### 2.4.3   Sample processing

Stark et al. (2001) have provided good information on how to collect and process invertebrate samples collected from rivers, and similar methods can be used to process wetland invertebrate samples. However, there are important differences between samples collected from rivers and wetlands. Firstly, wetland samples often contain much higher amounts of organic matter than river samples, making it time-consuming to sift through this organic material to find invertebrates. Secondly, many small, non-insect taxa tend to dominate wetland invertebrate communities (as opposed to the insect-dominated communities found in rivers), and such animals may be under-reported if samples are processed without a microscope. To minimise these problems, we developed a specific protocol to treat wetland samples prior to processing them.

The entire sample was sieved through a coarse (> 4.0 mm) sieve, and all material that passed through this was collected onto a set of nested sieves, with a 1.0-mm sieve on top of a 500-μm, 250-μm and 63-μm sieves. All material retained on the coarse sieve was placed on an inclined, boat-shaped tray, over which water ran (Fig. 2). Macrophyte stems, branches and other large organic matter were spread evenly across the tray and shaken gently in the water current to remove any small animals or other material associated with this large material. All fine material leaving the tray was then passed through the series of nested sieves. In this way, the sample was split into two sizes: a coarse size fraction > 1.0 mm and a finer size fraction < 1.0 mm (but greater than 63 μm). Both size fractions were then processed in their entirety, or sub-sampled so that either ½, ¼, ⅛ or 1/16 of the sample was processed, depending on the amount of material present. The material from each sieve (or subsample) was spread evenly across a small Bogorov tray (Winterbourn & Gregson 1989; Winterbourn et al. 2006) and examined under a dissecting microscope (up to 40× magnification) for invertebrates. A minimum of 400 invertebrates in each sample were identified, and the rest of the sample or subsample was scanned for uncommon taxa (Duggan et al. 2003). This process was repeated for both sieve sizes. All invertebrates were identified to as low a taxonomic resolution as possible, according to the availability of taxonomic keys and the practicality of identifying small taxa such as nematodes, tardigrades and microcrustacea (Suren et al. 2007).

Figure 2.   A boat-shaped tray that is used to wash any attached invertebrates from macrophytes and/or twigs that are retained on a 4.0-mm sieve. The tray is inclined and water is run over it. The material is then carefully shaken to dislodge attached invertebrates, which are washed into a collecting sieve in the sink (hidden).
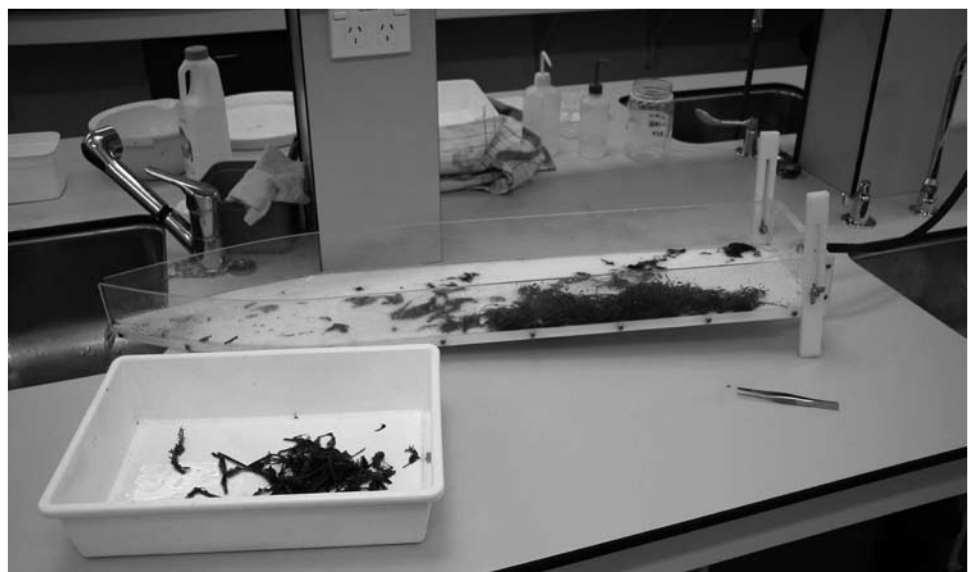
The large quantity of material that comprised the coarse and fine size fractions meant that up to 2 hours were needed to process each fraction, to be sure that the minimum 400 count was adhered to. This meant that it could take up to 4 hours to process a single invertebrate sample from a wetland that contained a large amount of organic matter and mud. These time estimates are close to values obtained by King & Richardson (2002), who found that it took c. 2.6 hours to process samples to a fixed 200 count, and 3.4 hours to process to a fixed 300 count. The long processing time has large implications for the design of any sampling programmes. Development of new techniques to speed up sample processing would consequently have obvious beneficial outcomes that would encourage monitoring wetlands using invertebrates. One such improvement would be to pass the sample through the nested sieves, but only process material trapped on the coarse (> 1 mm) sieve. If it could be demonstrated that there is no loss of information using this method, then considerable time savings could be made.

2.5   EXPERIMENTAL DESIGN

In view of the lack of information about aquatic invertebrate communities in New Zealand wetlands, this study set out to address the following questions:

1. Which invertebrate taxa are found in wetlands? (Addressed by the combination of all studies)

2. To what extent do invertebrate communities differ within and between wetlands? (Spatial study)

3. To what extent do invertebrate communities vary over time, e.g. both seasonally and annually? (Temporal study)

4. How are invertebrate communities affected by environmental variables between different wetlands at a national scale? (National survey)

The work was carried out progressively, so that the results of one study could feed into the sampling design for the following study.

The spatial sampling programme (section 3) was conducted to determine which habitats within a wetland should be sampled to obtain the best representation of the community. This study was carried out in four relatively pristine wetlands that had easy access, as sampling within each wetland was undertaken over a few days. The study investigated whether invertebrate communities varied more between wetlands than within wetlands. The findings from this had implications for future sampling protocols. If, for example, it was found that invertebrate communities varied greatly between different plant species within a pond, or varied between small ponds, large ponds and channels, then any sampling protocol would need to take this into consideration, e.g. by sampling only areas containing submerged vegetation (if these habitats were found to support more taxa than areas without), or by not sampling leads (if these contained only a few of the taxa found in other open-water habitats within a wetland). The rationale behind the spatial sampling programme was to develop a method that most effectively characterised the invertebrate communities in each wetland while collecting as few samples as possible.

