stenopterobia* sp. A; (n) *Kobayasiella* sp. A; (o) *Encyonemane gracile*; (p) *Stenopterobia curvula*; (q) *S. delicatissima*; (r) *S.* sp. A; (s) *Achnanthisidium minutissimum*; (t) *Diadesmis* sp.; (u) *Tabellaria flocculosa*. Scale bar: 20 μ m. Note that not all the species illustrated were encountered in samples from the spatial study, but were found in other pools in the wetland

present in less than 20% of samples. As expected, the most common taxa also tended to be the most abundant (Table 1). Total cell density was 8000–12 000 cells/mm². Mean sample species richness was 14.1.

Regardless of sample collection method or data type, there were significant differences in community similarities between samples within transects in both the *x* and *y* direction (Table 2). However, for quantitative and semi-quantitative samples, there were differences between the *x* transects (transects parallel to the pool margin at increasing distances from the margin), but not between the *y* transects (transects perpendicular to the pool margin). In other words, community similarities (and by inference, community composition) changed more going out into the pool, than along the pool margin. This difference was not quite significant using presence/absence data ($p = 0.057$), but there were still stronger differences between *x* than *y* transects (Table 2).

The raw data for individual diatom taxa revealed that the gradients of species composition out into the pool were caused by abundance changes in a few taxa. *Frustulia krammeri*, *Pinnularia cf. macilenta*, *Brachysira microcephala* and *Tabellaria flocculosa* were more abundant near the pool edge, while *Eunophora cf. oberonica* was more abundant farther out into the pool (Fig. 3). Mean densities of *Eunophora cf. oberonica*, *F. krammeri* and *P. cf. macilenta* also differed significantly

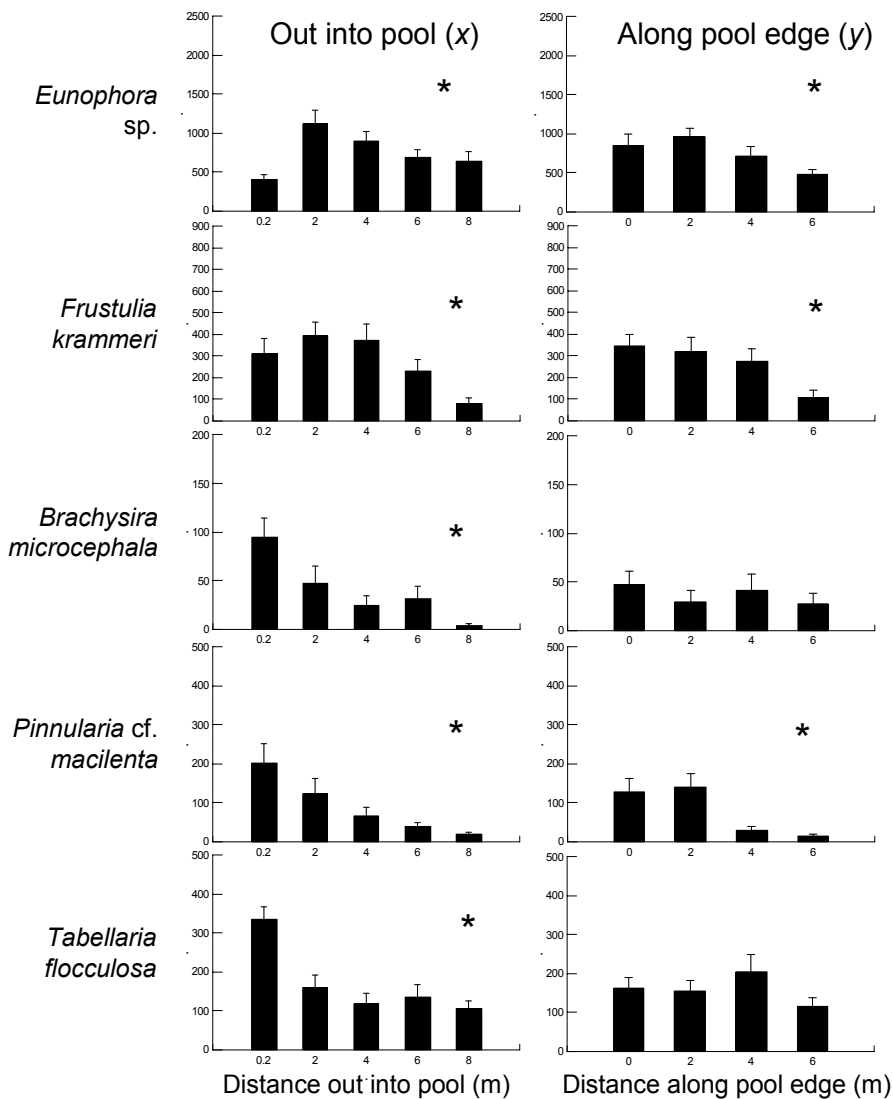


Figure 3. Examples of mean densities of diatom species (number of cells per mm²) determined from samples collected on an approx. 2-m grid in a single wetland pool, with five transects parallel to the pool edge and four transects perpendicular to the pool edge. Three separate samples were analysed at each sampling point. * indicates significant differences in abundances among transects (Kruskal-Wallis $p < 0.001$).

Table 2. Results of nested ANOSIMs (R statistic and p) to test for diatom community differences along the x and y transects of a sampling area in a wetland pool, comparing collection methods and data types. Statistically significant differences ($p < 0.05$) are highlighted in bold.

COLLECTION METHOD	DATA TYPE	ANOSIM ON SAMPLES NESTED IN X TRANSECTS				ANOSIM ON SAMPLES NESTED IN Y TRANSECTS			
		WITHIN TRANSECTS		BETWEEN TRANSECTS		WITHIN TRANSECTS		BETWEEN TRANSECTS	
		R	p	R	p	R	p	R	p
Quantitative	Counts	0.431	0.001	0.191	0.017	0.479	0.001	0.054	0.241
	Biovolume	0.496	0.001	0.169	0.050	0.521	0.001	0.012	0.385
Semi-quantitative (%)	Counts	0.420	0.001	0.250	0.004	0.512	0.001	-0.031	0.630
	Biovolume	0.429	0.001	0.236	0.010	0.493	0.001	-0.095	0.914
Non-quantitative	Presence/absence	0.170	0.010	0.145	0.057	0.188	0.003	0.010	0.426

between y transects, perpendicular to the pool edge (Fig. 3). Densities of 11 of the 15 most common taxa (i.e. the top 15 taxa in Table 1) differed significantly ($p < 0.05$) between x transects (parallel to the pool edge), while only four taxa differed along y transects (perpendicular to the pool edge). Results for percentage data were similar.

Nine of the 57 samples were collected from substratum that was noted as being less consolidated than the rest of the sampled area. Total cell densities were lower in these samples than in samples from the firmer substratum (Mann-Whitney U-test, $p = 0.001$). The difference was caused mainly by lower densities of the two most common species, *Kobaysiella* sp. A and *Eunophora* cf. *oberonica*. This contributed to the decline in abundance of *Eunophora* cf. *oberonica* on y transects (Fig. 3). Densities of *T. flocculosa* were greater in the softer substratum (Mann-Whitney U-tests, $p < 0.05$).

Up to 10 samples (average of five samples) were required to represent the mean densities of the eight most common taxa in the benthos of this pool, if samples were taken from the entire sampling area (Table 1). Estimates of sample numbers required to represent the less common species (i.e. not recorded in more than 20% of the samples) were much higher (e.g. >100 samples, Table 1). However, for a reliable estimate of mean species richness over the entire area (i.e. within 20% of the mean of all samples), only four samples were sufficient.

2.3.4 Discussion

In this single-pool study, we found: (1) stronger gradients in diatom community composition in transects out into the pool (parallel to the pool margin), than in transects perpendicular to the pool edge; (2) to accurately represent the densities of the eight most common diatom taxa across this whole area required about five samples. While the results apply only to the single wetland sampled, the patterns found in this study seem likely to apply more generally.

Community changes at the pool margin are intuitively reasonable because of habitat differences. For example, the filamentous diatom *Tabellaria flocculosa* is typically found associated with submerged vegetation, which was concentrated at the pool margin in this study and may have influenced diatom community composition in the surrounding benthos. Many *Pinnularia* species are typically found in aerophilic habitats (i.e. damp rather than inundated), which could explain higher densities near the edge, through migration from the shallow margin. Depending on aspect, the edge area may be subject to more shading and could be sheltered from, or exposed to, the effects of wave disturbances. Thus, a gradient in community composition away from the pool edge could equally apply at other sites. The community differences associated with changes in substratum texture were also reflected in other pools in the same wetland. For example, *Eunophora* sp. tended to occur in highest concentrations in stable, consolidated substratum (a matrix of cyanobacterial filaments and colonial forms) (Kilroy et al. 2006). A reasonable generalisation would therefore be that diatom community composition is likely to vary as substratum type varies.

Even with gradients associated with distance from the pool edge and substratum texture, only four to five samples, on average, were needed to represent species richness and the abundances of the common taxa. This is consistent with the findings of Weillhofer & Pan (2006), who recommended a composite of five samples to represent species richness across a wetland. In that case, hydrological gradients were highlighted as important in structuring community composition. In the present study, we considered permanent water bodies only and this suggests that a composite sample from four locations over the area of interest is more than adequate to represent benthic diatom diversity of that area.

Collection method (quantitative versus qualitative) and data type (counts versus biovolumes) did not affect the major conclusions about gradients of community composition (shown in Table 2), suggesting that the least time-consuming combination of sample collection and analysis (i.e. qualitative sample collection and analysis by presence/absence) may sometimes be sufficient to describe community structure.

This analysis suggested that the additional effort required to calculate biovolumes was not cost-effective. Other researchers have reported contrasting results. For example, Reavie et al. (2010) found that absolute abundances of river algae always had stronger relationships to water quality and landscape stressors than relative abundances, and biovolumes produced the strongest relationships for periphytic algae. Snoeijs et al. (2002) found that small and large taxa could show contrasting responses to the same environmental variables. In other studies, calculating biovolumes was judged to be unnecessary (Lavoie et al. 2006).

Our analysis demonstrated the value of considering the raw data (i.e. plots of densities of individual species) to help understand whole-community responses indicated by the results of multivariate analyses. In the case of the present series of wetland studies, a raw-data approach is precisely what should prove useful when evaluating taxa as potential indicators of change. This approach has recently been strongly advocated in the literature (Warton 2008).

Because permanently inundated wetlands are relatively stable environments compared to rivers, dramatic changes in algal densities are not expected (such as declines in abundance caused by scouring floods).² It is therefore reasonable to expect that in wetlands, relative abundances might consistently reflect community differences, and quantitative counts may not be essential. This is explored in the following section, in which changes over time are examined in four wetland pools with contrasting diatom community composition.

2.4 Detecting and explaining seasonal and long-term (years) changes in diatom communities

This section describes an investigation into temporal variability in diatom communities in four pools in the Bealey Spur wetland study area. The pools spanned a wide size range and had contrasting water chemistry (Table 3). We expected that a long sampling period would allow detection of both seasonal and non-seasonal changes over time, in both species populations and environmental variables. In section 2.3, spatial variability in diatom communities was equally well detected from both quantitative counts and relative abundance data. In this study, we focused on relative abundances because these data are easier to obtain. We were also able to check absolute densities and biovolumes.

The objectives were: 1) to determine which environmental variables best explained diatom community changes over time in four contrasting environments; and 2) to assess whether the temporal changes detected (both environmental and community) in a natural system need to be taken into account when designing monitoring programmes to evaluate changes in wetland aquatic environments.

² Note that declines in algal abundance in wetlands may occur in exceptional circumstances such as prolonged drought that can cause pools to dry out, or water temperatures to rise to levels that are detrimental to algae.

Table 3. Characteristics of the four wetland pools. Water chemistry variables are means of approximately fortnightly measurements over a two-year sampling period. NO₃-N = nitrate-nitrogen; DOC = dissolved organic carbon; DON = dissolved organic nitrogen; DOP = dissolved organic phosphorus; DRP = dissolved reactive phosphorus.

VARIABLE	UNIT	POOL			
		1	2	3	4
Pool area (approx.)	m ²	40	4400	700	1100
Water temperature	°C (logged at 30 min intervals)	10.1 ± 5.5 (mean ± s.d. for all pools)			
pH		5.3	6.2	6.2	6.4
Conductivity	µS/cm	6.0	8.6	5.9	11.1
Water colour (gilvin)*	g ₄₄₀ (absorbance at 440 nm)	4.07	1.73	0.98	1.96
DOC	g/m ³	15.4	10.5	5.8	8.0
Alkalinity	mg CaCO ₃ /L	0.45	3.08	1.55	4.36
Silica	g/m ³	0.43	0.31	0.32	0.41
DRP	mg/m ³	0.1	0.2	0.15	0.1
NO ₃ -N	mg/m ³	1.73	1.61	1.82	1.33
DOP	mg/m ³	1.25	0.55	0.4	1.02
DON	mg/m ³	280	215	175	206

* Gilvin is a measure of the absorbance (at 440 nm) of yellow substances in the water, and reflects the amount of dissolved humic material present.

2.4.1 Methods

Samples of the benthos in the four pools were collected in the same way as described in section 2.3, every two weeks for over two years (60 sample collections). On each occasion, five separate samples were collected 0.3–0.5 m from the pool margin, and composited into a single sample, which we expected to represent the abundances of the common taxa in the benthos (section 2.3). Care was taken to minimise disturbance to the area around each sample and samples were taken from pre-designated locations 1–3 m apart, to ensure that each spot was sampled only once (i.e. independence of subsamples). All samples were collected in a similar water depth (mean of 23–29 cm). A range of environmental variables was collected on each occasion (Table 3). For more details of collection and laboratory methods refer to Kilroy et al. (2008).

Sample preservation and processing was as described in section 2.3.

2.4.2 Data analysis

Non-metric multidimensional scaling (NMDS, PRIMER v. 6) was first used on all the data to assess differences in community composition among sites. In NMDS similarities (proportion of species in common in a pair of samples, weighted by their abundance) are calculated between all pairs of sites and then ranked. The sites can then be plotted in two (or three) dimensions to provide a visual representation of these rankings; i.e., if sample A is more similar to sample B than to sample C, then A will be positioned closer to B than to C. A stress value for each plot indicates how well the sample similarities are represented in two or three dimensions, with lower values indicating best fit, and up to about 0.15 a reasonable fit.

The data from each site were then analysed separately. The time-series of taxonomic and environmental data were reduced to 20 points by averaging samples over three consecutive sampling occasions. A season was then assigned to each combined sample, according to the months of sampling. Environmental variables used in the analysis were transformed where necessary.

We used ANOSIM to determine whether communities differed over time (comparing groups of five sequential points) or by season (comparing the points grouped according to season). Plots of the raw data were used to examine changes in the relative abundances of individual taxa over time. Non-parametric Kruskal-Wallis tests were run, with 'time' and 'season' as the grouping variables, to determine whether relative abundances of individual diatom species changed over the sampling period, or changed seasonally.

Linkages between community variation and environmental variables between sites and within sites (over time) were explored using the BIOENV routine in PRIMER v. 6. In this procedure, paired similarity matrices generated from the community data (Bray-Curtis similarities) and the environmental data (Euclidean distances) are compared, to find the combination of environmental variables that most closely corresponds to the community matrix. Bray-Curtis similarities were calculated from square-root transformed abundances standardised to % data, and the environmental data were normalised³ before generating the distance matrix. Significance ($p < 0.05$) of the coefficient R was tested using a resampling procedure in which new coefficients were generated from 99 random combinations of the community data. The probability of R being random was taken as the proportion of new coefficients exceeding R .

Species driving community composition differences among pools, and associated environmental variables, were identified by checking correlations with the axes of an NMDS plot.

To identify possible species-environment associations within pools and consistencies among pools, we generated Spearman rank correlation matrices between the relative abundances of individual taxa and environmental variables for each of the pools, and noted all correlations with $R > 0.5$. A total of 91 relationships were tested for each environmental variable (i.e. the sum of the number of species in each of the four pools).

2.4.3 Results

Thirty-one diatom species were included in the analyses (Table 4); other rare species were encountered periodically in low abundance. For examples, see Fig. 2.

Different communities inhabited each pool and samples were clearly separated on an NMDS plot, even using presence/absence data for taxa (Fig. 4). Communities in pools 2 and 3 were most similar, but were still highly significantly different (ANOSIM, $p < 0.001$).

Total diatom abundance and biovolume fluctuated seasonally only in pool 4, where maximum biovolume occurred in late winter-spring (Fig. 5). Temporal trends were evident in many taxa, with some examples of consistency among pools (Table 4). For example, the relative abundance of *Eunophora* sp. generally increased over time in pools 2 and 3, and *Kobayasiella parasubtilissima* first increased then declined over time in pools 2 and 3 (Fig. 6). Fewer species showed seasonal changes (Table 4, Fig. 6). The environmental variables also changed over time, and not necessarily seasonally (Fig. 7).

In pools 1, 2 and 3, different combinations of environmental variables were significantly linked to community composition over the two years, with dissolved organic carbon (DOC) and nitrate-N ($\text{NO}_3\text{-N}$) included in all three combinations (Table 5). No single variables were significantly related to community composition. In pool 4, no combinations of environmental variables were significantly related to community composition. Although we detected changes over time at all four sites (as assessed by significant separation of community composition over four sequential time periods), seasonal community changes were detected only in pool 2, and this signal was quite weak (i.e. low ANOSIM R -value, Table 5).

There were many strong correlations between environmental variables and the abundances of individual species in different pools (Spearman $R > 0.5$, data not shown). There were few consistencies among pools, although this would be expected given that the ranges of environmental variables varied among pools. However, temperature, which had a similar range in all four pools, showed some consistencies:

³ Environmental data are routinely normalised before generating a similarity matrix by calculating the mean for each variable then expressing each datapoint as a percentage deviation from the mean. Normalising the data means that all the variables are given equivalent weight. The procedure is necessary because different environmental variables are expressed in a range of units and their influence on similarity would be affected by the (arbitrary) unit used.

Table 4. Summary of changes over time and by season for individual diatom species in four wetland pools, as indicated by Kruskal-Wallis one-way ANOVA; i.e. ** $p < 0.05$; – indicates no changes over time or season; 0 indicates that the species was not detected in that pool.

SPECIES	POOL:	TIME				SEASON			
		1	2	3	4	1	2	3	4
<i>Achnanthes altaica</i>		0	0	0	–	0	0	0	–
<i>Achnantheidium minutissimum</i>		0	0	0	–	0	0	0	–
<i>Brachysira brebissonii</i>		–	–	**	–	–	–	–	–
<i>Brachysira microcephala</i>		0	0	–	**	0	0	–	–
<i>Brachysira wygaschii</i>		–	**	–	–	–	–	–	–
<i>Chamaepinnularia</i> sp.		**	**	–	–	–	–	–	–
<i>Diadesmis</i> sp.		0	0	0	**	0	0	0	–
<i>Encyonema neogracile</i>		–	–	–	–	**	–	–	**
<i>Encyonopsis</i> sp. A		**	0	0	0	–	0	0	0
<i>Encyonopsis</i> sp. B		0	0	0	**	0	0	0	–
<i>Eunophora berggrenii</i>		**	–	–	–	–	–	–	–
<i>Eunophora</i> cf. <i>oberonica</i>		–	**	**	–	–	–	–	**
<i>Eunotia bilunaris</i> v. <i>mucophila</i>		–	**	–	**	**	–	–	–
<i>Eunotia</i> sp. A [small].		**	–	–	–	–	–	–	–
<i>Frustulia krammeri</i>		–	–	**	–	–	–	–	**
<i>Frustulia magaliesmontana</i>		**	–	–	–	–	–	–	–
<i>Frustulia</i> sp. A		–	–	–	**	–	**	–	–
<i>Kobayasiella parasubtilissima</i>		–	**	**	**	–	–	–	–
<i>Kobayasiella</i> sp. A		**	–	**	–	**	**	–	–
<i>Kobayasiella</i> sp. B		–	0	0	0	–	0	0	0
<i>Navicula</i> sp. A		0	0	0	–	0	0	0	–
<i>Neidium iridis</i>		–	–	–	**	**	–	–	–
<i>Pinnularia biceps</i>		–	–	–	–	–	–	–	–
<i>Pinnularia</i> sp. A		–	–	–	**	**	**	–	–
<i>Pinnularia macilenta</i>		–	–	–	–	**	–	–	–
<i>Rossethidium</i> cf. <i>linearis</i>		0	0	0	5	0	0	0	–
<i>Stenopterobia curvula</i>		0	–	–	**	0	–	–	–
<i>Stenopterobia delicatissima</i>		**	–	–	**	–	–	–	–
<i>Stenopterobia</i> sp. A		–	–	–	**	–	–	–	–
<i>Tabellaria flocculosa</i>		0	–	–	**	0	–	–	–
<i>Tabellaria</i> sp.		0	–	–	**	0	–	–	–
Total number of species changing:		7	5	5	13	6	3	0	3

No correction was made for multiple tests in the ANOVAs because we were interested in comparing the numbers of significant relationships in each pool.

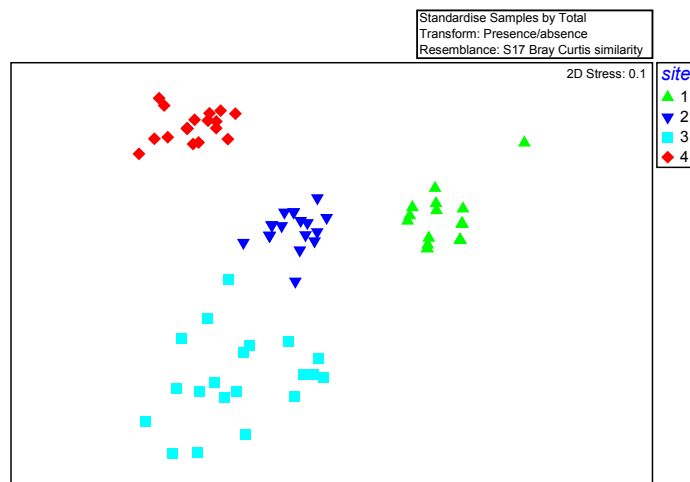


Figure 4. Non-metric multi-dimensional scaling plot of showing the relative similarities of diatom communities in four pools in the Bealey Spur wetland area from samples collected over a period of 2 years. Each point is the mean of samples collected over a month (three samples collected 2 weeks apart).