The temporal sampling programme (section 4) was carried out after completion of the spatial sampling programme. This investigated temporal changes to invertebrate communities, which also had implications for future survey work. If invertebrate communities vary temporally, then differences between dissimilar wetlands will be masked if samples are collected at different times. Such a scenario could complicate identification of factors regulating invertebrate communities in wetlands. If, however, invertebrate communities vary little over time, or if seasonal variation of individual taxa is similar between wetlands, then between-wetland similarity will remain relatively constant, irrespective of time. Under the latter scenario, surveys of multiple wetlands could be made over a longer time frame, as underlying differences between wetlands would transcend temporal fluctuations.

Finally, we surveyed wetlands throughout New Zealand (section 5) to determine how aquatic invertebrate communities varied in response to catchment, climate, geology, land cover and water quality. We also examined whether there were any regional differences between invertebrate communities. The design strategy for this survey drew on the findings from the spatial and temporal studies, and was focussed only on wetlands with minimal human impacts.

3. Spatial variability of wetland invertebrates—where should we sample?

Wetlands display a large diversity of aquatic habitats, including flowing and standing water, and vegetated and non-vegetated areas. Wetland vegetation can have a large impact on invertebrate communities, as many invertebrates are found on macrophytes, where they seek shelter from predators, and where they can obtain food in the form of algae, detritus and decaying macrophyte tissue. Invertebrate communities may also vary according to plant growth form (submerged, emergent or floating) or morphology (flat, cylindrical or complex). For example, dissected plants have larger surface areas than undissected plants, and thus provide more habitat for epiphytic invertebrates (e.g. Rooke 1986; Cheruvelil et al. 2002). A fundamental consideration for wetland invertebrate ecologists is, therefore, deciding where to sample in order to properly characterise the biodiversity of a particular wetland. Consequently, the first aim of this study was to investigate the spatial variability of wetland invertebrate communities in New Zealand.

3.1 METHODS

3.1.1 Study sites and field methods

Samples were collected from four lowland, coastal wetlands on the west coast of the South Island of New Zealand (Fig. 3). Three wetlands (Mahinapua, Shearer and Kakapotahi) were in Westland, while the Mangarakau Swamp was in the Tasman region. All of these wetlands were sited in areas where human disturbance was minimal. Two sites (Kakapotahi and Shearer) were classified as fens, while the other two sites (Mahinapua and Mangarakau) were classified as swamps. Four of the five open-water habitat types we identified (see section 2.3) were sampled, but each of the four habitats was found in only three of the four wetlands (Table 2).

The dominant terrestrial vegetation at Kakapotahi consisted of a mixture of *Apodasmia similis* rushland and *Gleichenia dicarpa*. The sedge *Baumea teretifolia* and flax *Phormium tenax* also had high cover throughout this wetland. Aquatic plants found in the open-water habitats included *A. similis*, *Glyceria fluitans*, *Myriophyllum robustum* and the tall sedge *Eleocharis sphacelata*, as well as a species of *Sphagnum* (Table 2). Terrestrial vegetation at Shearer was dominated by *G. dicarpa*, *Baumea arthrophylla* and the wire rush *Empodisma minus*. Vegetation in the main channel that meandered through this wetland was dominated by *B. arthrophylla* at its margins, and *E. sphacelata* and the bladderwort *Utricularia australis* in deeper water. Vegetation in the leads and ponds here was dominated by *B. arthrophylla*. Vegetation in the Mahinapua wetland was dominated by dense growths of *P. tenax* and *Carex sinclairii*, with species of *Coprosma* and kahikatea (*Dacrycarpus dacrydioides*) growing in the margins. Aquatic vegetation in the main channels and ponds at Mahinapua
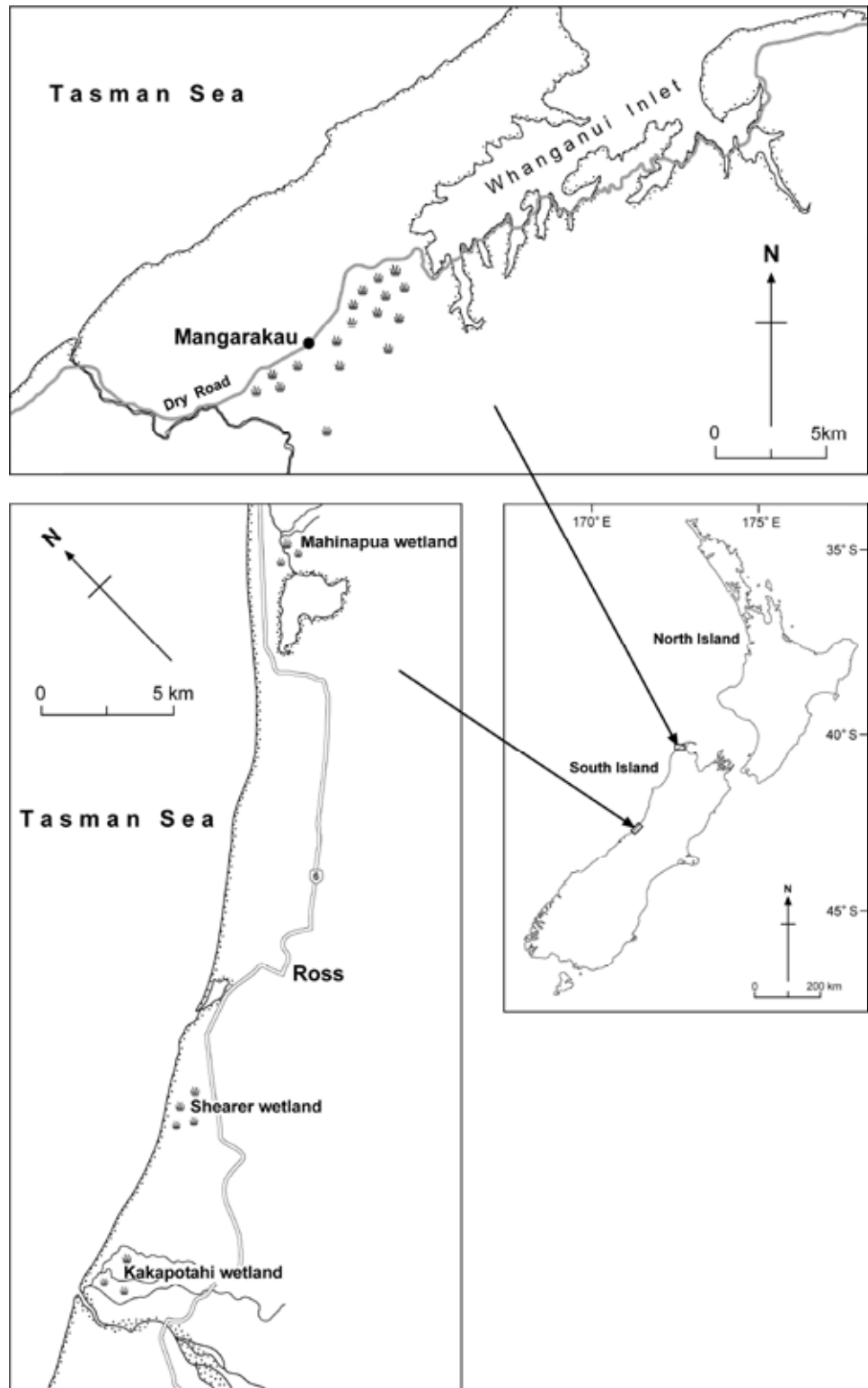
included *Aponogeton distachyus*, *Callitriche stagnalis*, *P. tenax* and *Myriophyllum propinquum*. Wetland vegetation at Mangarakau Swamp was dominated by species-rich sedgelands comprising four *Baumea* spp. and *Lepidosperma australe*, as well as *G. dicarpa*, *P. tenax* and *Typha orientalis*. The aquatic vegetation at this swamp included tussock (*Carex secta*), and marginal bands of *Baumea*, *E. sphacelata* and *T. orientalis*, with submerged species including two milfoils (*M. propinquum* and *M. robustum*) and *Potamogeton cheesemanii* (Table 2).

TABLE 2. SUMMARY OF THE HABITATS AND AQUATIC PLANTS FOUND IN THE FOUR WETLANDS SAMPLED IN THIS STUDY. THE NUMBER OF HABITATS SAMPLED WITHIN EACH WETLAND IS SHOWN, AS ARE THE NUMBER OF SAMPLES COLLECTED FROM EACH WETLAND, HABITAT AND AQUATIC PLANT TAXON (BRACKETS).

WETLAND (*n*)	HABITAT (*n*)	TAXON (*n*)	GROWTH FORM	MORPHOLOGY
Kakapotahi (25)	1 × main channel (20)	*Eleocharis* (4), *Apodasmia* (4)	Emergent	Cylindrical
	1 × lead (3)	*Glyceria* (4)	Floating	Flat
	2 × small ponds (2)	*Myriophyllum* (4), *Sphagnum* (4)	Submerged	Complex
		No vegetation (5)		
Mahinapua (11)	1 × main channel (5)	*Callitriche* (2), *Myriophyllum* (2)	Submerged	Complex
	1 × big pond (4)	*Phormium* (2)	Emergent	Flat
	1 × small pond (2)	*Aponogeton* (2)		
		No vegetation (3)		
Mangarakau (39)	2 × leads (14)	*Baumea* (14), *Eleocharis* (4)	Emergent	Cylindrical
	1 × big pond (21)	*Carex* (3), *Typha* (10)	Emergent	Flat
	2 × small ponds (4)	*Myriophyllum* (1),	Submerged	Complex
		Potamogeton (5)	Submerged	Flat
Shearer (19)	1 × main channel (12)	*Baumea* (5), *Eleocharis* (5)	Emergent	Cylindrical
	1 × lead (3)	*Utricularia* (2)	Submerged	Complex
	1 × big pond (4)	No vegetation (7)		

Because plant growth form (submerged, emergent or floating) or morphology (flat, cylindrical or complex) can influence invertebrate communities, we allocated plants within each wetland to their appropriate growth form or morphological characteristics (Table 2). We thus wanted to investigate how invertebrate communities varied with respect to the specific predictor variables of 'Wetland', 'Habitat', 'Plant taxon', 'Growth form', and 'Morphology'.

All samples were collected during November–December 2003, to minimise potential seasonal differences in invertebrate community composition. Invertebrates were collected from areas without vegetation and from different macrophytes within individual water bodies, and from different water bodies (e.g. large and small ponds, channels and leads) within each wetland (Table 2) using the protocols described in section 2.4.1. Invertebrate samples were preserved with IPA in the field, and were processed as described in section 2.4.3. Measurements of water chemistry (dissolved oxygen (DO), pH and conductivity) were made at each site using a Horiba® multiprobe. In addition, water samples were collected from each site, filtered through Millipore® GFF glass fibre filters and frozen for nutrient analysis. Upon thawing, samples were analysed for nitrate (NO_3-N), ammonium (NH_4-N), dissolved organic nitrogen (DON), dissolved reactive phosphorus (DRP) and dissolved organic phosphorus (DOP) using standard methods for the Lachat QuikChem Flow Injection Analyser (www.lachatinstruments/apps.asp; viewed December 2009).

3.1.2 Data analysis

Data for each of the water chemistry variables were analysed by Principal Components Analysis (PCA; McCune & Mefford 1997) to see how the spot water chemistry data differed between the wetlands. Individual variables were then correlated against resultant PCA axis 1 and 2 scores to see which were responsible for observed sample groupings. Differences in water chemistry data between the four wetlands were also investigated using ANOVA, and Tukey post-hoc tests (SPSS 2000) to determine where significant differences occurred.

Invertebrate data were analysed to see whether invertebrate communities varied more between wetlands than within wetlands. An ordination analysis (DECORANA, or detrended correspondence analysis—DCA) was used to investigate relationships between the different species assemblages found in each sample. This statistical technique graphically represents the location of samples based on their invertebrate communities, such that samples with similar communities appear close together on a graph, and samples with very different communities appear far apart from each other. Samples are plotted in (usually) two dimensions with arbitrary sample scores. A useful feature of ordination is also the ability to see which environmental and biological data are correlated to the ordination axes, and thus to particular sample groupings. The effects of the five predictor variables ('Wetland', 'Habitat', 'Plant', 'Growth form' and 'Morphology') on ordination scores were examined by Multi-response Permutation Procedures (MRRP; McCune & Mefford, 1997), a non-parametric procedure for testing the hypothesis of no difference between two or more groups of entities. The MRRP calculates the R statistic, which varies from 0 (all items within a group differ such that within-group variability is similar to that expected by chance) to 1 (all items within a group are identical, so within-group variability is much less than chance). Finally, all data were analysed using Multiple Regression Trees (MRT; De'ath 2002) to describe how the invertebrate community varied in response to the predictor variables of Wetland, Habitat, 'Plant, Growth form and Morphology. MRT uses selected predictor variables to predict a multivariate response variable, in this case invertebrate community composition. In this way, we could determine which of our measured environmental variables was causing the most variation in invertebrate community composition.