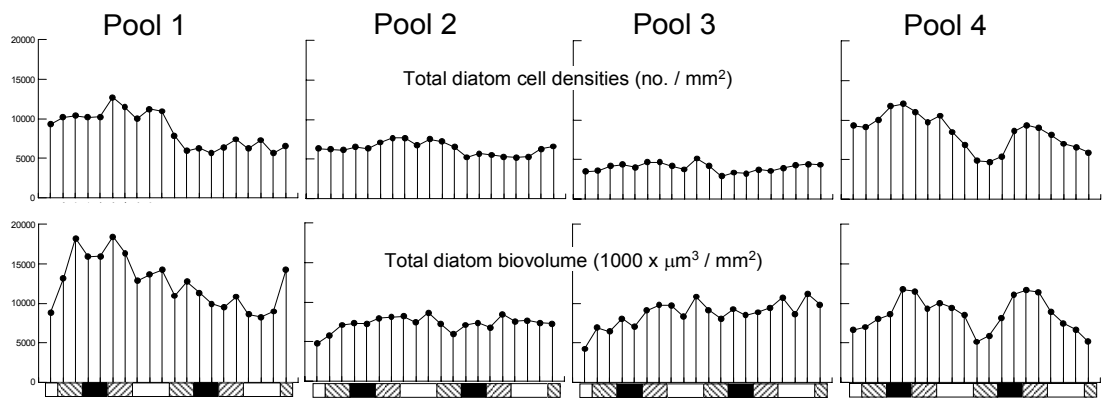


Figure 5. Time-series plots of total cell densities and total biovolumes of diatoms measured over 2 years in four shallow wetland pools. Pools 1–4 had progressively higher mean pH, from 5.1 to 6.5. Each point is the mean of three samples collected 2 weeks apart. On the x-axis (time), filled areas indicate winter, hatched areas indicate autumn and spring, and unfilled areas indicate summer.

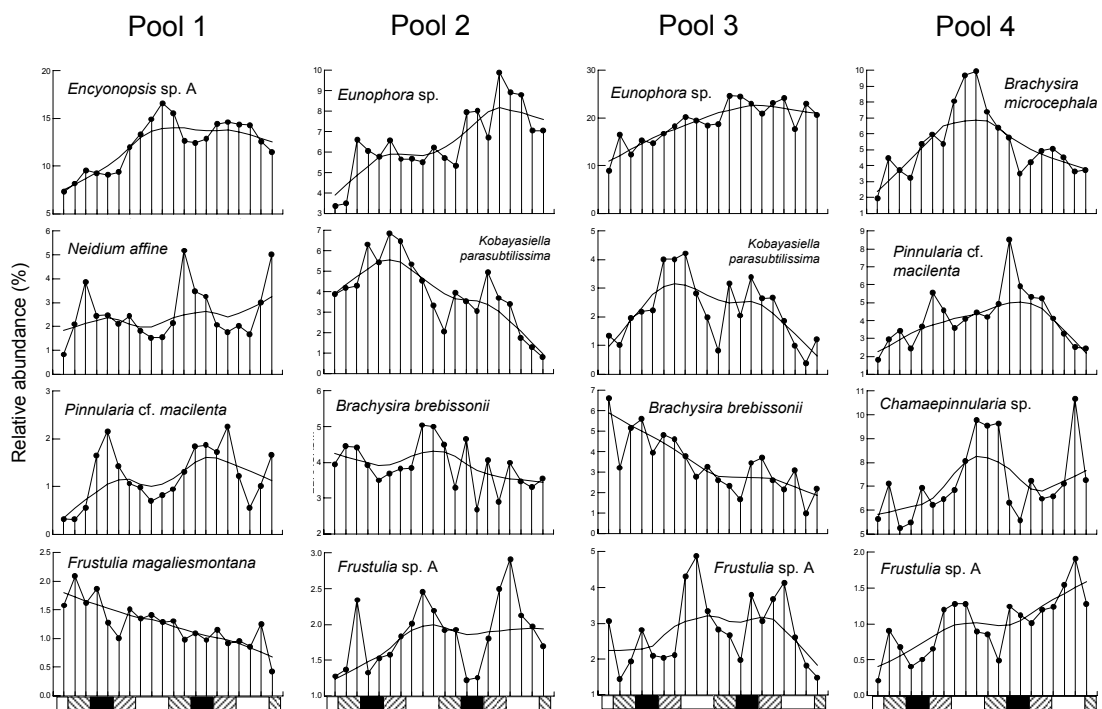


Figure 6. Time-series plots of relative abundances (%) of selected common diatom species measured over 2 years in four shallow wetland pools. Pools 1–4 had progressively higher mean pH, from 5.1 to 6.5. Each point is the mean of three samples collected 2 weeks apart. LOWESS smoothing lines have been fitted through the data to indicate overall trends (time), filled areas indicate winter, hatched areas autumn and spring, and unfilled areas indicate summer.

- Relative abundance of *Frustulia* sp. A was positively correlated with water temperature in both pools where it was common (pools 2 and 3) (and see Fig. 6).
- Relative abundance of *Pinnularia* cf. *macilenta* was negatively correlated with water temperature in all four pools.
- *Kobayasiella* sp. (the most numerically common species in all four pools) had strong negative correlations with temperature in pools 1 and 4, but not in pools 2 and 3. This species was also negatively correlated with dissolved reactive phosphorus (DRP) in pools 1 and 4, but not in pools 2 and 3.

The highest number of strong correlations (Spearman $R > 0.5$) with individual taxa was for DRP (16 cases out of a possible 91 combinations), followed by mean water temperature over 10 days (14 cases), and total dissolved phosphorus (TDP) (10 cases).

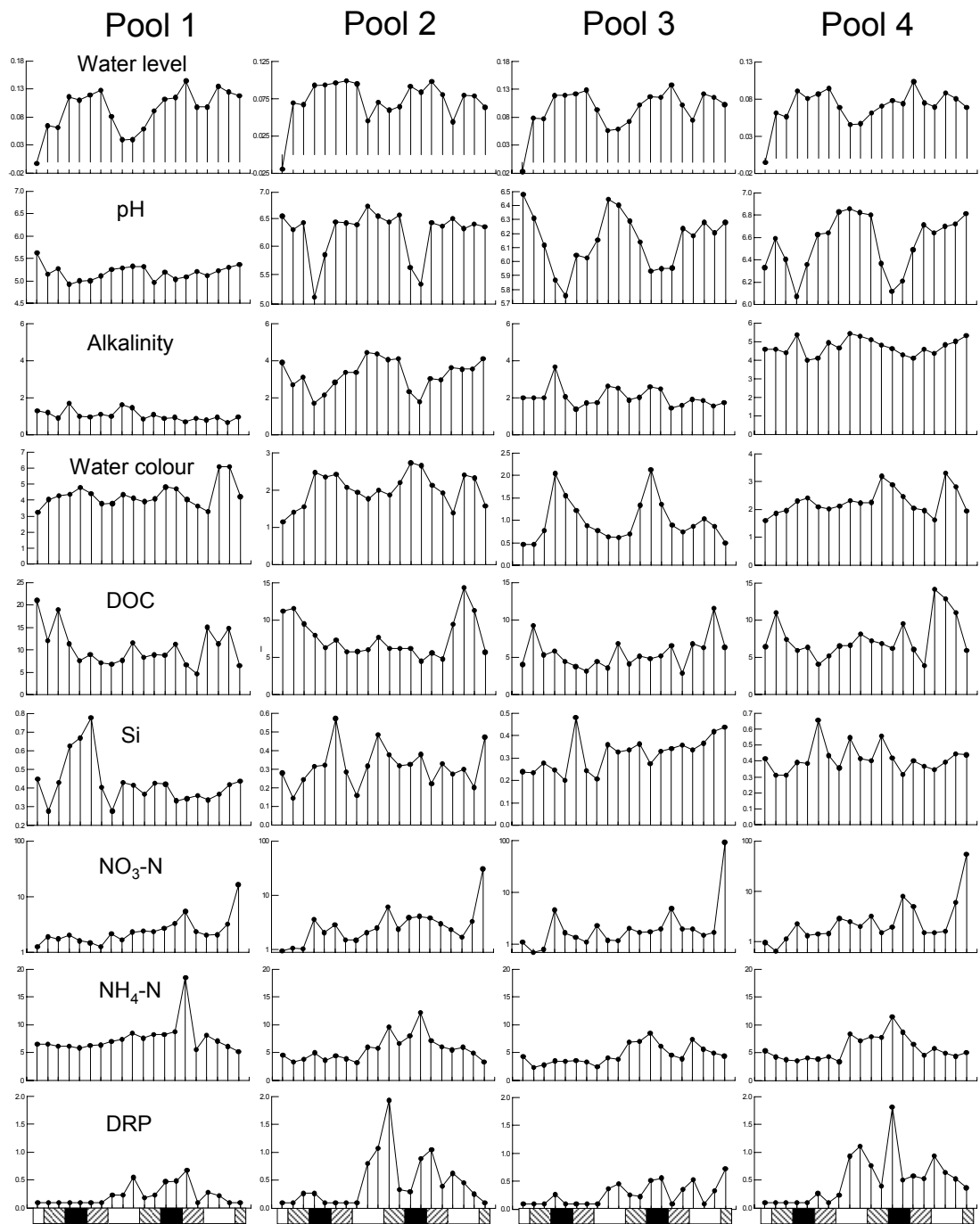


Figure 7. Plots of changes over time for selected environmental variables in each of the four wetland pools. Each point is the mean of three samples collected 2 weeks apart. On the x-axis (time), filled areas indicate winter, hatched areas indicate autumn and spring, and un-filled areas indicate summer. Water level in m relative to the level on the first sampling occasion. Other units as in Table 3. $\text{NH}_4\text{-N}$ is ammoniacal nitrogen.

In spite of mainly non-seasonal changes in environmental variables and diatom community composition, each pool retained its distinctive community composition over the 2-year sampling period. BIOENV run on the samples from all pools showed that similarities among the communities were best explained by a combination of alkalinity and gilvin (water colour) ($\rho = 0.801$, $p = 0.01$). Gilvin (see Table 3) was the best single explanatory variable ($\rho = 0.700$, $p = 0.01$), followed by pH ($\rho = 0.643$, $p = 0.01$). Several taxa were strongly correlated with these variables (Table 6).

Table 5. Results of BIOENV analyses linking diatom community composition (relative abundance) and environmental variables in four wetland pools, and ANOSIM to identify significant differences in community composition over time and by season. For BIOENV, results are reported for the best explanatory combination of variables, and the best single variable. DOC, dissolved organic carbon; DRP, dissolved reactive phosphorus.

POOL	ρ	p	NO. OF VARIABLES	VARIABLES	OVER TIME		BY SEASON	
					R	p	R	p
1	0.609	0.01	5	pH, gilvin, DOC, Si, NO ₃ -N	0.516	0.001	0.064	0.214
	0.360	0.13	1	gilvin				
2	0.501	0.01	4	water level, alkalinity, DOC, NO ₃ -N	0.235	0.004	0.151	0.044
	0.343	0.07	1	water level				
3	0.534	0.02	4	water level, DOC, NH ₄ -N, NO ₃ -N	0.285	0.050	-0.041	0.629
	0.216	0.36	1	NO ₃ -N				
4	0.414	0.14	4	pH, water level, DRP, NH ₄ -N	0.588	0.001	0.023	0.344
	0.323	0.07	1	DRP				

Table 6. Examples of taxa driving the diatom community differences between the four pools, with the environmental variables associated with high relative abundances of each, identified from correlations with the axes of an NMDS plot. Refer to Fig. 7 for the ranges of variables in each pool.

SPECIES	STRONGLY ASSOCIATED (SPEARMAN $R > 0.8$) WITH:	MODERATELY ASSOCIATED (SPEARMAN $R > 0.6 < 0.8$) WITH:
<i>Brachysira wygaschii</i>		low pH
<i>Encyonopsis</i> sp. A	low pH, high gilvin, low alkalinity	
<i>Encyonema neogracile</i>		low gilvin, higher alkalinity
<i>Eunotia bilunaris</i> v. <i>mucophila</i>	high gilvin	
<i>Frustulia</i> sp. A	low gilvin	
<i>Kobayasiella</i> sp. B		low pH, higher gilvin
<i>Neidium iridis</i>	high gilvin	
<i>Stenopterobia curvula</i>	high alkalinity	high pH
<i>Stenopterobia</i> sp. A	high alkalinity	high pH
<i>Chamaepinnularia</i> sp. A	high pH, high alkalinity	

2.4.4 Discussion

In this study, we detected seasonal and non-seasonal changes in the relative abundances of many individual diatom taxa over the two-year sampling period. Environmental conditions in the four pools also fluctuated over time, sometimes seasonally (Kilroy et al. 2008). Despite the changes, each pool retained its characteristic community composition. Although the analysis linked community composition to gilvin, alkalinity and pH in each pool, with only four pools to compare, the environmental variables driving differences in community composition and species abundances among pools cannot be identified definitively. In order to do this, samples covering a wider geographical area are required, and this is addressed in section 3.

Nevertheless, the above results suggest that gilvin and alkalinity should be considered for measurement in future surveys, as well as the routinely measured pH. Gilvin, in particular, could be an important variable in wetland ecosystems. High gilvin is linked to low pH, since humic material is generally acidic. High gilvin also attenuates light and filters out UVB radiation, both of which can influence benthic communities (see review in Hamilton et al. 2004). Furthermore, the humic content of water is influenced by hydrology and catchment characteristics (Findlay et al. 2001). Therefore, gilvin in wetland waters may be affected by changes within the catchment.

Measurement of chlorophyll *a* requires a spectrophotometer, but is straightforward. Accurate measurement of alkalinity involves a titration procedure, which is more time-consuming (and therefore costly).

Retention of community differences over time suggests that the time of sampling is not critical for detecting community differences between separate pools that have measurable differences in water chemistry. Thus diatom communities show the same consistency over time that has already been demonstrated for wetland invertebrate communities (Suren & Lambert 2010). Nevertheless, we found that water chemistry (including nutrient concentrations) was linked to community changes over time within pools more than would have been expected by chance. This indicates the potential importance of subtle changes in nutrient inputs in driving the community composition of these primary producers.

Within pools, although abundances of a few taxa showed seasonal patterns, and their abundances correlated with water temperature (both positively and negatively), temperature was never included in the combination of the best explanatory variables of community similarities. Therefore, the overall effect of temperature on community composition appears complex. Although water temperature was one of the environmental variables most often strongly correlated with temporal changes in individual diatom species, it was rarely the only environmental variable linked to species abundances.

Overall, these results suggest that detection of community shifts associated with anthropogenic changes in wetlands may be feasible within short to long time-frames (e.g. months to years). Conversely, we should also be able to detect changes associated with restoration efforts that affect water temperature (e.g. restoration of shade), nutrient supply, pH and other aspects of water chemistry.

Detecting relatively subtle community changes in an unimpacted wetland did require high sampling and analysis effort (intensive fortnightly sampling to generate mean values over time). Surveys to determine the success of restoration measures therefore need to be carefully designed to maximise efficiency. This is discussed in section 4.

3. Algal community composition in New Zealand lowland wetlands and relationships with environmental variables

3.1 Introduction

The studies described in section 2 were confined to pools in a single small fen system, and involved detailed species-level analyses of diatom communities. In this section, we describe a study that included bogs, fens and swamps (as defined by Johnson & Gerbeaux 2004) and covered the whole of New Zealand. The focus was on largely unmodified wetlands in lowland areas, because these are the most vulnerable to further degradation due to drainage and land-use changes (McGlone 2009). The aims of the study were:

- To describe, for the first time, the diversity of algae in New Zealand wetlands (bogs, fens and swamps; see Appendix 1), at a national scale; and
- To identify environmental and geographical drivers of differences in community composition of diatom and non-diatom algal community composition on a national scale.

Identification of geographical patterns is particularly important from a conservation perspective, and could highlight areas with special conservation value because of their distinctive communities. Separate analyses for the diatom and non-diatom algal community are of interest because components of the algae are frequently used separately as environmental indicators in fresh waters (e.g. diatoms: Kelly et al. 2008; Besse-Lotoskaya et al. 2011; cyanobacteria: Douterelo et al. 2004). Additional objectives aimed to contribute to future management and monitoring in wetlands by determining:

- Whether local environmental conditions/geographical patterns are better reflected by epiphytic or benthic samples; and
- Whether species-level identifications reflect environmental conditions/geographical patterns any better than do genus-level identifications.

Because few wetlands in lowland areas have completely escaped human-related impacts, we included independent assessments of wetland condition among the environmental variables considered. Two methods have been developed in New Zealand. One method (Clarkson et al. 2003) is based on field observations of five factors considered to affect wetland condition: 1) hydrological integrity; 2) physicochemical parameters; 3) ecosystem intactness; 4) browsing predation and harvesting regimes; and 5) dominance of native plants. Each indicator component is scored on a scale from 0 (most degraded) to 5 (unmodified or best condition). Scores for this Wetland Condition Index (WCI) range from 0 to 25. A second method (Ausseil et al. 2008, 2011) was developed by combining six spatial indicators of human activities (termed 'pressure measures') known to degrade wetland biodiversity and function, derived from national GIS (geographic information system) databases. The six indicators were: 1) proportion of natural vegetation cover; 2) proportion of human-made impervious cover; 3) number of introduced fish; 4) percentage cover by woody weeds (mostly willows); 5) presence of artificial drainage; and 6) a surrogate measure of land use intensity (nitrate leaching risk). After appropriate weighting, the pressure measures were transformed into an Index of Ecological Integrity (IEI), ranging from 0 (totally degraded) to 1 (pristine, no human-induced impacts). Both these methods primarily assess the landscape and catchment factors that are most likely to influence the naturalness and diversity of large organisms (plants, fish). However, both indices also include assessments of hydrological and nutrient regime disturbances that could affect algae.

Below, the methods and results of studies focusing on first non-diatom and then diatom algae are presented separately. The results of the studies are then discussed together.

3.2 Non-diatom algae

While diatoms are often favoured as the algal type that best reflects environmental conditions (e.g. U.S. EPA 2002), algal communities in wetlands can be diverse and variable, and are often dominated by other algal groups, particularly cyanobacteria and chlorophytes (Goldsborough & Robinson 1996). Apart from the desmids (which belong to the Chlorophyta), the non-diatom algal flora in New Zealand wetlands is relatively poorly known, and no information exists on the broad-scale distributions of these algae (including desmids), even at the genus level. Therefore, this survey represents a first attempt to examine national patterns in non-diatom algae in New Zealand wetlands.

3.2.1 Methods

Thirty-eight relatively unimpacted lowland wetlands throughout New Zealand (21 in the North Island and 17 in the South Island) were sampled once from between 2004 to and 2006. Wetlands included in the survey were selected following discussions with local Department of Conservation staff, who identified the 'best' (i.e. the least impacted) wetlands in their regions. The survey was restricted to western regions: (Northland, Auckland, Waikato, Taranaki, Wellington, West Coast, and Southland), because no lowland wetlands on the eastern side of the country (i.e. Bay of Plenty, Hawkes Bay, Marlborough, Canterbury and Otago) were judged to be sufficiently unimpacted. Stewart Island was treated as a separate region. The 38 wetlands sampled are listed in Appendix 1.

To quantify the level of human impacts on the wetlands, we carried out a field assessment was carried out to calculate the WCI (Clarkson et al. 2003) at the time of the survey. We also retrieved the IEI determined for each wetland using GIS- derived variables (Ausseil et al. 2008). The WCI and IEI indicated that some wetlands were potentially not in as good condition as originally thought (e.g., the lowest IEI was 0.2 and the lowest WCI was 16.9). However, all wetlands were retained in the dataset so as to provide optimum geographical coverage.

In each wetland, three representative, separate water bodies (i.e. sampling sites) were selected, making a total of 115 sites⁴. We collected two types of algae. Epiphytic algae were sampled by cutting off at least three sections (approx. 50 mm long) of submerged stems or leaves of the dominant aquatic macrophyte in each of the three water bodies. Periphyton was collected from the unvegetated substratum surface of each, by combining material from three cores (25 mm diameter) driven into the top 2 cm of the substratum. All samples were frozen within 6 hours of collection. Spot measurements of water pH and conductivity were made at each water body in each wetland using a Horiba® multiprobe, and water samples collected and filtered for nutrient analysis.

In the laboratory, thawed benthic samples were homogenised before subsampling for microscopic analysis. Epiphytic algae were scraped from plant surfaces using a fine blade and then mixed to a slurry. Aliquots of the algal slurry were pipetted into the chamber of an inverted microscope (Leica DMLS) and scanned at magnifications of up to 400×. Non-diatom algae taxa observed were listed and ranked in order of relative abundance on a scale of 1 (rare) to 5 (abundant/dominant) based on scans of up to 20 fields of views, and estimates of their relative biovolume within each field. Identifications were made to as low a level as possible using a range of texts (refer to references in Biggs & Kilroy 2000).

Water samples were analysed for nutrients (NH₄-N, NO₃-N, DRP, Total dissolved phosphorus (TDP), and total dissolved nitrogen (TDN)) using standard methods (APHA 1975; Diamond 2003).

⁴ In five wetlands we identified only two separate water bodies.

For each wetland modelled, catchment variables were extracted from GIS databases, including the Land Cover Database (LCDB) and Freshwater Environments of New Zealand (FENZ). Values extracted for land cover, geology and climate variables were based on polygons delineating a 1-km zone around the wetland area. Because these lowland wetlands were generally low-gradient areas within a low-gradient landscape, it was considered that 1 km was sufficient to cover any land-use influence on the wetland. Variables used are listed in Table 7.

3.2.2 Data analysis

The algae data were checked for differences between islands using species-level and genus-level data, and data reduced to the major algal groups. Differences between islands, regions, wetland type (at the wetland class level, see Appendix 1) and sample type (benthic or epiphytic) were checked using NMDS and ANOSIM. A cluster analysis of the local environmental variables (water chemistry) was performed to see whether any obvious geographical patterns were evident among the sites. Inter-island environmental parameters (log-transformed as necessary) were compared directly using *t*-tests. All environmental variables are listed in Table 7.

BIOENV was then run to look for associations between the local and modelled environmental variables and community composition. The analyses were run on both species and genera data. The analyses were performed on reduced datasets, excluding taxa scored as 'rare' at only one site, because these taxa were likely to have been present by chance rather than because of environmental conditions. We report the result for presence/absence data only because using the abundance data did not affect the results and made little difference to the ρ values generated in the analyses.

Table 7. Measured and modelled environmental variables used in the analyses of diatom and non-diatom algal community composition, with basic statistics (minimum, maximum and mean) for North and South Islands. The Easting (E) and Northing (N) for each site were also included as variables. DRP, dissolved reactive phosphorus; IEI, Index of Ecological Integrity; TDN, total dissolved nitrogen; TDP, total dissolved phosphorus; WCI, Wetland Condition Index.

VARIABLE	UNITS	EXPLANATION/NOTES	NORTH ISLAND			SOUTH ISLAND		
			MIN.	MAX.	MEAN	MIN.	MAX.	MEAN
IEI		Index of Ecological Integrity (Ausseil et al. 2011)	0.20	0.95	0.51	0.30	0.97	0.81
WCI		Wetland Condition Index (Clarkson et al. 2003)	16.9	23.3	19.9	18.8	24.7	23.1
pH		Measured at sample collection	4.1	9.0	6.6	3.9	7.7	5.3
Conductivity	$\mu\text{S}/\text{cm}$	Measured at sample collection	46.1	662.0	226.0	21.2	215.8	67.7
DRP	mg/m^3	Measured from water sample	0.5	530.0	17.9	0.3	3.4	1.3
$\text{NH}_4\text{-N}$	mg/m^3	Measured from water sample	1.0	295.0	18.8	4.0	1367.4	46.9
$\text{NO}_3\text{-N}$	mg/m^3	Measured from water sample	0.5	3122.0	15.0	0.7	18.0	3.9
TDN	mg/m^3	Measured from water sample	166.0	1420.0	580.6	101.1	1382.9	370.8
TDP	mg/m^3	Measured from water sample	1.0	590.0	36.9	0.8	31.6	5.9
AveTWarm	$^{\circ}\text{C}$	Mean air temperature in the warmest month	16.5	19.7	18.3	12.8	15.6	14.6
AveTCold	$^{\circ}\text{C}$	Mean air temperature in the coolest month	8.1	12.6	9.9	3.7	7.4	6.1
temp_range	$^{\circ}\text{C}$	Difference between the warmest and coolest month	6.7	9.9	8.3	7.3	10.4	8.5
Rainfall	mm	Mean annual rainfall	1131	2283	1452	1070	5656	3389
1km_native	%	Cover in 1 km buffer by native vegetation	14.6	94.0	63.4	0.0	96.2	22.1
1km_past	%	Cover in 1 km buffer by pasture	0.0	94.0	39.2	0.0	46.4	5.8
1km_Alluv	%	Proportion of 1 km buffer in alluvium	0.0	33.4	10.4	0.0	100.0	58.8
1km_Hsed	%	Proportion of 1 km buffer in hard sedimentary rock	0.0	88.1	12.8	0.0	14.2	2.2
1km_Peat	%	Proportion of 1 km buffer in peatland	0.0	73.5	13.2	0.0	96.6	21.1
1km_Sand	%	Proportion of 1 km buffer in sand	0.0	100.0	10.0	0.0	51.7	6.2
1km_Ssed	%	Proportion of 1 km buffer in soft sedimentary rock	0.0	77.5	12.9	0.0	0.0	0.0
1km_Sstone	%	Proportion of 1 km buffer in sandstone	0.0	60.1	9.7	0.0	1.8	0.1
1km_V_ash	%	Proportion of 1 km buffer in volcanic ash	0.0	100.0	18.4	0.0	0.0	0.0
1km_V_rock	%	Proportion of 1 km buffer in volcanic rock	0.0	76.1	10.5	0.0	38.7	4.0

Finally, we used regression trees (De'ath & Fabricius 2000) to determine whether taxonomic richness (the simplest measure of diversity) was associated with environmental conditions. This technique, the response variable data (in this case, species richness) are split into groups on the basis of their association with certain value ranges of one or more of the explanatory variables. Each group is defined by threshold values of the explanatory variable(s). For example, low species richness might be encountered mainly below a certain threshold of one environmental variable. The splitting process produces a 'tree' with branches and leaves, where the leaves are the final groups. Diagrams of the tree represent which environmental variables are associated with each group of the response variable. Parameters such as minimum number of members of each group are set before running the analysis. Trees are particularly useful for ecological analyses because: 1(a) they work equally well with both continuous and categorical variables (with the latter, they are termed classification trees); 2(b) they do not rely on data transformations or particular data distributions; and 3(c) they can be easy to use and the results are easy to interpret. For the present exploratory analysis, we used the CART (Classification and Regression Trees) routine in SYSTAT v. 11.

3.2.3 Results

We identified 206 taxa (to species level where possible) in 90 genera across all samples. Only 44 (21%) of the species were recorded in both islands, but 65 (72%) of the genera were common to both islands. Mean taxon richness per sample was 6.6 in the North Island and 8.1 in the South Island. The most common taxa overall were species (i.e. morphotypes, see Fig. 8) of the green filamentous alga *Oedogonium* (narrow forms, probably comprising several different species, which were indistinguishable without culturing the material, Novis 2003), and a colonial cyanobacterium *Aphanocapsa grevillei*. Examples are illustrated in Figs 8 and 9. Refer to Appendix 2 for a list of all taxa recorded.

When the data were grouped into seven major (informal) algal groups, the inter-island differences were obvious: a far higher proportion of records from the South Island samples were single-celled or colonial cyanobacteria (> 35%, compared with 3% in the North Island), and many more of the North Island records were filamentous green algae (> 38% compared with approx. 11% in the South Island) (Table 8). The North Island and South Island communities differed significantly (for species-level data, ANOSIM $R = 0.368$, $p < 0.0001$; for genus-level data $R = 0.389$, $p < 0.0001$). An NMDS plot showed almost complete separation of the two groups of samples (Fig. 10a), mirrored by separation on the basis of wetland type (Fig. 10b), and also reflected by a strong correlation of NMDS axis 1 scores (x -axis) with pH (Fig. 10a). The most common taxa were important in structuring community composition, with *Aphanocapsa* and *Stigonema* associated with low pH South Island sites, and *Spirogyra* and *Oedogonium* associated with higher pH North Island sites (Fig. 10b).

A cluster analysis using local environmental (water chemistry) variables divided the samples into two major groups, which largely corresponded to North Island samples and South Island samples (Fig. 11). Just eight of the 115 sites sampled clustered separately on the basis of unusual combinations of environmental conditions, such as extremely high TDN or TDP. Mean pH, conductivity, DRP, TDP, TDN and $\text{NO}_3\text{-N}$ all differed significantly between islands (Fig. 12).

A further difference between islands was that both mean IEI and mean WCI were significantly lower in the North Island than in the South Island (t -tests, $p < 0.0001$; Table 7), suggesting greater human impact in the North Island.

Almost 40% of taxa were only encountered in either epiphytic or benthic samples. While most of these were rare (found in just one or two samples), the cyanobacterium *Calothrix* cf. *fusca* was only identified in epiphytic samples (six samples) and the desmid *Closterium cynthia* was only identified in benthic samples (four samples). Overall, samples collected from sediment and plants at the same site differed, though with very high variability in both groups (ANOSIM $R = 0.064$, $p = 0.001$). Separate analyses of the data from epiphytic and benthic samples were therefore justified. On the basis of the clear separation between islands of both community composition and environmental conditions, subsequent analyses were conducted on data from the two islands separately.

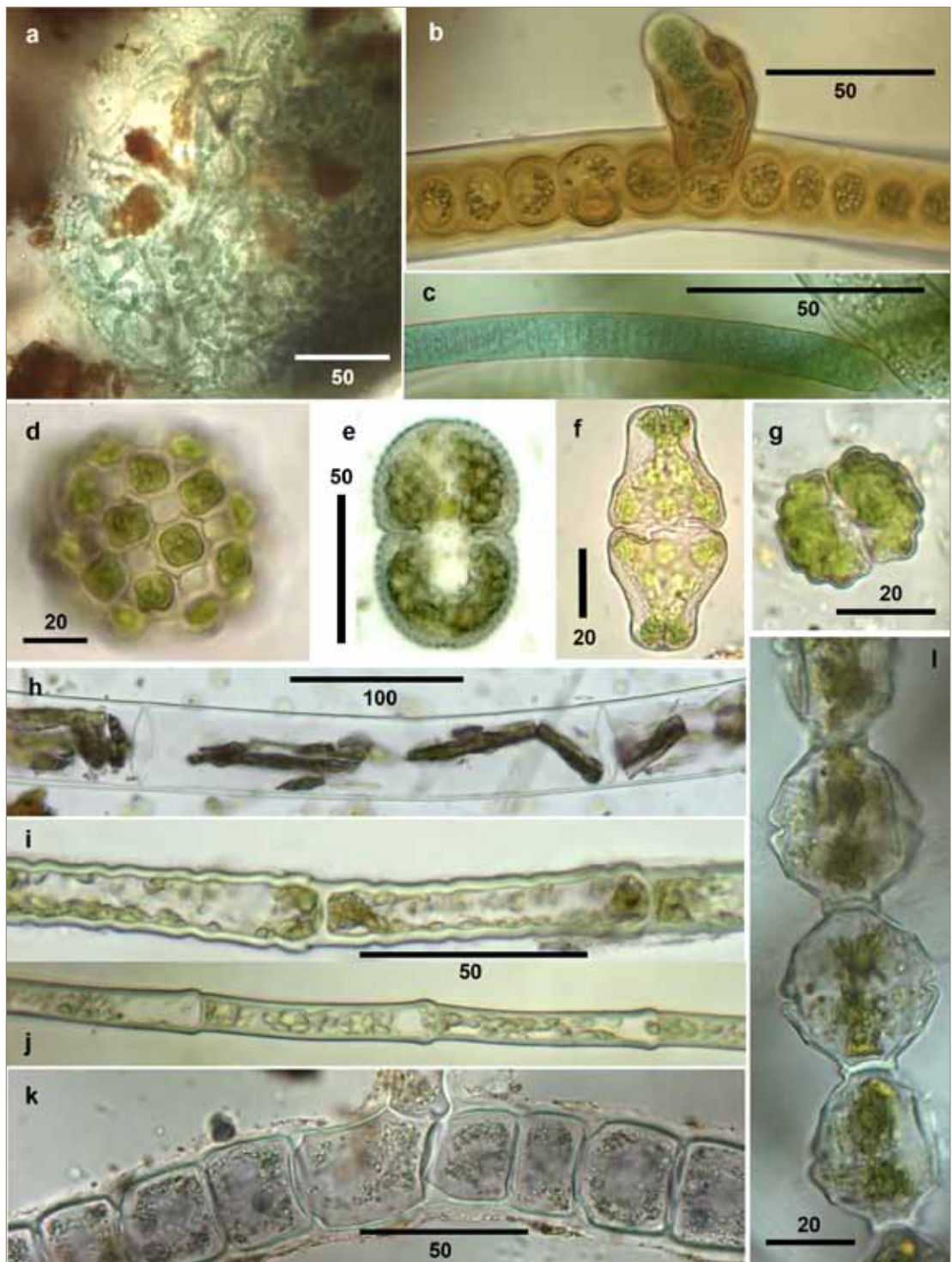


Figure 8. Examples of common non-diatom algae found at North Island wetland sites. Cyanobacteria (a) *Nostoc* sp.; (b) *Stigonema* sp.; (c) *Oscillatoria* sp.; Chlorophyta (d) *Coelastrum cambricum*; (e) *Cosmarium quadrifarium* v. *hexastichum*; (f) *Euastrum* cf. *sinusoum* v. *gemmulosum*; (g) *Euastrum* sp. A; (h) *Spirogyra* sp. mt 3; (i) *Oedogonium* aff. *wissmanii*; (j) *Oedogonium* sp. mt 1 (mt = morphotype—taxa recognised as possible species, but not able to be identified); (k) *Compsopogon* sp. (Rhodophyta); (l) *Desmidiium occidentale*. Scale bars in μm .

Relationships with environmental variables

After excluding rare taxa, the datasets included 152 species and 74 genera. In both islands, the combination of environmental variables most closely associated with species composition differed between epiphytic and benthic algae, but the single variables with the strongest correlation tended to be the same. Differences between species-level and genus-level data were generally minor (Table 9). In the North Island, the variables 1km_peat and pH best explained epiphytic community variation. In the South Island, the variables TDP and pH best explained

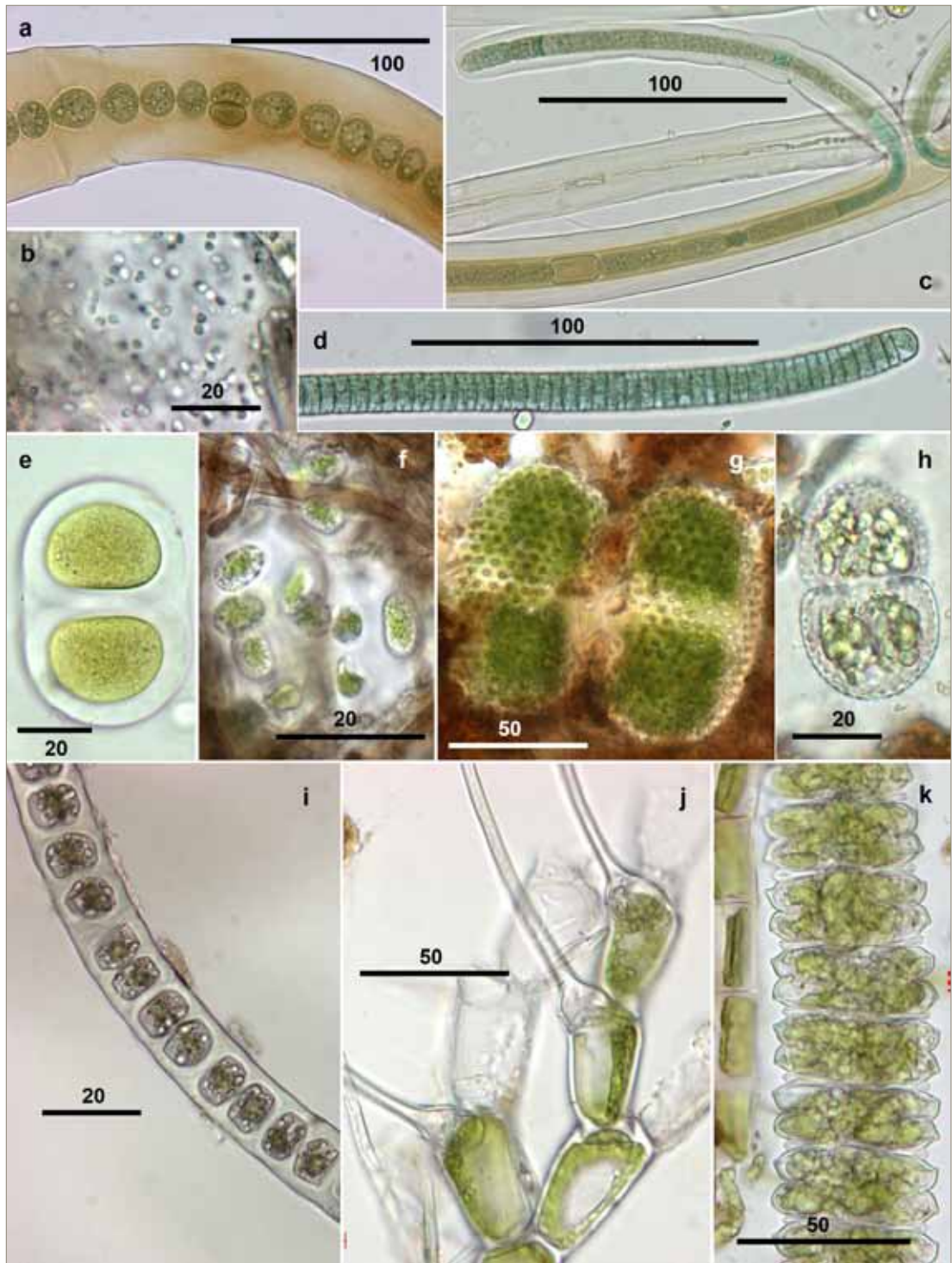


Figure 9. Examples of common non-diatom algae found at South Island wetland sites. Cyanobacteria (a) *Stigonema* sp.; (b) *Aphanocapsa grevillei*; (c) *Scytonema* sp.; (d) *Oscillatoria* sp.; (e) *Chroococcus* sp.; Chlorophyta (f) *Gloeocystis* sp.; (g) *Cosmarium* sp. (?quadrum); (h) *Cosmarium quadrifarium* v. *hexastichum*; (i) *Cylindrocapsa* sp.; (j) *Bulbochaete*; (k) *Desmidium*. Scale bars in μm . Photos: Donna Sutherland.

community variation for epiphytic and benthic samples, respectively. With the exception of benthic samples in the North Island, the correlations with environmental variables were statistically significant (Table 9).

Neither species-level nor genus-level data consistently produced stronger correlations with the available environmental variables. The utility of epiphytic versus benthic samples was also unclear, with epiphytic samples showing stronger correlations with environmental variables in the North Island, but benthic samples showing stronger correlations in the South Island (Table 9).

Table 8. Proportions of records of non-diatom algae in major taxonomic groups, subdivided into informal descriptive 'types', recorded in North Island and South Island samples.

TAXONOMIC GROUP	DESCRIPTION	NORTH ISLAND % SAMPLES	SOUTH ISLAND % SAMPLES
Cyanobacteria	Single-celled/colonial algae	3.0	35.2
	Trichomes (filaments)	19.2	23.3
Chlorophyta	Desmids (including filamentous forms)	26.2	23.5
	Single-celled/colonial algae (non-desmid)	12.7	6.6
	Green filamentous algae (non-desmid)	38.4	10.9
Rhodophyta	Red algae	0.3	0.0
Other	Iron bacteria/bryophyte protonema	0.3	0.4

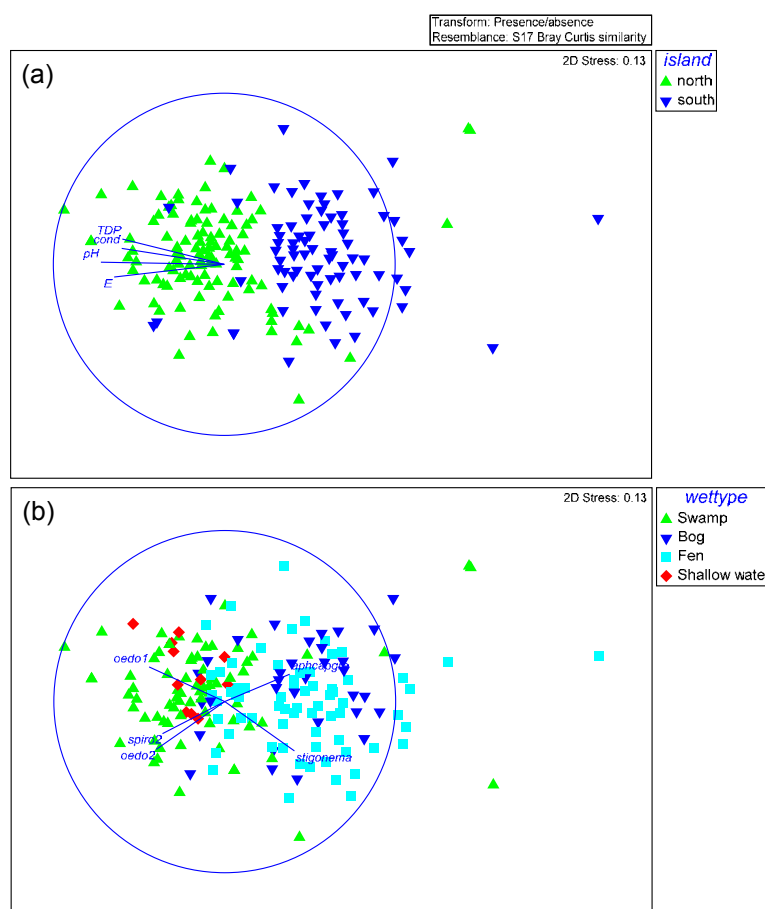


Figure 10. NMDS of all samples of non-diatom algae (species-level) showing (a) separation by island and (b) separation by wetland type. The overlaid vector plots show (a) environmental variables with Pearson rank correlations with the NDMS axes of >0.6; (b) species with Spearman rank correlations of >0.4. Note: some extreme outliers were removed from the dataset.

Taxonomic richness

Regression tree analysis on all the data resulted in a model explaining less than 25% of the variation in taxonomic richness across all samples (both islands). When data for epiphytic and benthic samples in each island were analysed separately, more variation was explained, but the trees were complex. The best model, for the South Island benthic samples, explained 62% of the variation in taxonomic richness. Highest taxonomic richness was, on average, found in samples where TDP was <4.82 mg/m³, 1km_pasture was <5% and pH was >4.6. Lowest taxonomic richness was found where TDP was >4.82 mg/m³ and 1km_water was <0.07%.

Group average

Normalise
Resemblance: D1 Euclidean distance

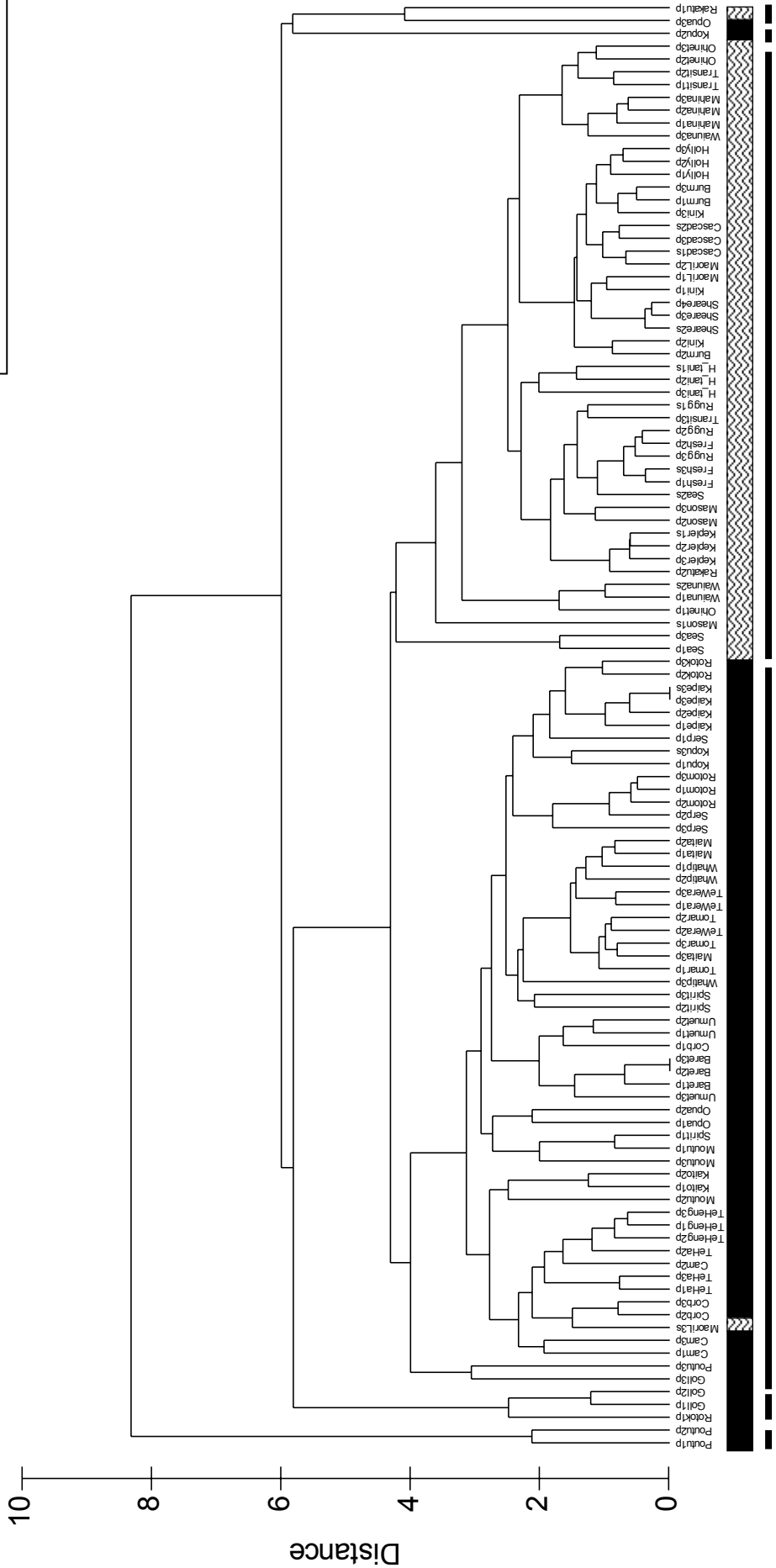


Figure 11. Dendrogram showing the results of a cluster analysis for all wetland sampling sites, based on seven measured environmental variables (water quality). The wide solid bar under the labels indicates Northisland samples, and the shaded bar South Island. The broken line at the bottom shows the separation of major groups. The short lines at the far left and right are outliers.