3.2 RESULTS

Spot water chemistry differed greatly between the four wetlands (Fig. 4). The Mahinapua wetland had higher DON, DRP and DOP levels than the other three wetlands (Table 3). Water pH was lowest in the Kakapotahi wetland and highest in the Mangarakau wetland, which also had the highest conductivity. Kakapotahi and Mahinapua had higher NO_3-N concentrations than Shearer and Mangarakau (Table 3).

A total of 75 invertebrate taxa were collected from the four wetlands. Mangarakau wetland supported the highest number of taxa (47), while Shearer supported the lowest (25). The fauna in all wetlands was numerically dominated by *Tanytarsus* and Orthoclad midges (17% and 8% of total density, respectively), and aquatic nematodes (12%). Five other taxa comprised > 5% of total density (harpactacoid copepods, the damselfly *Xanthocnemis zealandica*, ceratopogonid and tanypodinid midges, and the snail *Potamopyrgus antipodarum*). Forty-six taxa were collected only rarely, and occurred at < 5% of sites or had abundances of < 0.01% of total density. The most widespread invertebrates were orthoclad midges and aquatic mites, which were found in 91 of the 94 samples. Other widespread taxa were *Xanthocnemis*, ostracods and cyclopoid copepods, chironomid midges (including *Tanytarsus* and Tanypodinae), nematodes, hydroptilid caddisflies, Ceratopogonidae and Oligochaeta, which all occurred at > 70% of sites. Nineteen taxa were recorded in all four wetlands, while nine taxa were found in three wetlands and 16 taxa were found in two; 31 taxa were restricted to only one wetland. The Kakapotahi wetland supported the most
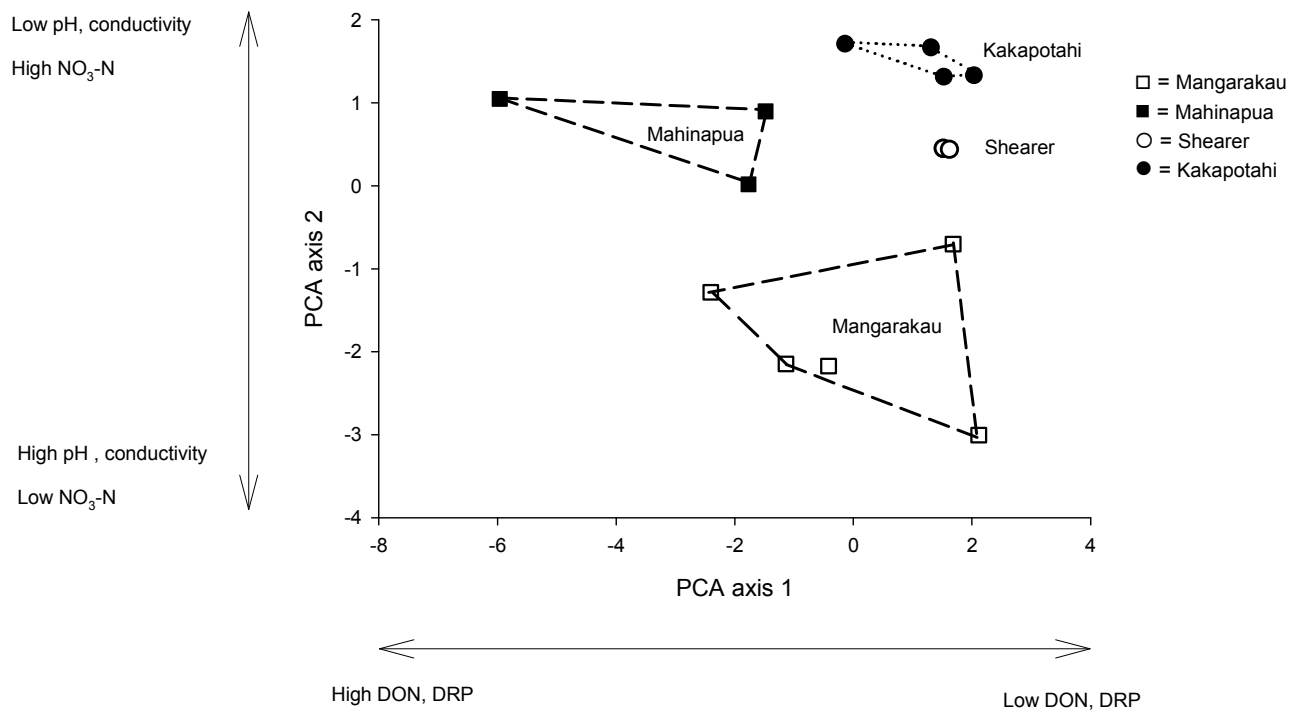
Figure 4. Results of a principle components analysis (PCA) of spot water chemistry data collected from the different habitats within each of the four wetlands surveyed for the spatial study. Significant factors of the PCA axis 1 and 2 scores are also shown. Note that samples from Shearer are superimposed on each other, so that only two of the three samples collected are shown.

TABLE 3. SUMMARY OF WATER QUALITY CONDITIONS (MEAN ± 1 SD) IN EACH OF THE FOUR WETLANDS SAMPLED IN THE SPATIAL STUDY.

DON = dissolved organic nitrogen; DRP = dissolved reactive phosphorus; DOP = dissolved organic phosphorus. Means with different superscript letters are significantly different from each other (Tukeys post-hoc tests, $P < 0.05$).

WETLAND	PH	CONDUCTIVITY (μS/cm)	NH_4-N (μg/L)	NO_3-N (μg/L)	DON (μg/L)	DRP (μg/L)	DOP (μg/L)
Kakapotahi	4.3 + 0.2[a]	56 ± 2[a]	7.1 ± 2.2[a]	5.2 ± 1.8[a]	173 ± 22[a]	0.54 ± 0.22[a]	1.8 ± 0.7[a]
Mahinapua	5.5 ± 0.2[b]	60 ± 10[a]	7.4 ± 0.7[a]	5.2 ± 2.1[a]	268 ± 31[b]	2.1 ± 1.0[b]	6.3 ± 1.8[b]
Mangarakau	6.4 ± 0.5[c]	98 ± 19[b]	9.8 ± 3.4[b]	3.5 ± 0.7[b]	204 ± 52[a]	0.57 ± 0.35[a]	3.5 ± 1.7[a]
Shearer	5.2 ± 0.01[b]	45 ± 5[a]	7.3 ± 1.3[a]	3.4 ± 0.1[b]	188 ± 7[a]	0.61 ± 0.10[a]	0.7 ± 0.9[a]

unique taxa (12), followed by Mangarakau (10) and Mahinapua (9). Shearer swamp supported only one unique taxon. Within each wetland, the number of taxa restricted to only one habitat varied from 26% to 47%, while the number of taxa found in all habitat types varied from 35% to 50%.

The four wetlands supported distinct invertebrate communities (Fig. 5). Samples from Kakapotahi and Shearer had low axis 1 scores, while Mangarakau had high axis 1 scores, which was positively correlated with the water quality variables of pH ($r^2 = 0.772$) and conductivity ($r^2 = 0.731$). Samples from the Mahinapua wetland had scores intermediate between the low pH fens and Mangarakau (Fig. 5). Correlations of invertebrate densities with the axis 1 and 2 scores showed that specific invertebrates were associated with different wetlands (Fig. 5). For example, 13 taxa had significant positive correlations ($r^2 > 0.3$, $P < 0.05$) to axis 1 scores, and were thus characteristic of sites with high axis 1 scores (i.e. were found at Mangarakau), while nine taxa had similarly significant negative correlations with axis 1 scores; i.e. were found at Shearer and Kakapotahi.
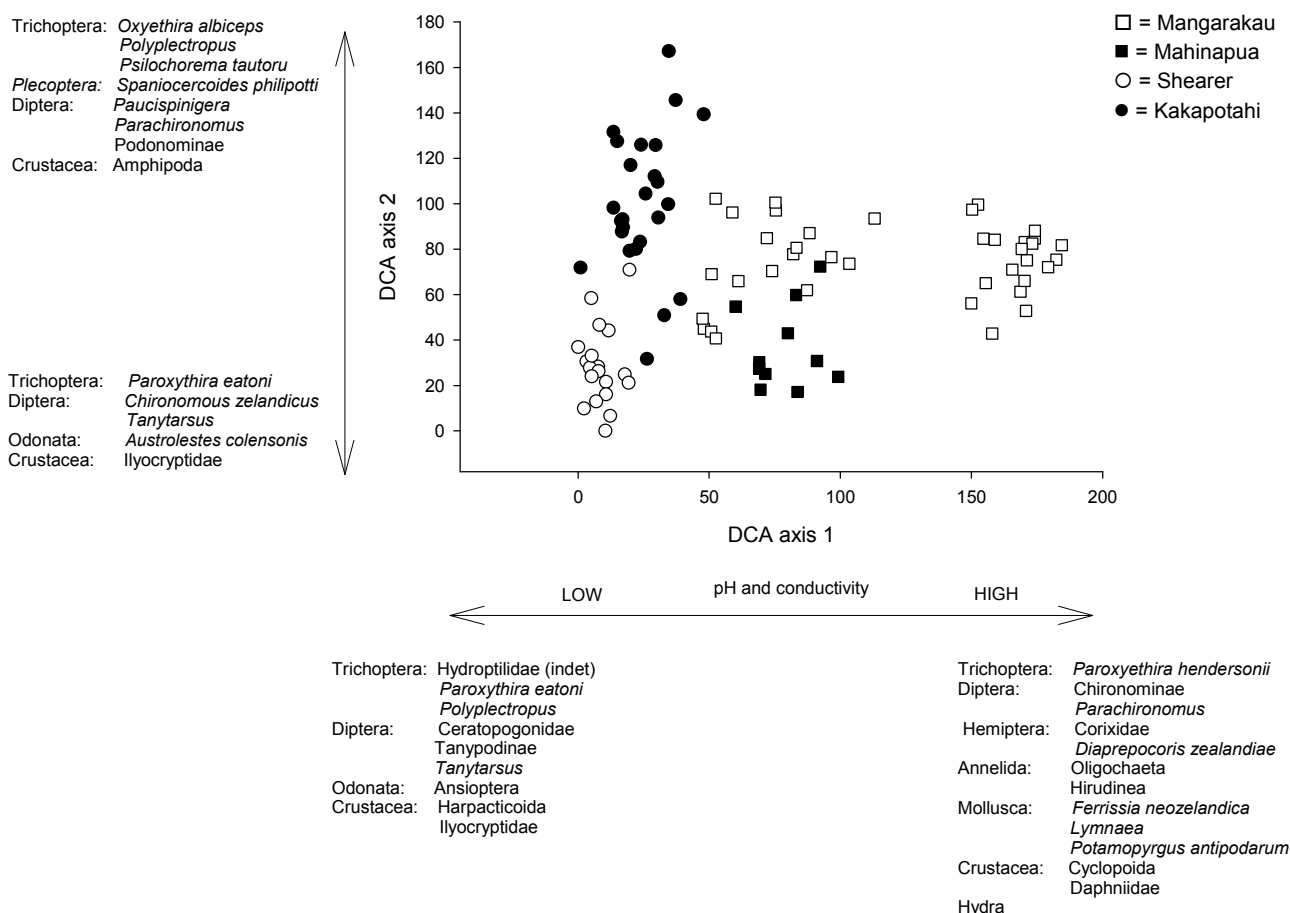
Figure 5. Results of the detrended correspondence analysis (DCA) of invertebrate communities collected from the different habitats within each of the four wetlands surveyed for the spatial study. The different taxa and water quality parameters that displayed significant correlations ($r^2 > 0.2$) to the axis 1 or 2 ordination scores are also shown.

Samples collected from Kakapotahi had higher axis 2 scores than samples collected from Shearer, with the other two wetlands having intermediate scores on this axis. No significant correlations ($P > 0.05$) were observed between axis 2 scores and any of the water quality variables, suggesting that this axis represented an unmeasured gradient. However, significant correlations existed between 13 invertebrate taxa and axis 2 scores (Fig. 5). Examination of densities of these taxa showed that only the caddisflies *Polyplectropus* and *Psilochorema tautoru* were restricted to Kakapotahi; densities of the other 11 taxa varied along this axis and were not restricted to one wetland.