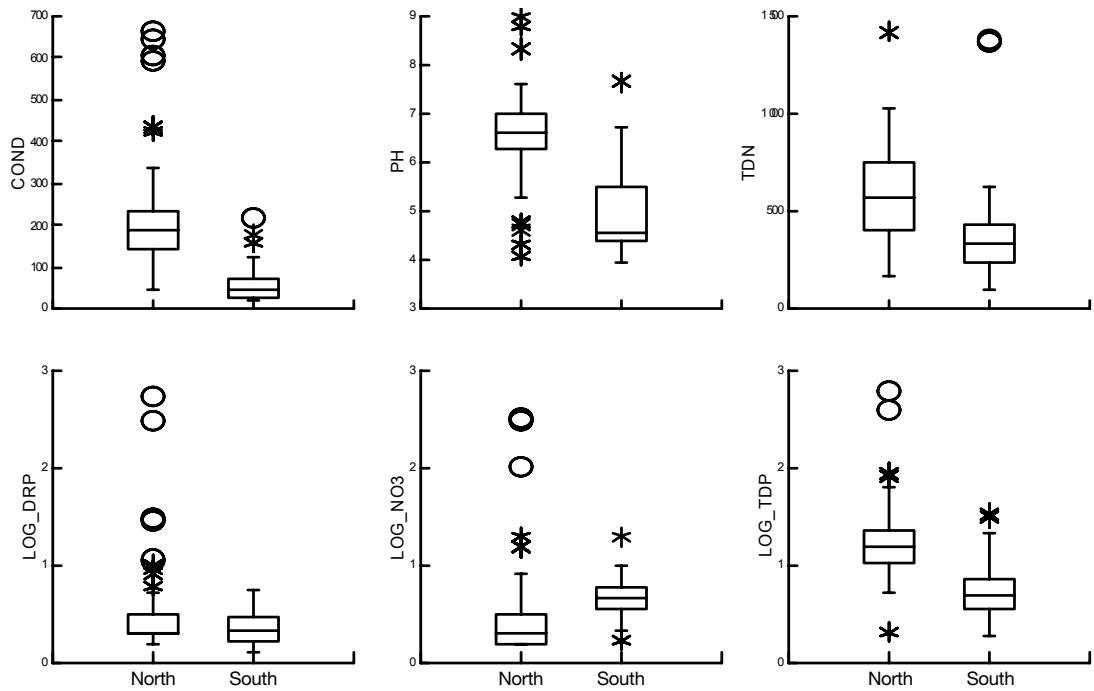


Figure 12. Box- plots of measured water quality variables that differed significantly between North Island and South Island wetlands sites (*t*-tests, $p < 0.0001$).

Table 9. Summary of results of BIOENV analyses between non-diatom algal community composition and environmental variables (local and catchment) in lowland wetlands in the North and South Islands. Results for epiphytic and benthic communities are presented, with community data at the genus and species levels. The single variable with the highest correlation (coefficient ρ) with community composition is shown, as well as the combination of variables with the highest correlation. Significant correlations are highlighted in bold. DRP = dissolved reactive phosphorus; E = Easting (location); IEI = Index of Ecological Integrity; TDN = total dissolved nitrogen; TDP = total dissolved phosphorus; WCI = Wetland Condition Index.

ISLAND	ALGAE TYPE	TAXONOMIC LEVEL	NO. OF VARIABLES	COEFFICIENT ρ	p	VARIABLES INCLUDED IN MATRIX
North	Epiphytic	Species	4	0.503		pH, TDP, AveTCold, 1km_peat
			1	0.440	0.01	1km_peat
		Genus	5	0.480	0.01	WCI, pH, TDP, AveTWarm, 1km_peat
	Benthic	Species	1	0.435	0.01	pH
			5	0.270		IEI, WCI, pH, 1km_native, 1km_peat
		Genus	1	0.152	0.24	1km_peat
			5	0.312		IEI, pH, TDN, alluvium, 1km_peat
1	0.145	0.38	1km_peat			
South	Epiphytic	Species	5	0.424		E, pH, DRP, NO ₃ -N, TDP
			1	0.307	0.01	pH
		Genus	4	0.478		pH, NO ₃ -N, TDP, 1km_native
	Benthic	Species	1	0.374	0.01	TDP
			5	0.537		WCI, E, pH, NO ₃ -N, TDP
		Genus	1	0.384	0.01	pH
			5	0.511		WCI, E, pH, NO ₃ -N, TDP
1	0.421	0.01	pH			

3.3 Diatoms

The objectives for our study of diatom communities across New Zealand wetlands were (a) to characterise communities across geographical and environmental gradients, and (b) to compare the ability of species-level and genus-level data to reflect environmental differences. We also compared the responses of epiphytic and benthic communities.

3.3.1 Methods

Sample collection was as described in section 3.2.1. Representative subsamples from each sample of epiphytic and periphytic algae were cleaned with sulphuric acid and hydrogen peroxide using the method described by Biggs & Kilroy (2000). Aliquots of the resulting rinsed suspension of diatom frustules were mounted on glass slides using Naphrax[®]. Counts of at least 400 valves were made at 1000× magnification, except where sparse samples precluded this. In the latter case, entire slides were scanned. All identifications were made to species level, or to morphospecies in cases of uncertain identities, using a range of literature, particularly Foged (1979) and Krammer & Lange-Bertalot (1991–1997). For a more comprehensive list of diatom identification texts refer to Kilroy (2007).

3.3.2 Data analysis

Rare species (i.e. occurring at ≤ 5 sites, with a total abundance across all sites of $\leq 5\%$) were omitted from the species-level analysis, as such uncommon species were likely to be present in the sample by chance. For the genus-level analysis we used all the data. Preliminary ANOSIMs on the community data showed significant differences between islands, though the differences were less clear-cut than for the non-diatom algae (see section 3.2.3). pH was the strongest explanatory variable for community differences across the whole dataset and within islands (e.g. benthic samples at species level, BIOENV $\rho = 0.412$, North Island; $\rho = 0.555$, South Island). To try to identify other factors that might be associated with community similarities, we divided the entire dataset into five groups on the basis of pH. The definition of groups was necessarily largely arbitrary because cluster analyses on the diatom communities did not reveal unambiguous separation of groups of sites related to pH. However, diatoms are well known to be highly sensitive to pH, with species exhibiting a wide range of responses (Batterbee et al. 2010). Selection of group boundaries was partly based on the results of a previous analysis (Kilroy et al. 2006) in the single wetland described in section 2, where we observed significant species turnover among pools with mean pH < 6 , 6.2–6.5 and > 6.8 . Boundaries were therefore set between these values, at pH 6.0 and 6.7. Two additional groups were added to cover the highest and lowest pH samples: < 4.5 and > 7.5 . Twenty datasets were therefore available for analysis: each of the five pH groups, with epiphytic or benthic samples, and species-level or genus-level relative abundances. For each dataset, we performed BIOENV, as described in section 2.4.2. In all analyses we used relative (%) abundances, square-root transformed to increase the weighting of rare taxa.⁵

Grouping of the sites on the basis of pH resulted in uneven splits between the North and South Island. Most of the South Island sites were in groups 1 and 2 (low pH), and most of the North Island sites were in groups 3, 4 and 5 (higher pH). However, an important question is whether North Island and South Island samples from sites with similar pH are consistently different, which would suggest geographical or temperature-driven differences. To test this, we performed NMDS on data for each pH group, to check for clustering of samples on the basis of region, and to identify both the diatom species and environmental variables associated with gradients in community composition.

Finally, as for the non-diatom algae, we used regression trees to explore associations between total taxonomic richness and environmental variables (see section 3.2.2).

⁵ Percentage data are often arc-sine transformed, or square-root arc-sine transformed, depending on the distribution of the data. In the present non-parametric analysis, the transformation simply serves to prevent the analysis being unduly influenced by some dominant taxa.

3.3.3 Results

A total of 260 diatom species⁶ in 61 genera were identified. 71 species and 10 genera were recorded only in the North Island, and 68 species and 11 genera were recorded only in the South Island. Thus, 48% of species and 67% of genera were recorded on both islands. Although *Eunotia*, *Nitzschia*, *Encyonema*, *Pinnularia* and *Brachysira* were among the eight most common genera on both islands, the two islands had completely different common species (Table 10). Common taxa are shown in Figs 13 and 14; refer to Appendix 3 for a complete list of taxa. Species richness for individual samples ranged up to 41 taxa (species level). Four samples contained no diatoms.

There were many rare species in the dataset: 95 taxa (36%) were removed from the species dataset using the criteria specified in section 3.3.2. Of these, 62 were recorded at one site only, mostly represented by a single individual.

There were no obvious patterns of species fidelity to either benthic or epiphytic habitats, and no difference between the habitats (ANOSIM $R = 0.000$, $p = 0.750$). Over 70% of species were recorded in both habitats. All the species recorded in just one habitat were included in the 95 rare taxa deleted from the species dataset.

Relationships with environmental variables

Community composition in each of the five pH groups was associated with a different set of environmental variables (Table 11). For pH groups 1, 2 and 3, there was generally some consistency in explanatory variables between the different datasets (i.e. epiphytic or benthic samples, at genus or species level) within each pH group. For pH groups 4 and 5, consistency was slight: each community dataset was explained best by a different combination of environmental variables, and usually the best single explanatory variable also differed (Table 11). Across all the datasets, both or either of the wetland condition indices (WCI or IEI) were included in 16 of the 20 best combinations of explanatory variables. Geological variables were included in 14 combinations, and nutrient measures were included in 13 (Table 11).

Table 10. The eight most common diatom species and genera (by % of occurrence) in the North Island (118 samples) and South Island (78 samples).

ISLAND	COMMON SPECIES	PROPORTION OF SAMPLES (%)	COMMON GENERA	PROPORTION OF SAMPLES (%)
North	<i>Eunotia implicata</i>	57	<i>Eunotia</i>	88
	<i>Encyonema neogratile</i>	50	<i>Nitzschia</i>	76
	<i>Aulacoseira</i> sp.	45	<i>Navicula</i>	69
	<i>Nitzschia perminuta</i>	41	<i>Encyonema</i>	64
	<i>Brachysira</i> sp. C	39	<i>Gomphonema</i>	63
	<i>Achnanthyidium minutissimum</i>	37	<i>Pinnularia</i>	56
	<i>Sellaphora pupula</i> (complex)	36	<i>Fragilaria</i>	53
	<i>Nitzschia palea</i>	31	<i>Brachysira</i>	49
South	<i>Frustulia magaliesmontana</i>	67	<i>Eunotia</i>	73
	<i>Frustulia crassinervia</i>	64	<i>Frustulia</i>	83
	<i>Kobayasiella</i> sp. A	62	<i>Brachysira</i>	72
	<i>Brachysira metzeltinii</i>	53	<i>Kobayasiella</i>	62
	<i>Frustulia saxonica</i>	44	<i>Pinnularia</i>	36
	<i>Frustulia</i> sp. A	40	<i>Eunophora</i>	31
	<i>Eunophora</i> spp.	31	<i>Encyonema</i>	31
	<i>Brachysira wygaschii</i>	29	<i>Nitzschia</i>	29

⁶ The term species here includes 'morphotypes' for taxa suspected to be separate species from their morphology, but which could not be identified.

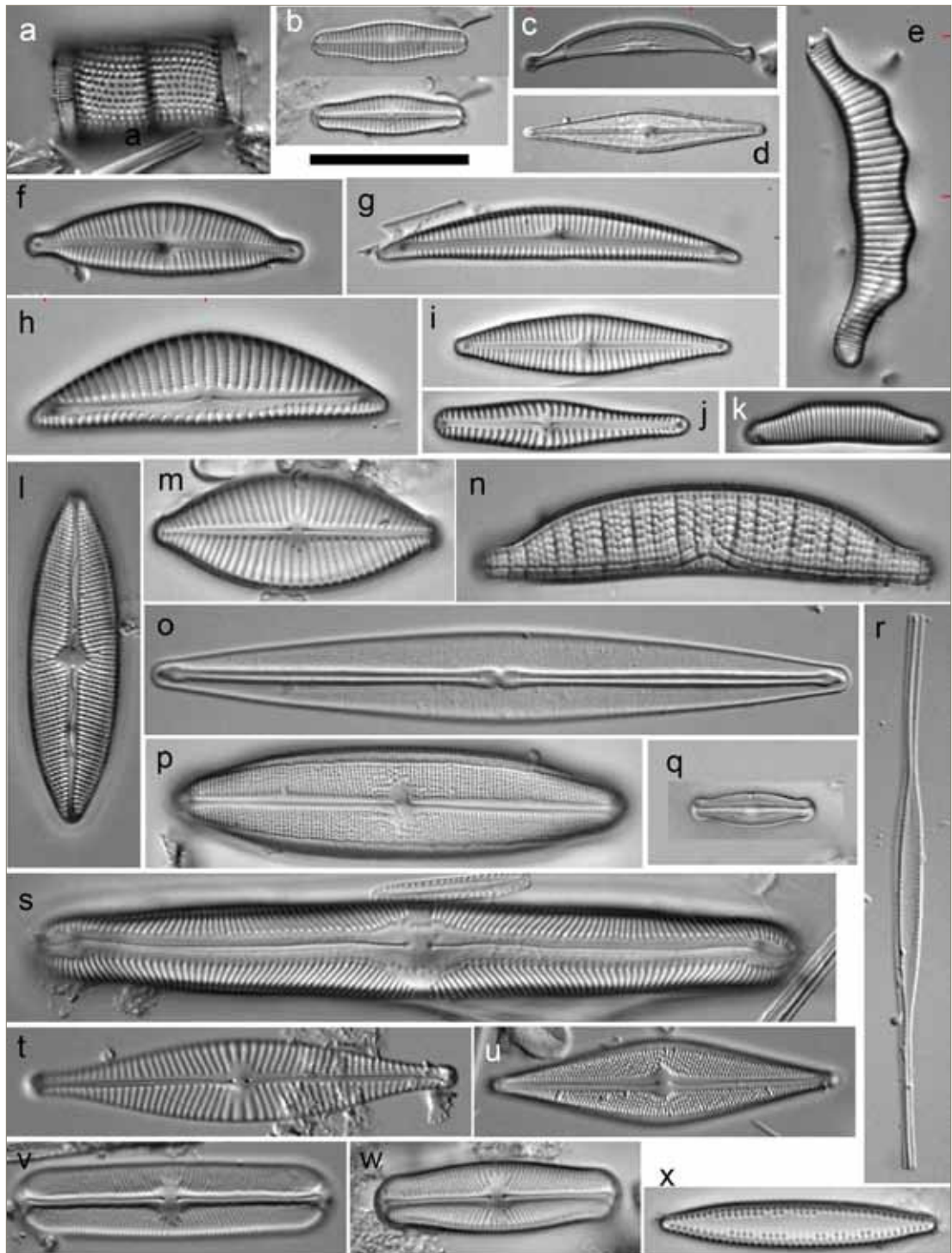


Figure 13. Diatoms encountered in samples from North Island wetlands. (a) *Aulacoseira* sp.; (b) *Rossithidium* sp.; (c) *Amphora veneta*; (d) *Brachysira* sp. C; (e) *Eunotia camelus*; (f) *Cymbella naviculiformis*; (g) *Encyonema neogracile*; (h) *Encyonema* cf. *mesianum*; (i) *Gomphonema gracile*; (j) *G. clavatum*; (k) *Eunotia implicata*; (l) *Mastogloia elliptica*; (m) *Placoneis placentula*; (n) *Epithemia turgida*; (o) *Frustulia cassieae*; (p) *Neidium iridis*; (q) *Navicula festiva*; (r) *Nitzschia acicularis*; (s) *Pinnularia graciliodes*; (t) *Navicula rhynchocephala*; (u) *Brachysira wygaschii*; (v, w) *Sellaphora pupula*; (x) *Tabularia variostrata*. Scale bar: 20 μ m.

For four of the five pH groups, benthic samples analysed to species level produced the strongest taxa–environment relationships, though all analyses of pH group 1 yielded strong associations (Table 11). The exception was group 3 (pH 6–6.7), in which all community–environment relationships were weak. To simplify the results, in the following we therefore discuss only benthic samples analysed to species level.

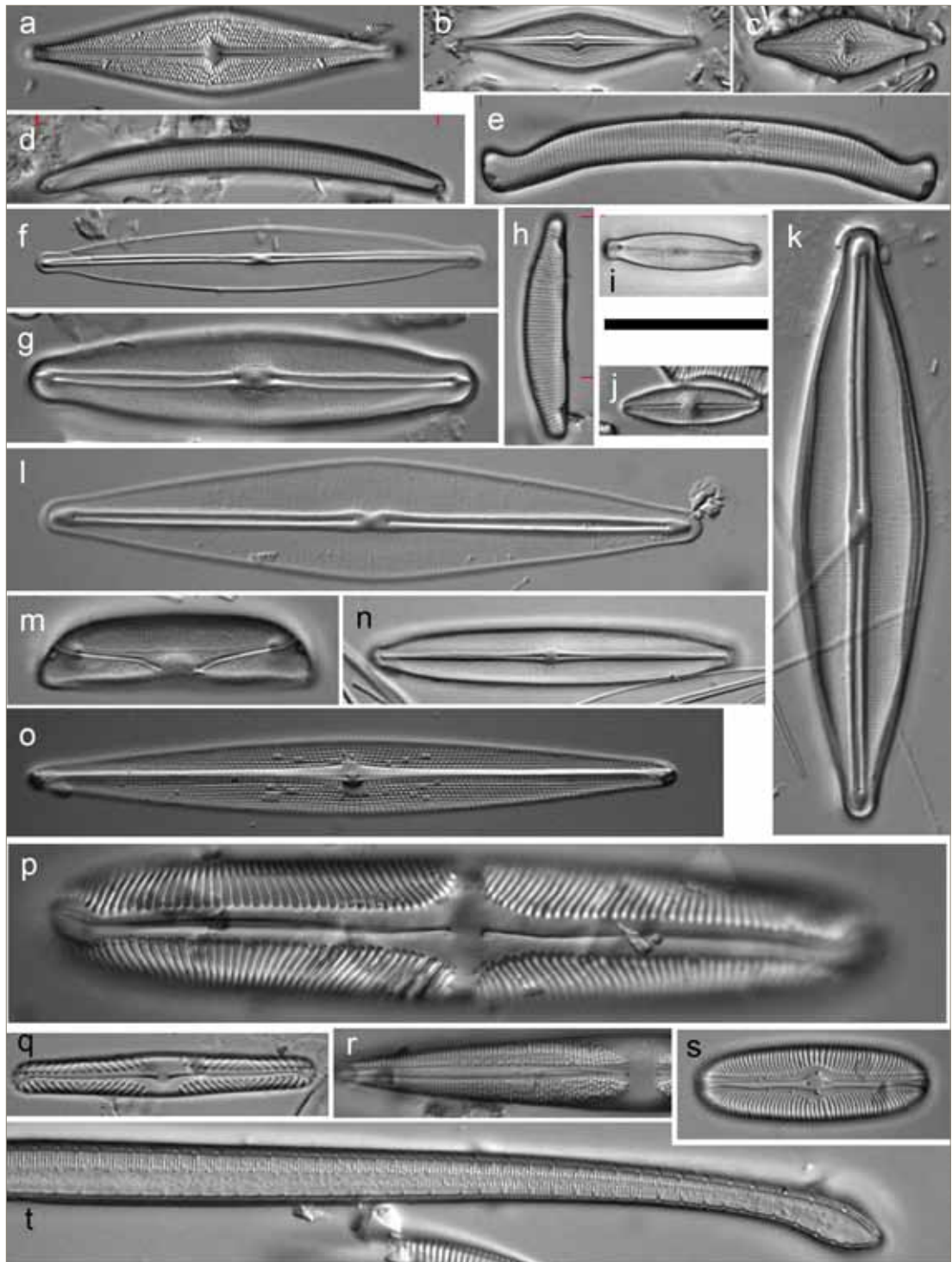


Figure 14. Diatoms encountered in samples from South Island wetlands. (a) *Brachysira wygaschii*; (b) *Brachysira metzeltinii*; (c) *Brachysira* sp. B; (d) *Eunotia bilunaris*; (e) *Eunotia* sp. (cf. *praerupta*); (f) *Frustulia* cf. *magaliesmontana*; (g) *Frustulia vulgaris*; (h) *Eunotia incisa*; (i) *Navicula festiva*; (j) *Nupela* sp. A; (k, l) *Frustulia saxonica* (k, with girdle band still attached); (m) *Eunophora* sp. (n) *Kobayasiella* sp. C; (o) *Navicula helvetica* var. *wolterecki*; (p) *Pinnularia divergens* var. *rhombundulata*; (q) *Pinnularia divergentissima*; (r) *Stauroneis frauenfeldiana*; (s) *Sellaphora bacillum*; (t) *Stenopterobia curvula*. Scale bar: 20 µm.