MRPP illustrated how the ordination scores differed according to the characteristics of the sampling site (Wetland, Habitat, Plant, Growth form and Morphology). Most of the differences between ordination scores occurred when samples were coded for different wetlands ($R = 0.198$), although the Habitat and Plant terms also resulted in relatively high within-group homogeneity ($R = 0.140$ and 0.107, respectively). The Growth form and Morphology terms showed little within-group homogeneity ($R = 0.03$ and 0.05, respectively), suggesting that invertebrate communities did not display any strong preference to plants on the basis of their growth form or morphology. The results of the multivariate regression tree generally confirmed these findings, showing that the Wetland term contributed most to the explanatory power of the model (58.2%), followed by Habitat (23.3%), Growth form (12.6%) and Morphology (5.8%). Unlike the

MRPP analysis, the Plant term contributed nothing to the observed variability in the invertebrate community. Such ambiguous results for the importance of the Plant term most likely reflect differences in the two techniques, and are best interpreted as meaning that differences in plant species have less influence on invertebrate composition than either differences between wetlands or habitats.

The number of taxa unique to specific habitats within each wetland was calculated. This showed that approximately 33% of taxa were found in only one habitat in each wetland, 23% were found in two habitats only, and 43% were found in three or more habitats. Based on this result, it was apparent that sampling just one habitat type within a wetland may not have completely characterised the invertebrate communities.

3.3   DISCUSSION

In this spatial study, it was found that invertebrate communities in these natural wetlands varied more between different wetlands than they did between habitats or plants within a wetland. Each of the four wetlands sampled supported distinctive invertebrate communities, presumably reflecting, in part, differences in water chemistry between these two wetland types (fens and swamps). For example, Mangarakau was less acidic and had higher conductivity than the other wetlands, and supported an invertebrate community very different from that in the more acidic wetlands. Molluscs in particular were commonly collected from Mangarakau, but were absent from the lower pH wetlands. Absence of molluscs from the low pH wetlands most likely reflects their inability to obtain enough free calcium for shell maintenance (Crumpton 1978) or the inability of snail eggs to develop in low pH water (Burton et al. 1985). Batzer et al. (2005) also reported a lack of molluscs in water with pH < 6.0 and a similar absence of molluscs has been observed in streams and lakes with low pH (Oekland & Oekland 1986; Oekland 1990).

Our finding of low variability in invertebrate community composition between plant types was somewhat surprising, especially in light of the review by Wissinger (1999), where it was suggested that wetland macroinvertebrates are responsive to variations in plant community structure. Our results suggested that within each of the four wetlands sampled, invertebrate community composition and percentage abundance were relatively similar between areas with and without vegetation. Kratzer & Batzer (2007) also found little variation in invertebrate communities in Okefenokee Swamp, Florida, USA, despite sampling five plant community habitats (marsh prairies, cypress forest, scrub-shrub thickets, deepwater lakes and boat trails) in six discrete areas of the swamp. They attributed this lack of variation to the fact that water quality did not vary greatly throughout the wetland, as a result of its source being almost entirely precipitation-based. If water chemistry is responsible for structuring invertebrate communities, then there are no biological reasons why invertebrate communities would change between different habitats within a wetland, as long as water chemistry within these habitats was similar. The corollary to this is that wetlands with different water chemistry would support different invertebrate communities, despite having similar habitats.

The results of this study suggest that most of the variation in invertebrate communities in wetlands on the west coast of the South Island occurs at the spatial scale of the wetland. Such a finding is likely to be similar throughout the country, assuming that water chemistry within a wetland is relatively uniform and reflective of the particular wetland's hydrosystem. Although invertebrate communities vary at the smaller spatial scale of habitat, plant species or morphology, these variations are not large enough to mask differences between individual wetlands. This means that it may not be necessary to sample a specific habitat or plant type within a wetland in order to properly characterise and compare invertebrate communities, as larger scale processes operating at the wetland level appear to control this. Rather, we suggest sampling from as wide a range of aquatic habitats that are found in a wetland as possible, given the time and cost constraints inherent in collecting too many samples. Furthermore, rather than concentrating on collecting samples from vegetated and non-vegetated areas, or a particular plant taxon, we suggest collecting samples from as many micro-habitats as possible within a water body, and pooling these, asssuming the water chemistry and hydrological variation is generally consistent across the wetland. Consequently, our subsequent sampling protocol was to identify different types of aquatic habitat within a wetland, and to try to sample a representative number of each. Within each habitat, two replicate samples were collected from a range of micro-habitats including vegetated and non-vegetated areas. Up to three water bodies within each wetland were chosen, giving a total of six samples per wetland.

4. Temporal variation—when should we sample?

The spatial study outlined in section 3 was carried out over a 3-week period during the austral spring (November–December 2003) to minimise potential seasonal effects that may have altered the invertebrate communities. However, future nationwide inventories of wetlands may need to consider potential interannual or seasonal variation in invertebrate communities that could obscure or exaggerate differences between wetlands.

If wetland invertebrates vary interannually or seasonally, and if this affects our ability to discriminate between different wetlands, then sampling may need to be restricted to particular seasons. Unless this is taken into account, it will be difficult to identify potential factors regulating invertebrate communities in perennial wetlands. In contrast, if invertebrate communities vary little over time, or if seasonal variation in the abundance of individual taxa is similar between different wetlands, then variation between wetlands will remain relatively constant. Under such a scenario, surveys of multiple wetlands encompassing a wide range of environmental conditions could be conducted at any time of the year, because the underlying differences between wetlands would transcend those caused by temporal fluctuations.

This second sampling programme investigated temporal variability in invertebrate communities and whether this would affect our ability to discriminate between wetlands. This consisted of two separate studies: the first study investigated interannual variation, while the second study investigated seasonal variation.

4.1 STUDY SITES AND METHODS

4.1.1 Interannual variation

The first study was conducted in the Bullock Creek wetland: an enclosed depression in a steep karst landscape in the Paparoa Ranges on the west coast of the South Island. The study area was a 100-ha palustrine fen within the wetland, surrounded by tall limestone cliffs and indigenous forest of southern beech (*Nothofagus* spp.) and podocarp conifers (Podocarpaceae). Climatic conditions in the area (obtained from a climate recording station at Westport, approximately 40 km north of the wetland) are characterised by cool seasonal temperatures (mean temperature = 12.5°C, mean winter minimum = 2.9°C, mean summer maximum = 25°C), and relatively high amounts of unpredictable rain (mean monthly rainfall = 170 mm).

Most of the wetland was converted into pasture for grazing by 19th-century settlers, and a network of drains was established during the first half of the 20th century (Fig. 6). The wetland is a mosaic of vegetation types, separated by the drainage network. In wetter areas remote from drains, the vegetation is dominated by native wetland species, including the sedges *Carex sinclairii* and *Baumea rubiginosa*, flax (*Phormium tenax*), and the peat-forming moss

Figure 6. Map showing the location of the Bullock Creek wetland, and diagrammatic representation of the main drainage network.

Suren & Sorrell—Aquatic invertebrate communities of lowland wetlands
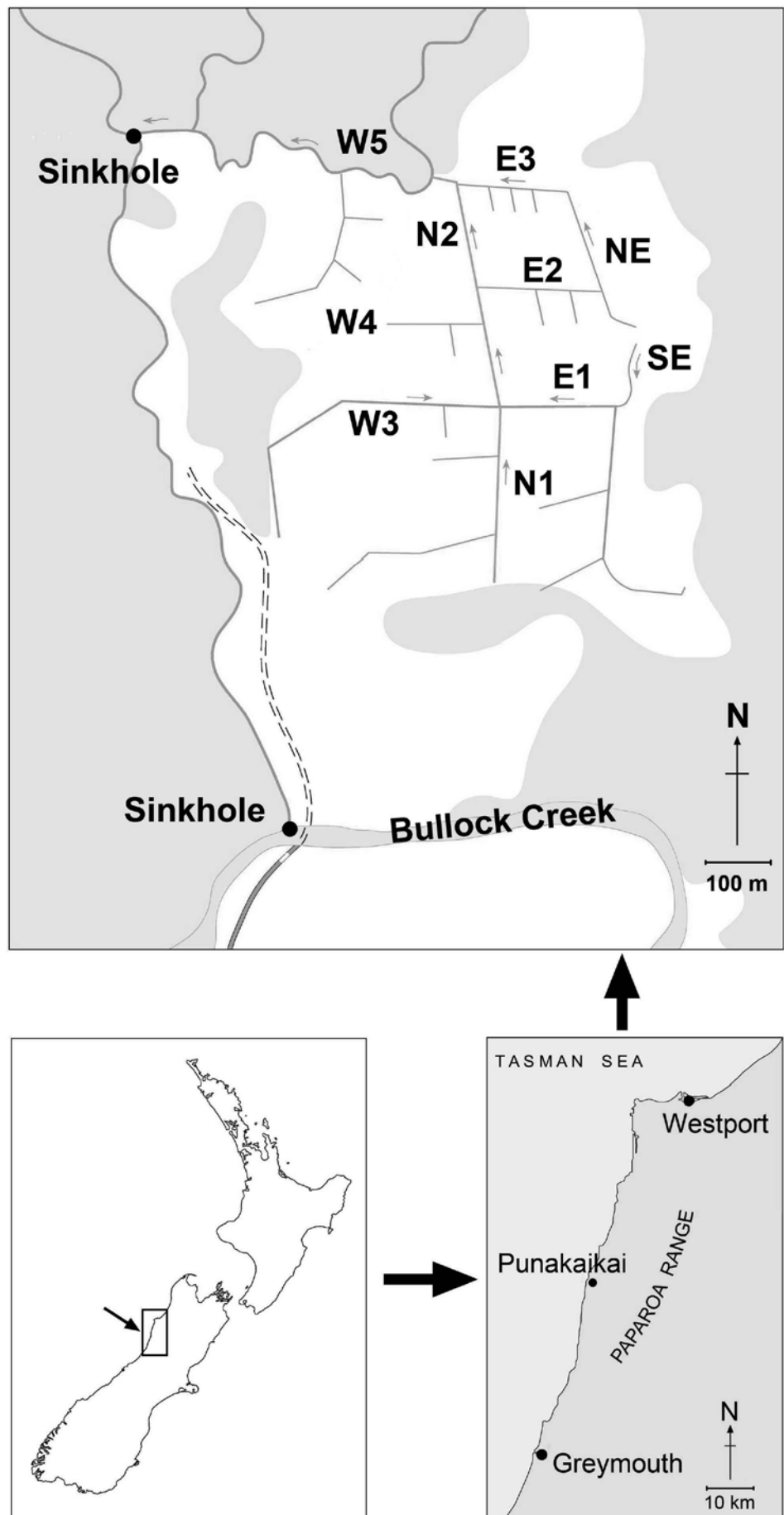
Sphagnum cristatum. In contrast, drier and more disturbed areas, especially areas close to drains, are dominated by alien pasture grasses and weeds (e.g. *Agrostis stolonifera*, *Holcus lanatus*, *Lotus pedunculatus*, *Ranunculus repens*). The entire site passed into public ownership in 1986, and is currently being managed and restored for conservation and biodiversity values by DOC.

A large central drain (N2) runs north through the wetland into the headwaters of Cave Creek (Fig. 6). These headwaters then flow west into a submergence. During base flow, water flows down this submergence, but during periods of high rainfall, the submergence is unable to cope with the volume of floodwater and the water flow reverses, flooding the fen and discharging south into Bullock Creek (Sorrell et al. 2007). Water input to the fen is therefore a combination of rainfall and overland floods, including the backflow from the sinkhole. A number of smaller side branches drain the western and eastern parts of the wetland. At the time of sampling, some drains (N1, W3, W4, E2) had steep, unvegetated banks with no instream macrophytes. Other drains (E1, E3, SE, NE and N2) were lined with overhanging vegetation and supported a range of aquatic macrophytes.