In pH group 1 (lowest pH, < 4.5), community composition was strongly associated with the 1km_{native} and IEI (Table 11) variables. On an NMDS plot, the two North Island sites in this group were well separated (i.e. dissimilar) from the South Island sites, which were mostly very similar to each other, except for Southland (Fig. 15). The South Island samples (at the top of the NMDS plot) were characterised by *Eunophora* cf. *oberonica*. and *Frustulia* sp. A along gradients of increasing NO₃-N and decreasing temperature.

Table 11. Summary results of BIOENV analyses of diatom community composition data (% abundances, species and genus level) for epiphytic and benthic samples from wetland pools. Significant correlations are highlighted in bold. DRP, dissolved reactive phosphorus; IEI, Index of Ecological Integrity; TDN, total dissolved nitrogen; TDP, total dissolved phosphorus; WCI, Wetland Condition Index.

pH GROUP	<i>n</i>	SAMPLE TYPE	TAXONOMIC LEVEL	NO. OF VARIABLES	BEST EXPLANATORY VARIABLES	ρ	p
1 (pH <4.5)	18 (2 N, 16 S)	Epiphytic	Species	2	IEI, 1km_native	0.868	0.01
			1	1km_native	0.849	0.01	
		Genus	2	IEI, 1km_native	0.825	0.01	
			1	1km_native	0.745	0.01	
	14 (2 N, 12 S)	Benthic	Species	5	WCI, IEI, DRP, AvTCold, 1km_native	0.922	0.01
			1	1km_native	0.891	0.01	
			Genus	2	IEI, 1km_native	0.865	0.01
			1	IEI	0.805	0.01	
2 (pH 4.5–6.0)	21 (6 N, 15 S)	Epiphytic	Species	4	WCI, IEI, 1km_alluv, 1km_Sstone	0.486	0.09
			1	IEI	0.438	0.06	
		Genus	5	IEI, pH, DRP, TDP, 1km_native	0.450	0.02	
			1	WCI	0.368	0.01	
	20 (6 N, 14 S)	Benthic	Species	4	IEI, pH, TDP, 1km_Sstone	0.654	0.01
			1	WCI	0.538	0.01	
			Genus	4	IEI, DRP, TDP, 1km_Sstone	0.570	0.01
			1	WCI	0.529	0.03	
3 (pH 6.0–6.7)	30 (25 N, 5 S)	Epiphytic	Species	5	WCI, NH ₄ -N, AvT_cold, 1km_Hsed, 1km_Sstone	0.292	0.36
			1	WCI	0.286	0.11	
		Genus	5	WCI, NH ₄ , AvT_cold, 1km_Hsed, 1km_sand	0.338	0.24	
			1	1km_sand	0.301	0.11	
	28 (23 N, 5 S)	Benthic	Species	4	DRP, AvT_cold, 1km_native, 1km_Hsed	0.333	0.16
			1	DRP	0.273	0.02	
			Genus	5	WCI, DRP, 1km_Hsed, 1km_sand, 1km_Ssed	0.347	0.03
			1	DRP	0.245	0.06	
4 (pH 6.7–7.5)	22 ((20 N, 2 S)	Epiphytic	Species	4	IEI, DRP, rainfall, 1km_Vash	0.529	0.01
			1	rainfall	0.333	0.01	
		Genus	5	WCI, NH ₄ , TDN, AvTwarm, 1km_V_ash	0.491	0.01	
			1	WCI	0.382	0.03	
	28 (18 N, 3 S)	Benthic	Species	4	WCI, IEI, NO ₃ -N, 1km_V_rock	0.570	0.01
			1	vol_rock	0.413	0.02	
			Genus	4	WCI, E, hard_sed, vol_rock	0.502	0.02
			1	IEI	0.299	0.23	
5 (pH >7.5)	9 (8 N, 1 S)	Epiphytic	Species	5	DRP, TDN, rainfall, sand, vol_rock	0.664	0.02
			1	sand	0.487	0.06	
		Genus	1	rainfall	0.693	0.04	
	10 (8 N, 2 S)	Benthic	Species	5	WCI, AvT_cold, sand, vol_ash, vol_rock	0.794	0.01
			1	vol_rock	0.500	0.15	
			Genus	5	pH, NH ₄ , AvT_warm, vol_ash, vol_rock	0.769	0.18
				1	vol_rock	0.699	0.05

Environmental variables linked to community structure in pH group 2 included WCI, IEI, TDP and geological variables, varying with sample type and taxonomic level (Table 11). An NMDS run on benthic samples analysed to species level showed that sites with high WCI and low pH, TDP and DRP tended to have higher relative abundances of *Frustulia magaliesmontana* and *Brachysira metzeltinii*. These sites included representatives from Northland, the West Coast and Stewart Island (Fig. 16). Communities at other Northland sites were characterised by higher abundances of *Stenopterobia curvula*, *Encyonema neogracile* and *Pinnularia divergens* associated with higher TDP and pH.

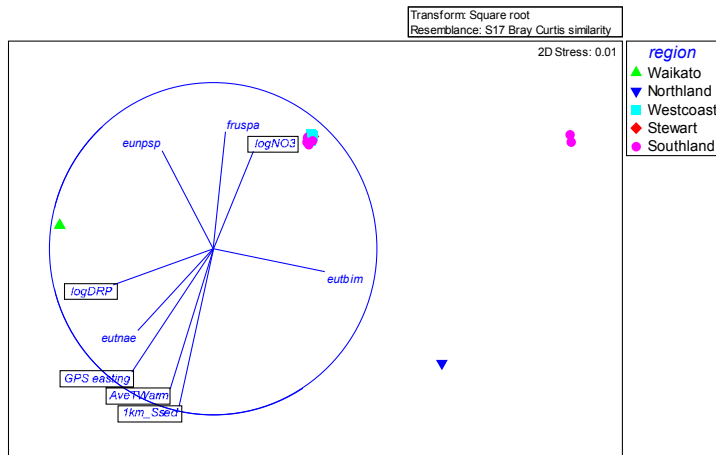


Figure 15. NMDS plot showing benthic samples analysed to species level in pH group 1 (pH < 4.5) separated by region. The vectors represent correlations of species (Spearman, >0.6) and environmental variables (Pearson, >0.65) with the NMDS axes.

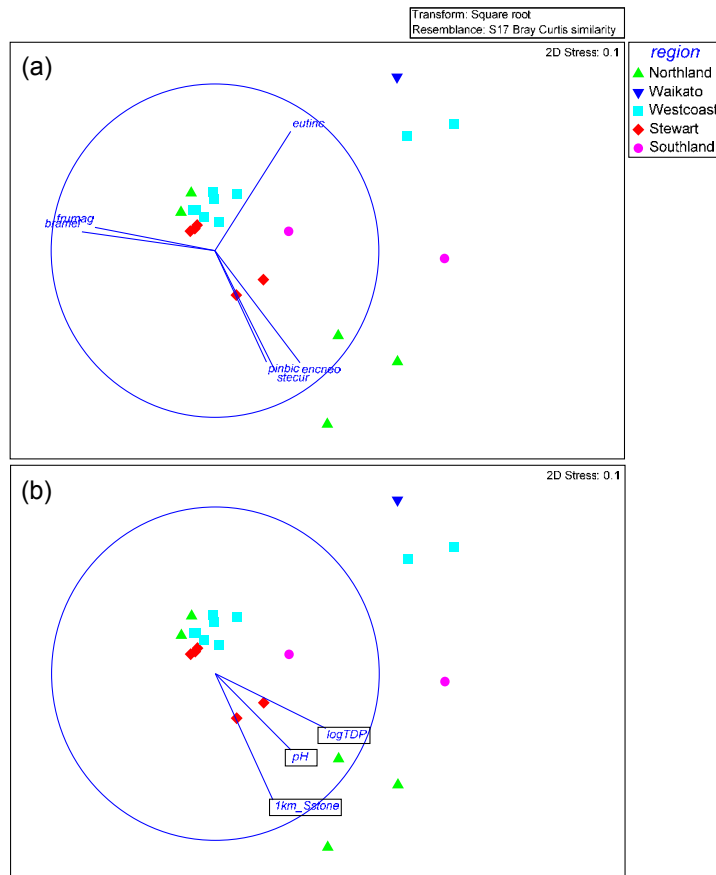


Figure 16. NMDS plot showing benthic samples analysed to species level in pH group 2 (pH 4.5–6.0) separated by region. The vectors represent correlations of (a) species (Spearman, > 0.6 [non-normally distributed data]) and (b) environmental variables (Pearson, >0.72 [normally distributed data]) with the NMDS axes.

The BIOENV result for pH group 3 (pH 6.0–6.7) suggested only weak correlations with the available environmental variables (Table 11). Furthermore, stress in the NMDS (see section 2.4.2) was high. Therefore, the species and environment associations suggested by Fig. 17 are unreliable. Communities in the five South Island sites in that group were not distinctive in composition compared to the North Island sites (Fig. 17).

Samples in pH group 4 (pH 6.7–7.5) were significantly associated with various combinations of IEI, WCI, nutrient concentrations, and % volcanic rock or % volcanic ash (Table 11). NMDS of benthic samples analysed to species level yielded high 2-D stress, but suggested possible associations of *Encyonema minutum* and *Gomphonema parvulum* with higher IEI, % native vegetation and % volcanic rock, and *Aulacoseira* sp. and *Brachysira* sp. C with lower values of these variables (Fig. 18).

With only 10 samples, the correlations in pH group 5 would have been strongly driven by the communities and environments of individual samples. For the benthic samples analysed to species level, samples at the bottom of the plot (including the two South Island samples) are characterised by higher WCI. North Island samples at the top left-hand side of the plot are simply associated with their more easterly location and warmer temperatures (Fig. 19).

Species richness

Total diatom species richness across all samples was associated with higher pH and temperature (AveTCold), with lower species richness at pH <5.4 and, where pH >5.4, higher species richness in areas with cooler winter temperatures. This analysis explained about 40% of the variation in species richness. A similar pattern was seen in South Island epiphyte samples. In South Island benthic samples, a combination of pH and WCI explained 69% of benthic taxonomic richness, with highest species richness at sites with pH >5.94 and WCI >18.8; in other words, in the higher pH sites, which were also the least impacted sites. In the North Island, the regression tree for benthic samples explained 60% of the variation in species richness, with highest species richness at sites with pH >5.85, and with relatively low water conductivity (<162 $\mu\text{S}/\text{cm}$). For epiphytic samples, again less than 40% of the variation was explained in the analysis. In both islands, lowest species richness was at low-pH sites, regardless of other characteristics.

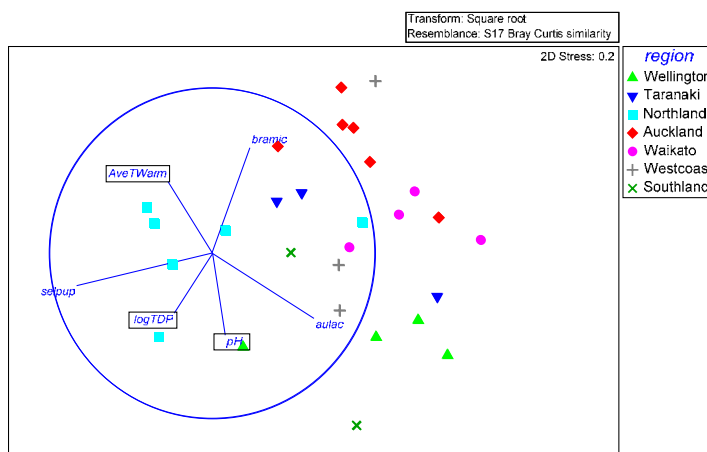


Figure 17. NMDS plot showing benthic samples analysed to species level in pH group 3 (pH 6.0–6.7) separated by region. The vectors represent correlations of species (Spearman, > 0.65) and environmental variables (boxed) (Pearson, > 0.43) with the NMDS axes.

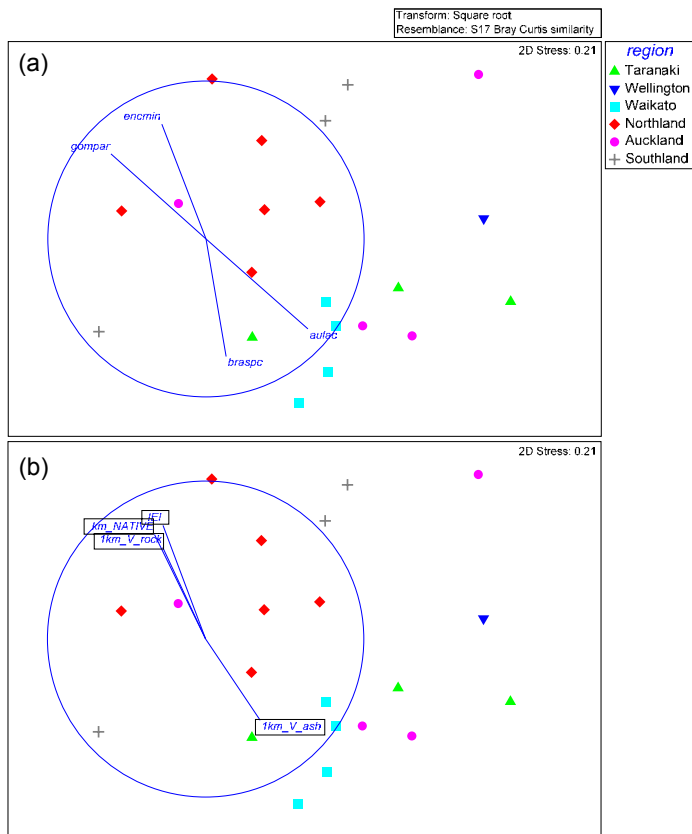


Figure 18. NMDS plot showing benthic samples analysed to species level in pH group 4 (pH 6.7–7.5) separated by region. The vectors represent correlations of (a) species (Spearman, >0.7) and (b) environmental variables (Pearson, >0.6) with the NMDS axes.

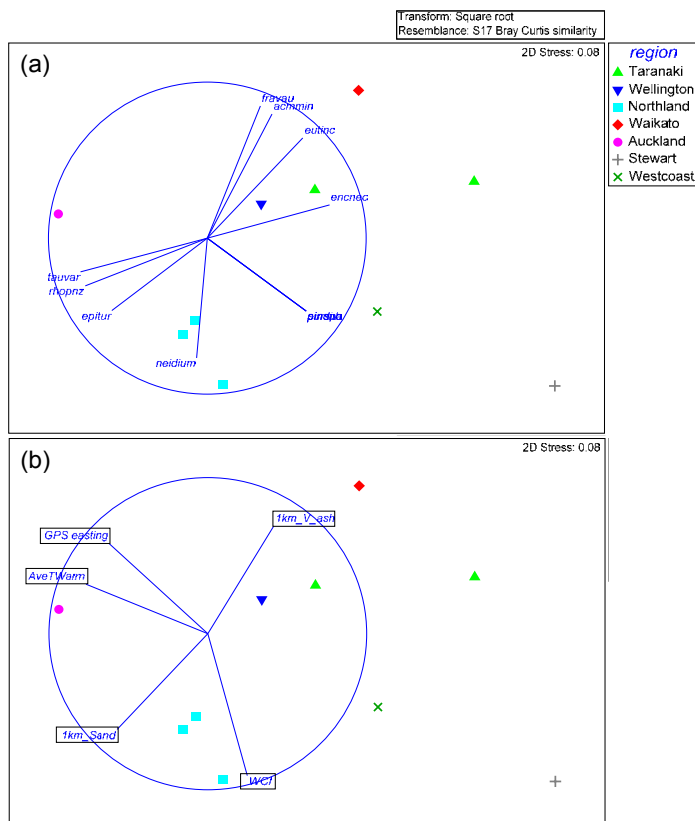


Figure 19. NMDS plot showing benthic samples analysed to species level in pH group 5 (pH >7.5) separated by region. The vectors represent correlations of (a) species (Spearman, >0.8) and (b) environmental variables (Pearson, >0.8) with the NMDS axes.

3.4 Discussion

3.4.1 Overall diversity of algae in lowland wetlands

Over 450 algal taxa (including >260 diatom taxa) were identified from 38 wetlands in lowland areas of New Zealand, suggesting great diversity of algae in these habitats. High diversity of benthic diatoms has also been recorded in other freshwater habitats (e.g. >360 taxa in 53 New Zealand lakes, Reid 2005). Foged (1979) identified almost 700 taxa of diatoms from 174 samples from multiple habitats (although this included some estuarine and marine taxa). Overseas, extremely high diatom diversity has been recorded from deep freshwater lakes. For example, 800 diatom taxa were distinguished from only 20 samples collected from three contrasting oligotrophic lakes in central Europe (Lange-Bertalot & Metzeltin 1996). Thus, the 260 diatom taxa found in our wetland dataset is not an unusually high number compared with other studies. Estimates of diversity of the non-diatom algae are more difficult to compare because of the difficulty of identification below genus level. However, diversity of both non-diatom and diatom algae was high compared to the diversity of other freshwater aquatic organisms in wetlands. For example, 133 invertebrate taxa were identified from samples from the same set of lowland wetlands (Suren & Sorrell 2010).

The identities of algae in these wetland samples appear quite distinctive from those of, for example, flowing waters. A formal comparison is currently lacking, although data from Kilroy et al. (2007) could be used to perform such a comparison for diatoms. Part of the difference is due to the often lower pH in wetland water compared with river water, leading to a much wider variety of desmid taxa, and species in the diatom genera *Brachysira*, *Eunotia*, *Frustulia* and *Pinnularia*. Furthermore, in rivers, flowing water and hard substrata favour domination by algal taxa that have attachments to the substrata, such as the filamentous green alga *Ulothrix*, or the stalked/attached diatoms *Gomphonema* spp. and *Rhiocosphenia abbreviata*.

Long-term hydrological stability in undisturbed, low-nutrient wetland pools is also likely to lead to higher proportions of diatom taxa that have geographically restricted distributions (Kilroy et al. 2007). Taxa encountered in the present dataset that have geographically restricted distributions were *Eunophora* cf. *oberonica* and *Frustulia* sp. A. Potentially restricted distributions in non-diatom algae are more problematic to demonstrate because of the difficulties in identification to species level without lengthy culture studies (Novis et al. 2008).

3.4.2 North Island–South Island differences

The most striking pattern seen for both the non-diatom and diatom community analyses was the strong separation of North and South Island samples. At first glance, this could suggest geographical patterns in species composition, driven by temperature gradients or spatial separation of species because of historical processes, in which restricted distributions could arise through inefficient dispersal mechanisms and geographical isolation. However, with few exceptions, sites on North and South Islands were distinctive from one another in their local water quality/chemistry characteristics. Therefore, the observed algal community differences between North Island and South Island wetland samples are likely to have arisen mostly because of local environmental differences. Large- and small-scale analyses elsewhere have established that diatom community composition is strongly influenced by local variables, including pH, conductivity, and nutrient concentration (e.g. Potapova & Charles 2002; Passy 2010). However, geographical/historical factors are also thought to contribute to community differences over medium to large spatial scales (Soininen et al. 2004).

The North Island–South Island environmental division arises because: 1) there are intrinsic geological (and hence water chemistry) differences between the two islands; and 2) land-use intensification on lowland areas of the North Island is generally higher than on lowland areas of the South Island (at least in the areas sampled). Water chemistry differences are seen clearly in, for example, the National River Water Quality Monitoring Network data (WQIS; <https://secure.>

niwa.co.nz/wqis/index.do). Land-use differences mean that even the 'best' (i.e. closest to pristine) lowland wetlands in the North Island on average tend to be more influenced by human activities than in the South Island. This was clear in our dataset, where wetland condition indices were lower in the North Island than in the South Island (see Table 7).

Another aspect of the North Island–South Island community differences was the greater discrepancy at species level observed in non-diatom communities (only 23% of species shared between islands) than in diatom communities (52% of species shared). One explanation for this discrepancy could be that the non-diatom algae in each island were analysed by different people. Even though the second analyst used the same sample treatment methodology as the first, and referred extensively to the first species list and reference photographs when assigning species identifications, there may have been operator bias. This is an acknowledged problem in many ecological assessments (e.g. Daniels & McCusker 2010, and see Birks 2010 for a review for diatoms).

Almost half of all diatom species identified were exclusive to one island. Analysis of samples by pH group did not suggest separation of communities on the basis of island. Thus, in pH group 2, communities from Northland clustered closely with those from the West Coast and Stewart Island, again suggesting that local environmental conditions were responsible for community structure, rather than geographical patterns. However, the numbers of samples available for direct comparison were small because most samples in each pH group tended to be from one island or the other.

3.4.3 The role of temperature

Temperature strongly influences algal growth rates and therefore might be expected to be responsible for community variation. In general, warmer temperatures and higher light levels favour the development of green filamentous algae (see review by DeNicola 1996), which could explain their predominance in North Island wetlands. Cyanobacteria are also cited as favouring warmer conditions (DeNicola 1996), which is inconsistent with their dominance in the South Island samples. However, cyanobacteria may dominate communities for other reasons. For example, tolerance of extreme environments (repeated freezing and thawing) explains why cyanobacteria probably dominate algal communities in freshwater ponds in Antarctica (Vincent & James 1996), despite temperatures well below those supporting optimum growth rates (Tang et al. 1997). Freshwater diatoms in general tend to be associated with cooler temperatures (DeNicola 1996). Strong community structuring as a result of temperature gradients has proved difficult to demonstrate for freshwater diatoms (e.g. Anderson 2000; and see comments in section 2.4.4), although Potapova & Charles (2002) identified responses to temperature as one of three important drivers of diatom community composition in rivers over large spatial scales in the USA. However, in that study the problem of temperature correlation with other environmental factors was acknowledged.

We faced the same problem in the present study: strong inter-island environmental differences meant that it was impossible to separate geographical, nutrient-driven or temperature-driven patterns in community composition. Identification of such patterns would require more precise comparisons of equivalent wetland types. For example, the latitude/longitude gradient cannot be separated from the temperature gradient in the present study because all the sites were in lowland areas. The existence of biogeographical species distributions could be tested by inclusion of an altitude gradient to allow comparison of wetlands with cooler temperatures across a wider latitudinal gradient.

A further consideration with regard to temperature is that mean annual air temperature in New Zealand's lowland regions ranges from about 8°C to 14°C, which may not be a broad enough range to expect major community changes. In contrast, the range in the USA was 2.4°C to 22.7°C (Potapova & Charles 2002).

3.4.4 Can algal communities indicate wetland condition?

One or other of the wetland condition indices (IEI or WCI) featured in many of the best combinations of environmental variables explaining community composition. For diatoms (analysed by pH group), 16 of the 20 combinations included one or both indices, and in seven cases, the indices were the best single explanatory variable. A recent study, using a wider range of wetlands (in terms of human impacts) on the West Coast, showed that diatom community composition did reflect wetland condition (as assessed by the IEI or WCI) in some cases, but generally in combination with other variables (pH or dissolved nutrients) (Suren et al. 2011). Because both indices include landscape-scale components that are unlikely to influence aquatic communities, the weak correlations were expected. However, human impacts on wetlands typically result in hydrological changes (which may affect pH, alkalinity and water colour) and increases in nutrient concentrations. Therefore, the associations of algal community composition with nutrients and pH shown in our broad-scale study suggest that some taxa may strongly indicate these changes. The potential use of indicator taxa is discussed briefly in section 5.

3.4.5 Diversity v. community composition

Taxonomic diversity, defined as the number of species or taxa present (taxon richness), or some metric derived from species numbers and their relative abundances, is frequently the biotic variable of interest when testing ecological theory (Pollock et al. 1998; Vyverman et al. 2007; Passy 2010). However, biodiversity *per se* is not necessarily critical, or even important, for conservation (e.g. Srivastava & Velland 2005). This was illustrated by the analyses on diversity in the present studies. Both the non-diatom and diatom datasets showed patterns in taxon richness. For example, taxon richness of the non-diatom algae tended to decline as phosphorus concentrations increased, suggesting declining diversity as land development (indicated by enhanced phosphorus levels) increased. In the diatoms, samples from low-pH sites generally had comparatively low species richness, which is consistent with a known pattern (DeNicola 2000; Pither & Aarssen 2005). If we were to rely only on diatom species richness to determine the conservation value of a site, this would suggest low-pH sites were least worthy of conservation. In fact, these are the sites most likely to contain unusual taxa (e.g. endemic or rare species) (Kilroy et al. 2007), and therefore arguably warrant the highest level of protection. Thus the identities of species, not just the numbers of species, define the uniqueness or conservation value of a habitat.

4. Management and conservation considerations and implications

4.1 Sampling algae in wetlands

In this section, we consider the results of the studies described in sections 2 and 3 and make some general recommendations for optimising sampling and processing effort when carrying out surveys of algae in wetlands.

Compared to sampling larger organisms such as fish or invertebrates, field sampling of algae requires minimal equipment. Detailed sampling protocols have been developed for stream algae (e.g. Biggs & Kilroy 2000) and in general the same principles apply to sampling wetlands (fens, bogs and swamps). There are also established guidelines for sampling algae in wetlands (i.e. U.S. EPA 2002). Wetlands are not expected to experience the highly variable algal biomass and community composition over space and time caused by flow fluctuations in rivers, which must be considered when sampling algae in rivers. On the other hand, substratum types may be more variable. For example, standard methods for the quantitative sampling of periphyton on rocks may be applied in a high proportion of rivers. In wetlands, the substratum of permanent water bodies may vary from shallow to deep, mineral or organic sediment, with mineral substratum ranging from very fine to coarse. In addition, macrophytes are often, but not always, present, with variable coverage. This variability makes quantitative sampling difficult in many situations.

The first thing to consider when planning a survey is its purpose. For example, a different approach is required for a survey to describe biodiversity compared to a survey aimed at detecting change following restoration. Whatever the purpose, we suggest that a standard set of questions need to be addressed, summarised in Table 12.

4.1.1 What type of algae should be sampled?

The choice is normally between diatoms and non-diatom algae, although many samples will contain both. Specific algal groups may be selected if the indicator approach is being taken (see section 4.2). The decision on which algal type to use in the study may be made at the sample processing stage rather than in the field. For a biodiversity survey, clearly all types of algae will be listed. For detecting change, in general diatoms are more suitable because: 1) they are easier to identify and enumerate (see U.S. EPA 2002); and 2) they are probably less subject to large seasonal variations. In the long-term temporal study (section 2.4), we were able to detect changes in abundances of some diatom species over time in an unmodified wetland, but communities remained characteristic of their particular combination of environmental conditions. A difficulty with non-diatom algae is that it is often necessary to culture live material in the laboratory in order to identify species, or to observe the reproductive structures in the field (e.g. see Novis 2003, 2004). On the other hand, there may be scope to use desmids in more detail because good identification guides to the New Zealand flora exist (Croasdale & Flint 1986, 1988; Croasdale et al. 1994). The first step would be to investigate how desmid community composition is related to various aspects of wetland condition.

4.1.2 When should samples be taken and how often?

Although not specifically tested in our studies, sampling in the summer months would be expected to yield relatively more non-diatom algae than in the cooler months, when diatoms may dominate (U.S. EPA 2002). Therefore, summer is expected to be most appropriate for biodiversity surveys. We saw in section 2.4 that diatom species may vary seasonally in abundance, but are usually present year-round.

Table 12. Summary of questions to be considered (with comments on options) when designing surveys for algae in wetlands.

QUESTION	OPTIONS	PURPOSE OF SURVEY	
		ASSESSING BIODIVERSITY	DETECTING CHANGE
1. What type of algae should be sampled?	<ul style="list-style-type: none"> • Diatoms • Non-diatom algae (possibly specific types) 	All types of algae, depending on the specific aims of the survey.	Focus on one type of alga: diatoms are recommended.
2. When should samples be taken and how often?	<ul style="list-style-type: none"> • Sampling time not important • Sample consistently within seasons • Annually, monthly, etc. 	A single sampling during the summer months will probably capture most taxa present.	To detect responses to changes expected in wetlands, annual sampling may be sufficient (same time of year). A series of temporally spaced samples at 1–4-week intervals is suggested.
3. What habitat/substratum should be sampled?	<ul style="list-style-type: none"> • Epiphytic • Benthic (soft substratum) • Benthic (hard substratum, wood) • Artificial substrata 	Collect from all available natural habitats.	Constrain sampling to a single habitat (substratum) type. Use artificial substrata to reduce variability when testing for effects of restoration measures (for example).
4. What sampling methodology should be used?	<ul style="list-style-type: none"> • Quantitative • Qualitative 	Quantitative or qualitative; generally % abundance is enough. Use rock scrapes, cores, grab samples, etc., depending on substratum.	Ideally quantitative (provides more options). Use rock scrapes, cores, depending on substratum.
5. How many samples should be collected?	<ul style="list-style-type: none"> • Range of numbers 	Ideally a composite of at least five samples from each area of interest.	Replicate composite samples, ideally collected over time rather than space. Minimum of three samples; more are better.
6. How should samples be preserved?	<ul style="list-style-type: none"> • Examine fresh • Freeze • Preservative (e.g. glutaraldehyde, lugols iodine) • Permanent mounts (diatoms) 	Ideally examine fresh samples, especially for filamentous algae. For long-term storage glutaraldehyde is the most stable. Use permanent mounts of diatoms for detailed ID to species level.	Use the most convenient method. Freezing may be required if samples are to be analysed for pigments. Lugols iodine is good for medium-term studies over time.
7. How should the samples be processed?	<ul style="list-style-type: none"> • Qualitative (presence/absence) • Semi-quantitative (abundance assessments, e.g. common, rare) • Quantitative counts (%) • Fully quantitative (cells per unit volume or area) 	Depends on resources; at least semi quantitative assessments recommended if at all possible (better than presence/absence).	Normally at least quantitative counts (% abundances). Use fully quantitative counts for experimental studies over time.
8. What taxonomic resolution is required?	<ul style="list-style-type: none"> • Species level (or in as much detail as possible) • Genus level 	Tailor detail to the objectives of the survey. Genus level may be useful.	More detailed identifications yield stronger environmental relationships.

For detecting change, the timing and frequency of sampling is more critical, and will depend on the expected magnitude and duration of the change. Algae are acknowledged to be good environmental indicators because they provide an integrated response to changes in water chemistry (U.S. EPA 2002), whereas the actual water chemistry changes themselves may be detectable only from multiple samples collected over time. For example, water quality changes resulting from alterations in land use may be subtle, and occur over long time frames. In these cases, annual sampling (at the same time of year) is likely to be sufficient to detect changes (or lack of changes).

Replicates are needed to detect changes over time. As described in section 2.4, these may be over smaller time periods rather than spatial replicates. Thus, for a robust year-to-year comparison, we recommend a series of three to five composite samples, collected 1–4 weeks apart at a standard time of year, to allow statistical comparison of samples in successive years. When sampling at close intervals over time, care should be taken to avoid sampling in areas disturbed during previous surveys. Ideally, sampling areas should be pre-defined at the start of the study.

As with all studies of change over time, ideally, the ‘impact’ (or restoration) would be matched with a similar ‘control’ site, for a Before-After-Control-Impact (BACI) experimental design.

4.1.3 What habitat/substratum should be sampled?

In the studies described in this report, sampling was restricted to the benthos (e.g. Fig. 20) and to epiphytic algae, growing attached to macrophytes. There are two other recognised wetland habitats: phytoplankton⁷ and metaphyton (floating mats of algae; Goldsbrough & Robinson 1996; U.S. EPA 2002). In addition, benthic algae may be divided into epipelton (growth on soft sediments), epilithon (growth on stones or other hard surfaces), and epidendron (growth on wood surfaces). The benthic samples collected in our studies were generally epipelton. We found a weak distinction between epiphytic and benthic samples for the non-diatom algae (section 3.2). On the other hand, epiphytic and benthic diatom communities did not differ when compared in a dataset covering a wide geographical area. At the same time, an intensive small-scale spatial study (section 2.3) indicated that gradients of population densities exist even on uniform substrata, and substratum consistency can affect community composition.

We therefore recommend that for biodiversity surveys, representatives of all available substrata should be sampled. To detect changes over time, it is important to sample from a single substratum type. It has been emphasised in other studies that substratum-specific sampling can better detect changes over time than composite samples from several substrata (U.S. EPA 2002).

In some cases, the use of artificial substrata might be appropriate. For example, in experiments aimed at detecting the effects of restoration measures, such as weed removal, we suggest placement of arrays of artificial substrata in impacted and control areas, followed by repeated sampling over an appropriate period before, during and after the expected change (i.e. a BACI



Figure 20. A benthic mat (mainly composed of *cyanobacteria trichomes*, but also containing diatoms and other algae) about to peel off and become metaphyton.

⁷ Phytoplankton is not considered in this report.

design). In a heterogeneous environment, this is probably the most efficient means of detecting changes. Pre-selection of numbered substrata ensures effective random sampling on each sampling occasion. Suitable substrata include: small unglazed tiles attached to a backing plate, and placed in a suitable water depth; or roughened plastic slides attached at various depths to stakes driven into the natural substratum.

4.1.4 What sampling methodology should be used?

The choices here are quantitative versus qualitative, and this depends on the purpose of the survey. The analyses described in section 2.4 suggest that to detect ecologically significant diatom community changes over time, semi-quantitative data (% abundances) are probably adequate, which require only qualitative sampling. For other algal types, quantitative sampling to obtain absolute abundances may be more appropriate if the effects of increasing or decreasing nutrient inputs are being considered.

The sampling methodology depends on the substratum or habitat being sampled. Because wetlands, by definition, are often 'wet areas or shallow water' (section 1), sample collection can normally be carried out from margins of pools or by wading in pools with hard substrata. Algae in stable wetland pools can be very sensitive to physical disturbances. Therefore, minimal disruption of the benthos is desirable. In some cases (e.g. the spatial study described in section 2.3), sampling from a floating platform or similar is the only option without completely destroying algal mats.

Quantitative sampling

For epiphytes, quantitative sampling is difficult and would require measurements of surface area or weight to standardise the samples. The time and effort required to do this is unlikely to be worthwhile unless a specific research question is being addressed.

Benthic algae can be sampled quantitatively more easily. Periphyton attached to hard surfaces (epilithon and epidendron) may be sampled using methods described for sampling in rivers (see Biggs & Kilroy 2000). Briefly, the area to be sampled is defined by a circle of known diameter (e.g. a sampling container lid) and the algae within the circle are carefully removed using a sharp blade or a toothbrush. Sample removal can be made easier by first removing all periphyton from outside the circle. Soft sediments (epipelon) may be sampled quantitatively using a small coring device, for example: a narrow syringe with the tip removed; a simple cylinder; or a short cylinder closed at the top (except for a hole to allow air to escape). In all cases it is important to: 1) standardise the depth to which you sample; 2) close off the end of the corer by sliding a flat surface underneath it while in the sediment; and 3) note the internal diameter and depth of the corer so that subsequent cell counts can be normalised to either a unit area of sediment surface or a unit volume of sediment. Metaphyton can be sampled in the same way.

Artificial substrata (see section 4.1.3) are also useful for sampling quantitatively, because tiles, slides, etc. have a uniform surface area. However, the algae growing on them may not be representative of natural communities and they are most appropriate in experiments to detect change.

Qualitative sampling

Qualitative sampling is essentially grab sampling, and any convenient sampling device can be used. For example: sample epiphytic algae by cutting sections from the plants of interest; sample benthic algae by scraping a spoon across a soft substratum surface, withdrawing substratum using a turkey baster, or scrubbing whole stones. When collecting composite samples (see below), it is a good idea to make each subsample roughly the same size so that the eventual sample is not biased.

4.1.5 How many samples should be collected?

Whatever the methodology, we recommend collecting composite samples from the area, habitat or substratum of interest (see section 2.3). Variability (patchiness) in species distributions will then be averaged out in the sample eventually processed. We recommend at least five subsamples per composite sample.

The number of composite samples collected depends on the objective of the survey.

- If samples are being collected with the objective of linking community composition with environmental variables, then a single composite sample is sufficient for each site. This was the approach taken in the New Zealand-wide study described in section 3. In that study, usually only three subsamples were composited. However, because we were comparing algal communities over wide environmental gradients, this was sufficient to distinguish among sites.
- If the objective is to describe biodiversity, then composite samples should be collected from each distinguishable habitat in each area of interest.
- Replicates are needed to detect changes over time. As noted above (section 4.1.2), these may be over smaller time periods rather than spatial replicates; for example, at least three composite samples, collected one to four weeks apart at a standard time of year.
- When using artificial substrata, arrays should be set up at several replicate locations in the control and treatment areas. On each sampling occasion, samples should be collected (randomly) from each array.

It is beyond the scope of this report to cover experimental design in detail, but case studies can be found in U.S. EPA (2002), and other examples are referred to in this report (e.g. Lane & Brown 2007; Weilhofer & Pan 2007).

4.1.6 How should samples be preserved?

The simplest and cleanest method for preserving samples is freezing. This has the disadvantage that soft filamentous algae may become misshapen and difficult to identify. Cyanobacteria handle freezing well, and desmids relatively well, as they have semi-rigid cell walls. Freezing affects the chloroplasts of diatoms but not the cell walls (frustules), which are used in identification. Therefore, if at all possible, samples with visible filamentous algae should be examined fresh. The advantage of freezing is that samples can also be used in other analyses, such as pigment concentrations.

Alternatively, probably the most effective long-term preservative is glutaraldehyde. A 2% solution should be sufficient to retain features such as cell shape and chloroplast structure of all algae. Note that glutaraldehyde is toxic and a strong irritant. Protective clothing and eye protection should be used when handling glutaraldehyde.

Lugol's iodine is also a useful preservative, but needs to be checked and replenished periodically (e.g. annually) as it is unstable. This is probably the best option for preserving samples in a study over medium time periods (months) because Lugol's is relatively non-toxic, and cells retain their form well.

Diatom samples can be preserved permanently as mounted slides following digestion of the organic component of the sample (see section 4.1.7).

4.1.7 How should the samples be processed?

Depending on the sampling method used (section 4.1.4), samples may be processed qualitatively (e.g. presence/absence of taxa), semi-quantitatively (e.g. assign taxa to abundance classes such as abundant, common, rare), quantitatively (cell counts to obtain % abundances of each taxon), or fully quantitative (cell counts on known volumes of sample to obtain counts per unit volume or area). Standard methods can be used to process algal samples from wetlands, such as those described in Biggs & Kilroy (2000). Benthic samples may be blended lightly with a hand blender to obtain a homogenous solution, subsamples transferred to a sample well (Utermöhl chamber) and then examined at magnifications up to 400× under an inverted microscope. Epiphytic samples will first need to be scraped from the surfaces of the collected plants.

If counts are being made from quantitative samples, a record must be kept of the original volume of the sample (after blending), the volume of all subsamples examined, and if appropriate, the number of fields of view from which counts were made (refer to Biggs & Kilroy 2000 for more details). Fully quantitative counts would normally be required in experimental studies over time (such as assessing the effects of restoration measures using natural or artificial substrata).

To examine diatom communities in more detail, samples would normally be acid-cleaned to remove organic material and allow a clear view of the diatom frustule, on which diatom taxonomy is largely based. However, diatom community composition can be assessed to a useful level using live material (Cox 1996). This was the method employed in the study described in section 2 of this report. In those studies, the samples contained relatively few taxa in samples collected within a small area or from the same area over time. In studies with broad geographical coverage, such as the New Zealand-wide study described in section 3.3, it is necessary to examine acid-cleaned material to distinguish many taxa. Suspensions of diatoms dried onto glass cover slips may be permanently mounted onto a microscope slide, for examination under oil at magnifications up to 1000×. For details of the method, refer to Biggs & Kilroy (2000). Acid-cleaning diatoms means that live and dead cells can no longer be distinguished. This could be a potential problem for detecting changes in wetlands where shifting pH or nutrient concentrations might favour different species, but the original populations could still be present in the soft sediment. In a study of this issue in streams, Gillett et al. (2009) concluded that the communities assessed from live material and from acid-cleaned material were indistinguishable. However, it is not known whether this would hold for wetland samples, in which dead cells are not washed away by the current.

In section 3.2, the data were reduced to presence/absence for the analyses. This was the most straightforward way to align two datasets collected at different times, and reduction of the data made little difference to the results. Normally, if counts are not being done, it is a good idea to carry out a visual relative abundance assessment. For example, an 8-point system (from 1 = rare to 8 = dominant) was described by Biggs & Kilroy (2000). Simpler systems with, for example, five categories (rare, occasional, common, abundant, dominant) are also useful for distinguishing taxa that are rare (that may have ended up in the sample purely by chance) or more abundant (indicating that they are an actively growing part of the community). Balmer (2002) stressed the importance of taking abundance into account in ecological surveys.

4.1.8 What taxonomic resolution is required?

The results of the New Zealand-wide study of pristine lowland wetlands (section 3) suggested that analysis of samples to species level where possible generally produced the strongest relationships with environmental variables. For both the non-diatom and diatom datasets, a far higher proportion of genera were shared between the islands, than were species. At the same time, similar environmental relationships (albeit weaker) were still picked up by the genus-level analyses. Thus, while identification to species is desirable, genus-level identifications are still useful. This is advantageous, because identification to genus level is far easier than to species level, and can be done by relatively inexperienced analysts, using fresh material. Further investigations into the discriminatory power of species-level versus genus-level analyses would be useful in future projects aimed at detecting changes in wetland aquatic communities following, for example, restoration measures.

4.2 Using algae as indicators of wetland type and condition

Methods to assess the overall condition of wetlands in New Zealand have already been developed (see section 3.1) but use of small organisms to evaluate the integrity of the aquatic component of wetlands has only recently been considered. In particular, a Macroinvertebrate Community Index (MCI), developed specifically for wetlands, is under development (Suren et al. 2010). Algae probably respond to a suite of environmental variables that differs from that relevant to invertebrates. Algae also at least partly underpin invertebrate biomass. Therefore, algae and invertebrates may provide complementary information, and can be used together as management tools for documenting restoration success or evaluating impacts on wetlands. Comparisons of the effectiveness of algae and invertebrates for indicating environmental changes in rivers have most often found that the two approaches are complementary (Newall et al. 2006; Dohet et al. 2008; Justus et al. 2010; Torrisi et al. 2010).

To date, algal community composition has not been evaluated as an indicator of ecological integrity in New Zealand freshwaters in general, although algal biomass and broad visual categories are recommended as indicators of river eutrophication (e.g. Biggs & Kilroy 2000), or for tracking restoration efforts in streams (Parkyn et al. 2010). The variability of periphytic algal biomass over time has been cited as a disadvantage for its use as an indicator of ecological integrity in rivers, but use of taxonomic composition was not considered (Schallenberg et al. 2011). Algae in wetlands are not subject to the scouring floods that occur in many rivers and therefore present an obvious means for tracking ecological changes. Furthermore, algal community composition is increasingly being used as an indicator of wetland condition overseas (e.g. Stevenson 1998; Lane 2007; Lane & Brown 2007; Lougheed et al. 2007; Gaiser 2009).

The studies described in sections 2.4, 3.2 and 3.3 have demonstrated that it is feasible to link certain algal species (especially diatoms) with particular ranges of local environmental variables such as pH, alkalinity, gilvin (water colour, see section 2.4.4), and nutrient concentrations, all of which could be affected as a result of human activities within or in the vicinity of wetlands. We found clear North Island–South Island community differences, which can be largely attributed to differences in local environments. Because these studies focused on wetlands judged to be least disturbed in their region, data on algal associations across the full range of aquatic conditions in wetlands in individual regions are not yet available. An exception is the West Coast: analysis of diatom communities across a wide range of wetlands showed complete turnover of species across environmental gradients (Suren et al. 2011). While pH was the dominant driver of community composition, dissolved nutrients, and the measured wetland condition indices (IEI and WCI) were also related to the abundances of some taxa. Such linkages were partly driven by the fact that low-pH wetlands also tended to be those with high condition scores. If such wetlands are hydrologically disturbed, an early effect is a shift in pH (Lundin & Bergquist 1990), which would be expected to lead to rapid shifts in diatom community composition. The strong association between algal community composition (particularly diatoms) and water pH and nutrient concentrations also implies that algae could be added to the suite of metrics used to classify the wetland types described by Johnson & Gerbeaux (2004).

Preliminary analyses have shown that some diatom taxa occur almost exclusively in low-pH bogs and fens assessed to be in pristine condition (using the IEI and WCI scoring methods); examples are *Eunophora* sp. (a genus apparently confined to New Zealand and Tasmania/southeastern Australia) and *Frustulia* sp. A (yet to be described; see Kilroy 2007). Both these taxa are therefore potentially good indicators of wetlands in good condition. We expect that with more data from a wider range of wetlands, other taxa will be identified as indicators of both good and poor condition.

A range of methods exist for identifying indicator species, often with different definitions of what an ‘indicator’ is. For example, Halme et al. (2008) considered that presence of an indicator species should be associated with the presence of other species, in particular rare or threatened species

(‘target’ species) that may be less easy to observe. Goodsell et al. (2009) discussed the use of indicators that specifically signal certain levels of (or absence of) environmental contaminants, and concluded that biological indicators in these applications may be of limited use. We suggest taking a flexible approach, such as that proposed by Dufrene & Legendre (1997). These authors proposed a method for identifying and quantifying (by an ‘Indicator Value’) taxa that are associated with pre-defined groups of sites or environmental conditions. Taxa with high indicator potential could then be targeted in studies to track, for example, the effects of restoration efforts. Relevant taxa in the region of the restoration work could be identified in pilot studies.

4.3 Implications for conservation

The results of the studies in Section 2 provide some useful insights into how to best design monitoring and management studies aimed at, for example: 1) detecting responses to known stresses on the aquatic communities in wetland pools; 2) tracking changes resulting from restoration efforts; or 3) investigating linkages between aspects of biodiversity and environmental gradients.

The other studies described in this report represent a first attempt to place some geographical and environmental context around existing knowledge of algae in wetlands, particularly for lowland wetlands. From a conservation perspective, this contributes to filling a significant knowledge gap with respect to New Zealand’s biodiversity. In New Zealand’s 2000 Biodiversity Strategy, freshwater algae were described as suffering from ‘major gaps in our knowledge’ (DOC/MfE 2000), and there was no indication that the situation had changed after five years (Green & Clarkson 2005). Furthermore, freshwater algae are not considered at all on New Zealand’s list of threatened species. Given the specific mention by Green & Clarkson (2005) of wetlands as requiring representative protection, this report, along with the accompanying studies on invertebrates (Suren & Sorrell 2010), is highly relevant.

Our studies indicate that algal diversity in wetlands is high compared with the diversity of other taxonomic groups, such as invertebrates (e.g. Suren & Sorrell 2010). The diatom flora contains as yet undescribed species that are not known from other regions, and therefore may be endemic (Kilroy 2007), as well as recently described new species (e.g. Beier & Lange-Bertalot 2007). Furthermore, there are distinct differences between algal communities in relatively unimpacted wetlands in the North and South Islands. Our analyses suggest that this pattern is largely attributable to strong inter-island environmental differences, and this is supported by the presence of similar species in low-pH environments in Northland and Stewart Island. However, there is scope to test this result more thoroughly by comparing larger groups of samples from wetlands of a similar type defined by pH (e.g. fens, swamps).

The lists of algae in Appendices 2 and 3 provide a baseline for further studies. For example, a primary aim would be to develop algae-based indices of wetland condition (aquatic component) that complement the wetland MCI, currently under development. The dataset (including full taxonomic details for the named taxa) is available from the Freshwater Biodata Information System (FBIS) to facilitate use of the data in future analyses, such as comparison with taxa lists from other habitats.

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Appendix 1

Sampling sites included in the analysis of algal communities of largely unimpacted wetlands in lowland areas of New Zealand

IEI = Index of Ecological Integrity; WCI = Wetland Condition Index. Cond. = conductivity.

Wetland type was assigned at the time of sampling on the basis of pH and vegetation, according to Johnson & Gerbeaux (2004).

REGION	WETLAND	APPROX. LOCATION		TYPE	IEI	WCI	pH		COND. ($\mu\text{S}/\text{cm}$)		
		EASTING	NORTHING				MIN.	MAX.	MIN.	MAX.	
Northland	Spirits Bay	2494544	6749157	Swamp	0.95	23.3	5.7	6.6	168	437	
	Te Wherari	2483178	6748084	Swamp	0.60	18.8	6.8	6.9	256	259	
	Moututangi	2528271	6702348	Fen	0.56	20.7	4.1	5.3	158	190	
	Kaipuha	2577640	6636160	Bog	0.64	18.4	5.8	6.1	78	101	
Auckland	Maitahi	2578372	6592702	Fen	0.39	21.8	6.2	6.6	184	187	
	Tomarata	2658864	6554928	Fen	0.25	18.8	6.2	7.0	203	211	
	Kaitoke	2729769	6550353	Fen	0.93	22.8	6.3	6.3	608	646	
	Poutu	2604418	6536440	Swamp	0.37	22.3	7.6	9.0	239	662	
	Te Henga	2640528	6479924	Swamp	0.66	18.8	6.8	7.1	207	217	
	Whatipu	2643446	6461717	Swamp	0.92	18.8	7.0	8.8	217	335	
	Waikato	Kopuatai	2738495	6418670	Bog	0.23	17.8	4.3	4.3	54	54
Waikato	Opuatia	2692809	6416379	Bog	0.23	21.0	6.2	6.9	105	217	
	Lake Rotokauri	2704025	6379422	Swamp	0.20	16.9	6.0	7.6	144	187	
	Rotomanuka	2714052	6361936	Shallow water		18.0	6.5	6.9	178	195	
	Serpentine	2714012	6359105	Shallow water	0.20	17.9	7.8	7.0	120	121	
	Te Haora	2663098	6337812	Swamp		22.0	6.4	6.5	185	228	
	Taranaki	Umutekai	2608401	6234882	Swamp	0.20	18.8	6.0	7.4	136	142
Taranaki	Barrett Lake	2600176	6234416	Swamp	0.24	20.3	7.2	7.6	153	158	
	Corbett Lake	2589052	6219298	Swamp	0.34	20.8	6.7	7.6	119	127	
	Wellington	Cameron	2665958	5981892	Swamp	0.76	21.3	6.6	6.6	328	435
Wellington	Gollans	2666955	5981325	Swamp	0.69	19.4	6.4	7.6	207	233	
	West Coast	Mahinapua	2340258	5823910	Fen	0.56	22.8	5.4	5.9	56	77
West Coast	Shearer	2326411	5807742	Fen	0.89	24.3	5.3	5.4	42	44	
	Ohinetamatea	2256108	5739839	Swamp	0.80	21.3	6.2	7.7	47	54	
	Lake Kini	2237289	5728433	Bog	0.80	24.2	4.5	6.2	21	34	
	Heretaniwha	2231312	5728234	Fen	0.79	24.7	4.4	4.4	50	159	
	Maori Lakes	2195310	5700571	Fen	0.79	24.7	4.6	5.6	28	29	
	Burnmeister	2166072	5680810	Fen	0.80	24.2	4.3	4.5	28	30	
	Cascade	2138637	5672897	Fen	0.95	24.2	5.1	5.1	20	20	
	Southland	Waiuna Lagoon	2122952	5643966	Swamp	0.81	18.8	6.6	6.7	87	93
	Southland	Hollyford	2112219	5635845	Fen	0.79	24.7	4.3	4.4	22	29
		Transit Bay	2093206	5609579	Fen	0.96	18.8	4.5	6.1	46	52
Kepler Mire		2095183	5507509	Bog	0.30	19.3	4.0	4.1	27	34	
Lake Rakatau		2086198	5501282	Fen	0.95	24.2	4.8	6.7	30	216	
Awarua		2159434	5397414	Swamp	0.41	20.0	4.2	4.2	141	178	
Stewart Is.		Ruggedy Flats	2109396	5370261	Bog	0.97	24.2	4.6	4.6	60	60
Stewart Is.	Freshwater	2115130	5364497	Bog	0.97	24.0	4.4	4.6	75	91	
	Mason	2117142	5353962	Bog	0.97	24.2	5.6	5.7	126	136	

Appendix 2

Non-diatom algal taxa identified from 200 samples from 40 New Zealand wetlands

The number of records in the North and South Islands are shown. Note that many identifications to species level are tentative (indicated by 'cf.'). mt = morphotype (taxa suspected to be separate species from their morphology, but which could not be identified).

TAXONOMIC GROUP	DESCRIPTION	GENUS	SPECIES	VARIETY/ NOTES	NUMBER OF RECORDS		
					NORTH IS.	SOUTH IS	
Cyanobacteria	Single-celled and colonial	<i>Aphanocapsa</i>	<i>grevillei</i>		16	35	
		<i>Aphanocapsa</i>	<i>parasitica</i>			5	
		<i>Aphanothece</i>	<i>microscopica</i>			6	
		<i>Aphanothece</i>	<i>nidulans</i>			12	
		<i>Aphanothece</i>	sp.			1	
		<i>Aphanothece</i>	<i>stagnina</i>			8	
		cf. <i>Gloeocapsopsis</i>				9	
		<i>Chroococcus</i>	<i>giganteus</i>		2		
		<i>Chroococcus</i>	<i>minutus</i>			13	
		<i>Chroococcus</i>	<i>obliteratus</i>			3	
		<i>Chroococcus</i>	sp.			1	
		<i>Chroococcus</i>	<i>turgidus</i>			19	
		<i>Gloeocapsa</i>	aff. <i>decorticans</i>			3	
		<i>Gloeocapsa</i>	cf. <i>granosa</i>			13	
		<i>Gloeocapsa</i>	sp. 1		1	8	
		<i>Gloeocapsa</i>	sp. 3			6	
		<i>Gloeothece</i>	aff. <i>rupestris</i>			2	
		<i>Gloeothece</i>	sp. 1			6	
		<i>Gloeothece</i>	sp. 2			29	
		<i>Gloeothece</i>	sp. 3			3	
		<i>Gloeothece</i>	sp. 4			3	
		<i>Gloeothece</i>	<i>subtilis</i>			2	
		<i>Merismopedia</i>	cf. <i>marssonii</i>			7	
		<i>Merismopedia</i>	<i>elegans</i>			4	
		<i>Merismopedia</i>	<i>glauca</i>			6	
		<i>Microchaete</i>	sp.		1		
		<i>Rhabdoderma</i>			1	12	
		<i>Rhabdogloea</i>	sp.			4	
		<i>Synechococcus</i>	cf. <i>mundulus</i>			5	
		Trichomes (filaments)	<i>Anabaena</i>	sp.		12	1
			cf. <i>Arthrospira</i>				1
			<i>Aulosira</i>	sp. 1			5
			<i>Calothrix</i>	cf. <i>fusca</i>		5	1
	<i>Calothrix</i>		sp. 1		1	1	
	<i>Cylindrospermum</i>		sp.		1		
	cf. <i>Trichormus</i>		sp.			14	
	<i>Coleodesmium</i>				2		
	<i>Fischerella</i>		sp.		1	3	
	<i>Hapalosiphon</i>		<i>brasiliensis</i>			4	
	<i>Hapalosiphon</i>		<i>hibernicus</i>			9	

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TAXONOMIC GROUP	DESCRIPTION	GENUS	SPECIES	VARIETY/ NOTES	NUMBER OF RECORDS	
					NORTH IS.	SOUTH IS
		<i>Hapalosiphon</i>	<i>intricatus</i>			10
		<i>Hapalosiphon</i>	sp. 1			1
		<i>Hapalosiphon</i>	<i>pumilis</i>		8	
		<i>Hassalia</i>				1
		<i>Jaaginema</i>	cf. <i>gracile</i>			1
		<i>Jaaginema</i>	cf. <i>metaphyticum</i>			2
		<i>Leibleinia</i>	<i>epiphytica</i>			3
		<i>Leptolyngbya</i>	<i>angustissima</i>			5
		<i>Leptolyngbya</i>	<i>fragilis</i>			5
		<i>Leptolyngbya</i>	<i>frigida</i>			11
		<i>Leptolyngbya</i>	sp.		3	
		<i>Lyngbya</i>	<i>martensiana</i>			1
		<i>Microcoleus</i>				1
		<i>Nostoc</i>	<i>commune</i>		6	8
		<i>Nostoc</i>	<i>punctiforme</i>		2	7
		<i>Oscillatoria</i>	cf. <i>bornetii</i>		1	
		<i>Oscillatoria</i>	cf. <i>princeps</i>		1	
		<i>Oscillatoria</i>	cf. <i>sancta</i>		20	
		<i>Oscillatoria</i>	cf. <i>tenuis</i>		19	1
		<i>Oscillatoria</i>	<i>curviceps</i>		15	
		<i>Oscillatoria</i>	<i>simplicissima</i>		2	1
		<i>Oscillatoria</i>	sp.		2	
		<i>Phormidium</i>	<i>aerugineo-caerulea</i>		3	3
		<i>Phormidium</i>	cf. <i>granulatum</i>			2
		<i>Phormidium</i>	cf. <i>retzii</i>		2	
		<i>Phormidium</i>	cf. <i>simplicissimum</i>			1
		<i>Phormidium</i>	cf. <i>tenuis</i>		5	
		<i>Phormidium</i>	sp.		3	1
		<i>Pseudanabaena</i>	cf. <i>minima</i>			7
		<i>Pseudanabaena</i>	sp. 2			1
		<i>Pseudanabaena</i>	sp. 3			1
		<i>Schizothrix</i>	sp. 1			3
		<i>Scytonema</i>	cf. <i>millei</i>		10	3
		<i>Scytonema</i>	<i>hofmannii</i>			1
		<i>Stigonema</i>			9	27
		<i>Symploca</i>	<i>muscorum</i>	cf.	2	
		<i>Tolypothrix</i>	sp.		3	8
		cf. <i>Trichormus</i>	sp.			14
		<i>Tychonema</i>				1
Chlorophyta	Desmids (including filamentous forms)	<i>Actinotaenium</i>	<i>cucurbita</i>		1	
		<i>Bambusina</i>	<i>brebissonii</i>			5
		<i>Closterium</i>	cf. <i>ehrebergii</i>		2	
		<i>Closterium</i>	cf. <i>acerosum</i>	<i>elongatum</i>	4	
		<i>Closterium</i>	cf. <i>archerianum</i>		2	
		<i>Closterium</i>	cf. <i>gracile</i>		5	
		<i>Closterium</i>	cf. <i>intermedium</i>		1	1
		<i>Closterium</i>	cf. <i>lunula forma</i>		6	
		<i>Closterium</i>	cf. <i>moniliferum</i>		2	

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TAXONOMIC GROUP	DESCRIPTION	GENUS	SPECIES	VARIETY/ NOTES	NUMBER OF RECORDS	
					NORTH IS.	SOUTH IS
		<i>Closterium</i>	<i>cf. strigosum</i>		3	
		<i>Closterium</i>	<i>closteriodes</i>		2	1
		<i>Closterium</i>	<i>cynthia</i>		3	1
		<i>Closterium</i>	<i>dianae</i>	<i>pseudodiana</i>	10	2
		<i>Closterium</i>	<i>kuetzingii</i>		3	
		<i>Closterium</i>	<i>lineatum</i>		1	
		<i>Closterium</i>	<i>parvulum</i>			1
		<i>Closterium</i>	<i>ralfsii</i>		5	
		<i>Closterium</i>	<i>venus</i>	<i>venus</i>	1	1
		<i>Cosmarium</i>	sp.		3	
		<i>Cosmarium</i>	<i>cf. calcareum</i>		1	
		<i>Cosmarium</i>	<i>cf. askenasyi</i>		1	
		<i>Cosmarium</i>	<i>cf. biculatum</i>		2	
		<i>Cosmarium</i>	<i>cf. cymatium</i>		1	
		<i>Cosmarium</i>	<i>cf. impressulum</i>		1	
		<i>Cosmarium</i>	<i>cf. magnificum</i>		1	
		<i>Cosmarium</i>	<i>cf. nitidulum</i>		8	
		<i>Cosmarium</i>	<i>cf. obtusatum</i>		1	
		<i>Cosmarium</i>	<i>cf. punctulatum</i>		1	
		<i>Cosmarium</i>	<i>cf. pyramidatum</i>		1	
		<i>Cosmarium</i>	<i>cf. quadratum</i>		3	
		<i>Cosmarium</i>	<i>cf. subgranatum</i>		6	
		<i>Cosmarium</i>	<i>margaritatum</i>		2	
		<i>Cosmarium</i>	<i>norimbergense</i>			8
		<i>Cosmarium</i>	<i>pseudopyramidatum</i>			1
		<i>Cosmarium</i>	<i>quadrifarium</i>	<i>hexatichum</i>	7	3
		<i>Cosmarium</i>	<i>quadriverrucosium</i>		2	4
		<i>Cosmarium</i>	sp.		3	1
		<i>Cylindrocystis</i>	<i>crassa</i>	<i>crassa</i>		3
		<i>Desmidium</i>	<i>baileyi</i>	<i>coelatum</i>	4	
		<i>Desmidium</i>	<i>baileyi</i>	<i>undulatum</i>	4	
		<i>Desmidium</i>	<i>occidentale</i>		2	1
		<i>Desmidium</i>	<i>swartzii</i>		5	
		<i>Desmidium</i>	<i>coarctatum</i>		1	6
		<i>Euastrum</i>	<i>cf. denticulatum</i>		1	
		<i>Euastrum</i>	<i>cuneatum</i>		2	1
		<i>Euastrum</i>	<i>didelta</i>	<i>bengalicum</i>		2
		<i>Euastrum</i>	<i>euteles</i>			3
		<i>Euastrum</i>	<i>insulare</i>	<i>insulare</i>		7
		<i>Euastrum</i>	<i>irregulare</i>		1	4
		<i>Euastrum</i>	<i>sinuosum</i>	<i>gemmulosum</i>	9	7
		<i>Euastrum</i>	sp. B		1	
		<i>Euastrum</i>	<i>sphyroides</i>			1
		<i>Euastrum</i>	<i>subobatum</i>	<i>subobatum</i>		1
		<i>Gonatozygon</i>	<i>aculeatum</i>		4	
		<i>Gonatozygon</i>	<i>brebissonii</i>		2	
		<i>Hyalotheca</i>	<i>dissiliens</i>	<i>tatrica</i>	14	
		<i>Micrasterias</i>	<i>thomasiana</i>	<i>notata</i>	2	2

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TAXONOMIC GROUP	DESCRIPTION	GENUS	SPECIES	VARIETY/ NOTES	NUMBER OF RECORDS	
					NORTH IS.	SOUTH IS
		Netrium	cf. oblongum			3
		Netrium	digitus	<i>lamellosum</i>	1	
		Netrium	digitus	<i>naegelii</i>	7	
		Netrium	digitus	<i>digitus</i>		3
		Netrium	digitus	<i>latum</i>		5
		<i>Netrium</i>	<i>digitus</i>	<i>parvum</i>		1
		<i>Pleurotaenium</i>	cf. <i>tridentulum</i>	<i>tridentulum</i>		4
		<i>Pleurotaenium</i>	<i>ehrenbergii</i>		9	
		<i>Pleurotaenium</i>	<i>minutum</i>	<i>minutum?</i>		19
		<i>Pleurotaenium</i>	<i>nodosum</i>		1	
		<i>Pleurotaenium</i>	<i>rectum</i>			1
		<i>Pleurotaenium</i>	<i>trabecula</i>	cf. <i>elongatum</i>	6	
		<i>Spirotaenium</i>	sp.		1	
		<i>Spondylosium</i>	cf. <i>pulchrum</i>			1
		<i>Staurastrum</i>	<i>muticum</i>		8	
		<i>Staurodesmus</i>	<i>glaber</i>	<i>limnophilus</i>		1
		<i>Staurodesmus</i>	<i>inflexum</i>			6
		<i>Staurodesmus</i>	<i>mamillatus</i>	<i>mamillatus</i>		3
		<i>Staurodesmus</i>	<i>megacanthus</i>	<i>megacanthus</i>		2
		<i>Staurodesmus</i>	<i>osvaldii</i>			2
		<i>Staurodesmus</i>	<i>phimus</i>	<i>phimus</i>		1
		<i>Staurodesmus</i>	<i>skujae</i>			2
		<i>Staurodesmus</i>	sp.			1
		<i>Tetmemorus</i>	<i>granulatus</i>		1	9
		<i>Tetmemorus</i>	<i>laevis</i>	<i>laevis</i>		13
		<i>Xanthidium</i>	<i>armatum</i>	<i>armatum</i>		2
		<i>Xanthidium</i>	<i>bifidum</i>	<i>latodivergens</i>		1
		<i>Xanthidium</i>	cf. <i>hastiferum</i>		8	
		<i>Xanthidium</i>	<i>intermedium</i>			1
		<i>Xanthidium</i>	<i>simplicins</i>			3
		<i>Xanthidium</i>	<i>variabile</i>	<i>variabile</i>		6
Chlorophyta	Single-celled/colonial (non-desmid)	<i>Ankistrodesmus</i>			2	1
		<i>Chaetosphaeridium</i>	<i>glovesum</i>			4
		<i>Chlorella</i>	sp.			5
		<i>Coelastrum</i>	<i>cambricum</i>		8	
		<i>Coelastrum</i>	cf. <i>microporum</i>		6	
		<i>Coelastrum</i>	very large, six-sided		1	
		<i>Colonial green</i>			6	
		<i>Dictyosphaeridium</i>	<i>ehrenbergianum</i>		1	4
		<i>Elakatothrix</i>	<i>gelatinosa</i>			4
		<i>Gloeocystis</i>	sp.		29	14
		<i>Gloeocystis</i>	<i>vesiculosa</i>			6
		<i>Kirchneriella</i>	sp.		1	1
		cf. <i>Nephrocytium</i>			2	
		<i>Oocystis</i>	cf. <i>lacustris</i>		2	
		<i>Palmella</i>	cf. <i>mucosa</i>		3	6
		<i>Pediastrum</i>	cf. <i>boryanum</i>		10	
		cf. <i>Pseudulvella</i>			1	
		<i>Scenedesmus</i>	sp.		23	

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Appendix 2 continued

TAXONOMIC GROUP	DESCRIPTION	GENUS	SPECIES	VARIETY/ NOTES	NUMBER OF RECORDS	
					NORTH IS.	SOUTH IS
Chlorophyta	Green filamentous algae (non-desmid)	<i>Bulbochaete</i>	<i>cf. praereticulata</i>		1	
		<i>Bulbochaete</i>	mt 1		23	8
		<i>Cylindrocapsa</i>	<i>cf. conferta</i>		3	13
		<i>Cylindrocapsa</i>	<i>cf. geminella</i>		1	2
		<i>Cylindrocapsa</i>	sp.			4
		<i>Cylindrocapsopsis</i>	<i>indica</i>		3	
		<i>Klebsormidium</i>	sp.		2	1
		<i>Microspora</i>	sp.		12	4
		<i>Mougeotia</i>	sp.		38	12
		<i>Oedogonium</i>	sp. mt 1	narrow forms	60	8
		<i>Oedogonium</i>	sp. aff. <i>wissmanii</i>		30	
		<i>Oedogonium</i>	sp. mt 2	wide forms	38	6
		<i>Rhizoclonium</i>	sp.		2	
		<i>Spirogyra</i>	sp. mt 1	wide forms	24	
		<i>Spirogyra</i>	sp. mt 2	narrow forms	24	10
		<i>Spirogyra</i>	sp. mt 3	pigmented	6	
		<i>Stigeoclonium</i>	sp.		9	
				<i>Ulothrix</i>	mt 2	
		<i>Zygnema</i>	mt 2			2
Rhodophyta	Red algae	<i>Batrachospermum</i>	sp.		1	
		<i>Compsopogon</i>	sp.		1	
Other	Non-algae periphyton	Iron bacteria			2	
		Bryophyte protonema				3

Appendix 3

List of diatom taxa identified from 200 samples from 40 New Zealand wetlands

Includes the number of records in the North and South Islands. Note that many identifications to species level are tentative (indicated by 'cf.').

TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
achbia	<i>Achnanthydium</i>	<i>biasolettiana</i>	cf.		1
achbio	<i>Achnanthydium</i>	<i>biorettii</i>			2
achcle	<i>Achnanthes</i>	<i>clevei</i>			2
achdao	<i>Achnanthydium</i>	<i>daonensis</i>			1
achexi	<i>Achnanthes</i>	<i>exigua</i>		6	
achhun	<i>Achnanthes</i>	<i>hungarica</i>		15	
achinf	<i>Achnanthes</i>	<i>inflata</i>		2	
achlat	<i>Achnanthes</i>	<i>laterostrata</i>			1
achmar	<i>Achnanthydium</i>	<i>marginulata</i>	cf.		1
achobl	<i>Achnanthes</i>	<i>oblongella</i>		18	3
achros	<i>Achnanthydium</i>	<i>rossii</i>			1
achspb	<i>Achnanthydium</i>	sp. B			1
achsub	<i>Achnanthes</i>	<i>subatomoides</i>		2	2
achswz	<i>Achnanthydium</i>	<i>pseudoswazi</i>			1
acmmin	<i>Achnanthydium</i>	<i>minutissimum</i>		45	8
acmnod	<i>Achnanthydium</i>	<i>nodosa</i>			1
actaot	<i>Actinella</i>	<i>aotearoia</i>			5
actspb	<i>Actinella</i>	sp. B			2
adlafsp	<i>Adlafia</i>	sp.			5
ampina	<i>Amphora</i>	<i>inariensis</i>	cf.	1	
ampova	<i>Amphora</i>	<i>ovalis</i>		4	5
ampven	<i>Amphora</i>	<i>veneta</i>		2	
anosph	<i>Anomoeoneis</i>	<i>sphaerophora</i>		3	
astfor	<i>Asterionella</i>	<i>formosa</i>		4	
aulac	<i>Aulacoseira</i>	spp.	[all species]	57	10
brabre	<i>Brachysira</i>	<i>brebissonii</i>		3	12
braexi	<i>Brachysira</i>	<i>exilis</i>	cf.	2	1
bramel	<i>Brachysira</i>	<i>metzeltinii</i>		7	41
bramic	<i>Brachysira</i>	<i>microcephala</i>	cf.	16	5
braspb	<i>Brachysira</i>	sp. B			5
braspc	<i>Brachysira</i>	sp. C		47	11
brawyg	<i>Brachysira</i>	<i>wygaschii</i>		8	23
calbud	<i>Caloneis</i>	<i>budensis</i>	cf.	1	
calpat	<i>Caloneis</i>	<i>patagonica</i>			1
calsil	<i>Caloneis</i>	sp.		17	4
calsma	<i>Caloneis</i>	small fine			1
cavsp	<i>Cavinula</i>				1
cent	<i>Centric</i>	[unknown genus]			2
chasoe	<i>Chamaepinnularia</i>	<i>soehrensii</i>			6
chasp	<i>Chamaepinnularia</i>	sp. A		1	17
cocpla	<i>Cocconeis</i>	<i>placentula</i>		23	7

Continued on next page

Appendix 3 continued

TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
cracus	<i>Craticula</i>	<i>cuspidata</i>		7	
cyaple	<i>Cyamatopleura</i>	<i>solea</i>			1
cyclnz	<i>Cyclostephanos</i>	<i>novae zelandiae</i>		8	
cycmen	<i>Cyclotella</i>	<i>meneghiniana</i>		5	
cycste	<i>Cyclotella</i>	<i>stelligera</i>		21	
cymasp	<i>Cymbella</i>	<i>aspera</i>		12	
cymben	<i>Cymbella</i>	<i>bengalensis</i>	cf.	2	
cymcis	<i>Cymbella</i>	<i>cistula</i>		3	
cymcus	<i>Cymbella</i>	<i>cuspidata</i>		4	1
cymcym	<i>Cymbella</i>	<i>cymbiformis</i>			1
cymehr	<i>Cymbella</i>	<i>ehrenbergii</i>		1	
cymkap	<i>Cymbella</i>	<i>kappii</i>		19	2
cymnav	<i>Cymbella</i>	<i>naviculiformis</i>		13	5
cymtum	<i>Cymbella</i>	<i>tumida</i>			1
dahie	<i>Diatoma</i>	<i>hiemale</i>			3
diaten	<i>Diatoma</i>	<i>tenuis</i>			2
didsp	<i>Diadesmis</i>	sp.		7	2
dilspa	<i>Diatomella</i>	sp. A			1
dipova	<i>Diploneis</i>	<i>ovalis</i>		14	3
encelg	<i>Encyonema</i>	<i>elginensis</i>			3
encmes	<i>Encyonema</i>	<i>mesianum</i>	cf.	3	4
encmin	<i>Encyonema</i>	<i>minutum</i>		33	9
encneo	<i>Encyonema</i>	<i>neogracile</i>		59	18
encsil	<i>Encyonema</i>	<i>silesiaca</i>			1
enctur	<i>Encyonema</i>	<i>turgida</i>			1
enpaeq	<i>Encyonopsis</i>	<i>aequalis</i>		5	
enpdif	<i>Encyonopsis</i>	<i>difficilis</i>	cf.		6
enphau	<i>Encyonopsis</i>	<i>hauckii</i>	cf.	12	5
epiadh	<i>Epithemia</i>	<i>adnata</i>		3	
episor	<i>Epithemia</i>	<i>sorex</i>			1
epitur	<i>Epithemia</i>	<i>turgida</i>		16	3
ettz	<i>Eunotia</i>	sp. Z		15	
euninc	<i>Eunotia</i>	<i>incisa</i>			1
eunint	<i>Eunotia</i>	<i>intermedia</i>		2	
eunpsp	<i>Eunophora</i>	<i>oberonica</i>	cf.	3	
eutbib	<i>Eunotia</i>	<i>bilunaris</i>	<i>bilunaris</i>		24
eutbil	<i>Eunotia</i>	<i>bilunaris</i>	<i>linearis</i>	18	13
eutbim	<i>Eunotia</i>	<i>bilunaris</i>	<i>mucophila</i>	4	
eutcam	<i>Eunotia</i>	<i>camelus</i>		34	22
eutexi	<i>Eunotia</i>	<i>exigua</i>		20	2
eutfor	<i>Eunotia</i>	<i>formica</i>			3
eutimp	<i>Eunotia</i>	<i>implicata</i>		61	20
eutinc	<i>Eunotia</i>	<i>incisa</i>	cf.	22	16
eutint	<i>Eunotia</i>	<i>intermedia</i>		6	
eutlin	<i>Eunotia</i>	<i>lineolata</i>		27	3
eutmei	<i>Eunotia</i>	<i>meisteri</i>			10
eutmob	<i>Eunotia</i>	<i>monodon</i>	<i>bidens</i>		4
eutmon	<i>Eunotia</i>	<i>monodon</i>		6	
eutnae	<i>Eunotia</i>	<i>naegellii</i>		9	13

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TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
eutpal	<i>Eunotia</i>	<i>paludosa</i>			18
eutpec	<i>Eunotia</i>	<i>pectinalis</i>	cf.	6	
eutpol	<i>Eunotia</i>	<i>polydentula</i>		3	
eutpra	<i>Eunotia</i>	<i>praerupta</i>	cf.		9
eutrho	<i>Eunotia</i>	<i>rhomboides</i>		1	2
eutser	<i>Eunotia</i>	<i>serpentina</i>		22	
eutsp	<i>Eunotia</i>	sp. L		1	
eutspb	<i>Eunotia</i>	sp. B			5
eutspc	<i>Eunotia</i>	sp. C			1
eutspS	<i>Eunotia</i>	sp. S			1
eutsub	<i>Eunotia</i>	<i>subaequalis</i>	cf.	3	
eutsud	<i>Eunotia</i>	<i>sudetica</i>	cf.	1	
eutunk	<i>Eunotia</i>	sp. U			1
eutven	<i>Eunotia</i>	<i>veneris</i>	cf.	4	
frabre	<i>Fragilaria</i>	<i>brevistriata</i>		2	6
fracap	<i>Fragilaria</i>	<i>capucina</i>		10	11
fracas	<i>Fragilaria</i>	<i>cassieae</i>		2	
fracro	<i>Fragilaria</i>	<i>crotonensis</i>		11	2
fraell	<i>Fragilaria</i>	'elliptica'		24	4
fracfam	<i>Fragilaria</i>	<i>famelica</i>	cf.	2	1
fracfas	<i>Fragilaria</i>	<i>fasciculata</i>			1
franan	<i>Fragilaria</i>	<i>nanana</i>		10	
frapar	<i>Fragilaria</i>	<i>parasitica</i>		5	5
fraten	<i>Fragilaria</i>	<i>tenera</i>			1
fravau	<i>Fragilaria</i>	<i>vaucheriae</i>		27	7
frfexi	<i>Fragilariforma</i>	<i>exigua</i>		14	5
frfvir	<i>Fragilariforma</i>	<i>virescens</i>		1	
fruaot	<i>Frustulia</i>	<i>aotearoa</i>			4
frucas	<i>Frustulia</i>	<i>cassieae</i>	cf.	24	12
frucras	<i>Frustulia</i>	<i>crassinervia</i>		9	50
frugon	<i>Frustulia</i>	<i>gondwana</i>			10
frukra	<i>Frustulia</i>	<i>krammeri</i>	cf.	9	
frumag	<i>Frustulia</i>	<i>magaliesmontana</i>	cf.	15	52
frumao	<i>Frustulia</i>	<i>maoriana</i>	cf.	4	11
frupan	<i>Frustulia</i>	<i>pangaeopsis</i>			3
frusax	<i>Frustulia</i>	<i>saxonica</i>		16	34
fruspa	<i>Frustulia</i>	sp. A			32
fruvul	<i>Frustulia</i>	<i>vulgaris</i>		2	2
geipal	<i>Geissleria</i>	<i>paludosa</i>	<i>palustris</i>	2	
gomacu	<i>Gomphonema</i>	<i>acuminatum</i>		12	3
gomaff	<i>Gomphonema</i>	<i>affine</i>	cf.	7	
gomang	<i>Gomphonema</i>	<i>angustatum</i>		34	7
gomcla	<i>Gomphonema</i>	<i>clavatum</i>	cf.	11	4
gomgra	<i>Gomphonema</i>	<i>gracile</i>		31	4
gomlag	<i>Gomphonema</i>	<i>lagenula</i>			3
gomlan	<i>Gomphonema</i>	<i>lanceolatum</i>	cf.	4	
gomlat	<i>Gomphonema</i>	'laterostriata'			1
gommin	<i>Gomphonema</i>	<i>minutum</i>			4
gomnav	<i>Gomphonema</i>	<i>naviculiformis</i>		1	

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Appendix 3 continued

TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
gompar	<i>Gomphonema</i>	<i>parvulum</i>		30	2
gomspa	<i>Gomphonema</i>	sp. A			1
gomsph	<i>Gomphonema</i>	<i>sphaerophorum</i>			1
gomspla	<i>Gomphonema</i>	cf. <i>mexicanum</i>		4	
gomtru	<i>Gomphonema</i>	<i>truncatum</i>	var.	8	2
gyracu	<i>Gyrosigma</i>	<i>acuminatum</i>		3	
hanamp	<i>Hantzschia</i>	<i>amphioxys</i>		1	
hanelo	<i>Hantzschia</i>	<i>elongata</i>			3
kobaya	<i>Kobayasiella</i>	sp. A		12	48
kobplo	<i>Kolbesia</i>	<i>plonensis</i>		7	
lutmut	<i>Luticola</i>	<i>mutica</i>		5	2
massmi	<i>Mastogloia</i>	<i>elliptica</i>		5	
masspa	<i>Mastogloia</i>	sp.		3	
melvar	<i>Melosira</i>	<i>varians</i>		1	1
mercir	<i>Meridion</i>	<i>circulare</i>			1
navcap	<i>Navicula</i>	<i>capitatoradiata</i>		8	1
navcin	<i>Navicula</i>	<i>cinctaeformis</i>		4	
navcra	<i>Navicula</i>	<i>cryptonella</i>	cf.	3	
navcry	<i>Navicula</i>	<i>cryptocephala</i>		27	4
navdig	<i>Navicula</i>	<i>digitatoradiata</i>	cf.	2	
navfes	<i>Navicula</i>	<i>festiva</i>		8	5
navgre	<i>Navicula</i>	<i>gregaria</i>		1	
navhel	<i>Navicula</i>	<i>helvetica</i>	<i>wolterecki</i>	5	4
navhuc	<i>Navicula</i>	<i>hungarica</i>	<i>capitata</i>		3
navlan	<i>Navicula</i>	<i>lanceolata</i>		11	1
navlat	<i>Navicula</i>	<i>laterostriata</i>	cf.		1
navlep	<i>Navicula</i>	<i>leptostriata</i>		10	
navmin	<i>Navicula</i>	<i>minima</i>		2	
navmis	<i>Navicula</i>	<i>minsculoides</i>	cf.	1	
navper	<i>Navicula</i>	<i>peregrina</i>		3	
navqua	<i>Navicula</i>	<i>quadripartita</i>	cf.	2	
navrad	<i>Navicula</i>	<i>radiosa</i>		23	5
navrap	<i>Navicula</i>	<i>radiosa</i>	<i>parva</i>	9	2
navrec	<i>Navicula</i>	<i>recens</i>	cf.	1	
navrhy	<i>Navicula</i>	<i>rynchocephala</i>		18	7
navsp	<i>Navicula</i>	sp.			1
navsubr	<i>Navicula</i>	<i>subrynchocephala</i>			2
navtel	<i>Navicula</i>	<i>tellenoides</i>	cf.	1	
navtri	<i>Navicula</i>	<i>tridentula</i>			1
navtrv	<i>Navicula</i>	<i>trivialis</i>			1
navvar	<i>Navicula</i>	<i>variostrata</i>			1
navven	<i>Navicula</i>	<i>veneta</i>	cf.	12	4
navvir	<i>Navicula</i>	<i>viridula</i>	<i>rostellata</i>	1	
neiaff	<i>Neidium</i>	<i>affine</i>	<i>amphyrhynchus</i>	2	
neiamp	<i>Neidium</i>	<i>ampliatum</i>			8
neiiri	<i>Neidium</i>	<i>iridis</i>		10	1
nitaci	<i>Nitzschia</i>	<i>acicularis</i>		5	1
nitacpe	<i>Nitzschia</i>	<i>acidoclinata/</i> <i>perminuta</i>	[species complex]	49	14

Continued on next page

TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
nitamp	<i>Nitzschia</i>	<i>amphibia</i>		10	
nitaod	<i>Nitzschia</i>	<i>acicularioides</i>	[fine]		1
nitbac	<i>Nitzschia</i>	<i>bacillum</i>	cf.		1
nitdeb	<i>Nitzschia</i>	<i>debilis</i>		1	
nitdim	<i>Nitzschia</i>	<i>dissipata</i>	<i>media</i>		1
nitdis	<i>Nitzschia</i>	<i>dissipata</i>		7	4
nitfat	<i>Nitzschia</i>	<i>fine fat</i>			4
nitfil	<i>Nitzschia</i>	<i>filiformis</i>		1	2
nitgra	<i>Nitzschia</i>	<i>gracilis</i>		10	10
nitinc	<i>Nitzschia</i>	<i>incognita</i>		10	2
nitint	<i>Nitzschia</i>	<i>intermedia</i>		2	1
nitlin	<i>Nitzschia</i>	<i>linearis</i>		6	
nitlis	<i>Nitzschia</i>	<i>linearis</i>	<i>subtilis</i>	21	
nitnan	<i>Nitzschia</i>	<i>nana</i>		18	2
nitpal	<i>Nitzschia</i>	<i>palea</i>		37	
nitrec	<i>Nitzschia</i>	<i>recta</i>	[long]	5	
nitrev	<i>Nitzschia</i>	<i>reversa</i>	cf.	4	
nitsub	<i>Nitzschia</i>	<i>subacicularis</i>	cf.	3	
nitter	<i>Nitzschia</i>	<i>terrestris</i>		1	
achimp	<i>Nupela</i>	sp. A			5
nupela	<i>Nupela</i>	sp. B			1
pinang	<i>Pinnularia</i>	<i>angustistriata</i>		4	6
pinbar	<i>Pinnularia</i>	<i>barberiana</i>	cf.	6	
pinbic	<i>Pinnularia</i>	<i>biceps</i>	cf.	4	7
pindec	<i>Pinnularia</i>	<i>decrescens</i>	<i>rhombarea</i>		1
pindis	<i>Pinnularia</i>	<i>divergentissima</i>	<i>triundulata</i>	2	3
pindiv	<i>Pinnularia</i>	<i>divergens</i>	<i>rhombundulata</i>	8	8
pingib	<i>Pinnularia</i>	<i>gibba</i>	cf.	16	3
pingra	<i>Pinnularia</i>	<i>graciloides</i>		12	
pininf	<i>Pinnularia</i>	<i>interruptiformis</i>		13	7
pinlat	<i>Pinnularia</i>	<i>lattevitata</i>			1
pingib	<i>Pinnularia</i>	[large sp. cf. <i>gibba</i>]		3	
pinmaj	<i>Pinnularia</i>	<i>major</i>			2
pinmic	<i>Pinnularia</i>	<i>microstauron</i>		1	2
pinnem	<i>Pinnularia</i>	<i>neomajor</i>	cf.	4	4
pinpir	<i>Pinnularia</i>	<i>perirrorata</i>		10	3
pinrnz	<i>Pinnularia</i>	<i>rhomboelliptica</i>	<i>novaezealandiae</i>		1
pinseg	<i>Pinnularia</i>	<i>segariana</i>	cf.	1	
pinslb	<i>Pinnularia</i>	cf. <i>lange-bertalotti</i>			1
pinsmsp	<i>Pinnularia</i>	sp.	small, unknown		2
pinsp	<i>Pinnularia</i>	sp.	unknown species	4	
pinspA	<i>Pinnularia</i>	sp. A		1	2
pinspd	<i>Pinnularia</i>	sp. D			7
pinpsm	<i>Pinnularia</i>	sp. small		2	
pinsto	<i>Pinnularia</i>	<i>graciloides</i>		4	
pinsub	<i>Pinnularia</i>	<i>subcapitata</i>		18	8
pinsug	<i>Pinnularia</i>	<i>subgibba</i>			3
pinsur	<i>Pinnularia</i>	<i>subcapitata</i>	<i>rostrata</i>		2
pintra	<i>Pinnularia</i>	<i>transversa</i>			4

Continued on next page

Appendix 3 continued

TAXON CODE	GENUS	SPECIES	VARIETY/NOTES	NUMBER OF RECORDS	
				NORTH IS.	SOUTH IS.
pintro	<i>Pinnularia</i>	<i>tropica</i>	cf.	1	
pinvir	<i>Pinnularia</i>	<i>viridis</i>		27	4
plaelg	<i>Placoneis</i>	<i>elginensis</i>		4	3
plapla	<i>Placoneis</i>	<i>placentula</i>		3	
plalan	<i>Planothidium</i>	<i>lanceolatum</i>		10	3
plaper	<i>Planothidium</i>	<i>peragalli</i>			3
reisin	<i>Reimeria</i>	<i>sinuata</i>			1
rhcabb	<i>Rhoicosphenia</i>	<i>abbreviata</i>		1	
rhomus	<i>Rhopalodia</i>	<i>musculus</i>		7	1
rhopnz	<i>Rhopalodia</i>	<i>novae zelandiae</i>		20	
rospet	<i>Rossithidium</i>	<i>petersenii</i>		26	10
rospus	<i>Rossithidium</i>	<i>pusillum</i>		12	5
selame	<i>Sellaphora</i>	<i>americana</i>		16	
selbac	<i>Sellaphora</i>	<i>bacillum</i>			2
sellae	<i>Sellaphora</i>	<i>laevissima</i>			1
selpup	<i>Sellaphora</i>	<i>pupula</i>		43	12
selspa	<i>Sellaphora</i>	sp. A		1	
selwit	<i>Sellaphora</i>	<i>wittrockii</i>			3
staanc	<i>Stauroneis</i>	<i>anceps</i>		7	2
stacon	<i>Staurosira</i>	<i>construens</i>		1	
stajav	<i>Stauroneis</i>	<i>javanica</i>		1	
stakre	<i>Stauroneis</i>	<i>kreigerii</i>		2	1
stcruc	<i>Staurosirella</i>	spp.		8	8
stapac	<i>Stauroneis</i>	<i>pachycephala</i>		7	
stapho	<i>Stauroneis</i>	<i>phoenicenteron</i>		17	6
staski	<i>Stauroneis</i>	<i>frauenfeldiana</i>			1
stecur	<i>Stenopterobia</i>	<i>curvula</i>		14	8
stedel	<i>Stenopterobia</i>	<i>delicatissima</i>		1	7
stespa	<i>Stenopterobia</i>	sp. A		18	2
surang	<i>Surirella</i>	<i>angusta</i>			1
surlin	<i>Surirella</i>	<i>linearis</i>		4	
surspa	<i>Surirella</i>	sp. A			10
surspc	<i>Surirella</i>	sp. C			3
surspd	<i>Surirella</i>	huge twisted			1
surten	<i>Surirella</i>	<i>tenera</i>	cf.	3	
synacu	<i>Synedra</i>	<i>acus</i>		6	3
syndel	<i>Synedra</i>	<i>delicatissima</i>		19	1
synrum	<i>Synedra</i>	<i>rumpens</i>		7	
synubi	<i>Synedra</i>	<i>ulna</i>	<i>biceps</i>	15	
synuln	<i>Synedra</i>	<i>ulna</i>		24	5
tabflo	<i>Tabellaria</i>	<i>flocculosa</i>		33	22
tauvar	<i>Tabularia</i>	<i>variostrata</i>		9	1