Invertebrates were sampled from ten sampling stations within the Bullock Creek wetland (Fig. 6) using a sweep net (300-μm mesh) that was repeatedly jabbed into vegetation or moved around the bottom of each drain to collect the benthos. Care was taken to empty the net regularly as it filled. Samples were collected for approximately 2 minutes from an area of c. 2 m^2 in each drain. Samples were collected during each summer (December–January) from 1999 to 2003. Invertebrate samples were preserved using 100% IPA, and processed as previously described (section 2.3.3). Measurements of water chemistry (DO, pH and conductivity) were made at each drain on each sampling occasion using a Horiba® multiprobe (Sorrel et al. 2007). Waterway width, depth and bank height were measured at five locations within each waterway. The depth of organic matter was measured by pushing a steel rod into the substrate until it hit solid material underneath. Five sediment samples were collected from each waterway and ashed (550°C: 8 h) to determine the % organic matter content. The remaining inorganic fraction was then passed through a series of nested sieves (4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.064 mm) for size analysis. The substrate size was expressed as the D_{16}, D_{50} and D_{84}, which represented the 16th, 50th and 84th percentile, respectively.

All invertebrate data were examined for normality and fourth-root transformed where necessary—log transformation was not as effective at normalising the data. We used non-metric multidimensional scaling (NMDS) ordination to see whether invertebrate communities in the drains differed between sampling locations, and whether these differences persisted over time.

4.1.2 Seasonal variation

Samples for the second study were collected from Mahinapua and Shearer wetlands, which are situated approximately 10 km and 30 km southwest of Hokitika, respectively. These wetlands were also included in the spatial variability study (section 3; Fig. 3). Climatic conditions in the area (obtained from a climate recording station at Ross, approximately equidistant from both wetlands) are characterised by cool seasonal temperatures (mean temperature = 15.7°C, mean winter minimum = 4°C, mean summer maximum = 30°C), and relatively high amounts of unpredictable rain (mean monthly rainfall = 277 mm).

Duplicate invertebrate samples were collected semi-quantitatively every 3–4 months over an 18-month period from each of three open-water habitats within each wetland using the same hand-held sweep net as used in other studies (sections 3 and 4.1.1). All samples were preserved immediately following collection using 100% IPA. Spot measurements of water chemistry (temperature, pH and conductivity) were made at each habitat within each wetland using a Horiba® multiprobe. Water level was monitored against a known benchmark placed at a discrete point in the main channel in each wetland.

Taxonomic richness was calculated for each sample, as was the percentage abundance of the 11 most common taxa, each of which contributed > 2% to total density. A repeated measures ANOVA (SPSS 2000) was used to assess whether selected invertebrate metrics differed between the wetlands and over time. The wetland × time interaction term showed whether the metrics behaved in the same way in both wetlands. A repeated measures ANOVA was also used to determine whether measured water quality metrics differed between each wetland over time. An ordination was then carried out on the percentage abundance data, and resultant ordination scores were assigned to each wetland and to each sampling occasion. These scores were then analysed to see whether samples differed more as a result of differences between wetlands or sampling occasions.

4.2   RESULTS

4.2.1   Interannual variation

Ordination of the invertebrate data collected from the waterways of the Bullock Creek wetland gave three distinct clusters, with samples from the stream site (W5) having lower axis 1 scores than samples collected from the drains, and samples collected from unvegetated drains in the wetland (N1, N2, W3 and W4) having higher axis 2 scores than samples collected from vegetated drains (E1, E2, E3, NE and SE) (Fig. 7). These differences persisted throughout the 4 years, despite evidence of some interannual variation in the invertebrate communities within each habitat, as shown by small shifts in ordination scores. However, at no time did the invertebrate communities converge in their species composition.

Densities of individual invertebrate taxa differed significantly between sites. Insects such as Ephemeroptera (*Deleatidium*, *Neozephlebia* and *Zephlebia*), Trichoptera (*Costachorema*, *Psilochorema*, *Oxyethira* and *Pycnocentria*) and Diptera (*Austrosimulium*, Eriopterini and Orthocladiinae) were significantly more common in the stream site (W5); dipterans (Chironominae, *Chironomus* and *Paradixa*), the amphipod *Paraleptamphopu*s, copepods, the hempiteran *Microvelia*, and Collembola were significantly more common in the acidic drains; and Mollusca (*Potamopyrgus* and *Sphaerium*), microcrustacea (Cladocera and Ostracoda), nematodes and the corixid *Sigara* were more common in the more circum-neutral vegetated drains. The occurrence
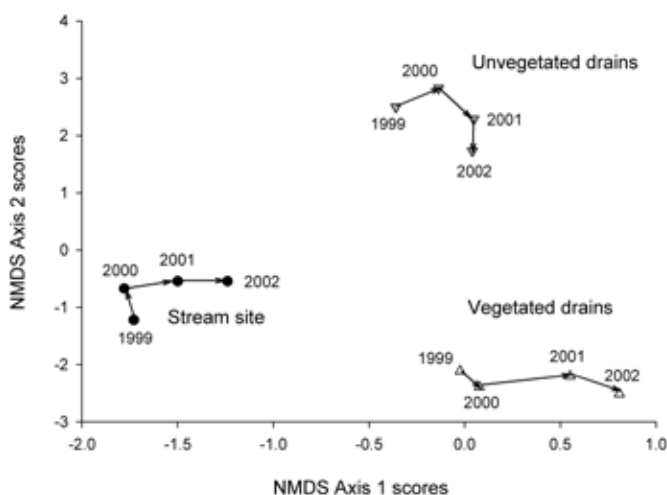

Figure 7.   Non-metric multidimensional scaling (NMDS) ordination of invertebrate communities collected from drain bottoms in the Bullock Creek wetland, showing the three discrete groups found in the drains, and temporal differences in calculated NMDS scores for the different groups. For clarity, only the centroids of each group have been shown. Note that although community composition changed over time, at no time did we lose the ability to discriminate between the three sampling locations.

of Ephemeroptera and Trichoptera, and *Austrosimulium* and Eriopterini at the stream site is not surprising, as these invertebrates are common to streams throughout the West Coast region. The fauna of the drains was more typical of that found in wetlands throughout the country, being dominated by midges, molluscs, micro-crustacea and nematodes.

These consistent differences in invertebrate communities between the sampling sites during the 4-year period most likely reflected differences in water chemistry and physical habitat conditions (Table 4). Water pH, in particular, varied greatly between the waterways within Bullock Creek: the stream site (W5) and the vegetated drains (most of which were found in the northeastern part of the wetlwnd) had relatively neutral pH, while the unvegetated drains (most of which were in the southwestern part) had lower pH. Such a large pH variability within a wetland appears unusual: indeed, this wetland had the highest within-wetland pH variability (with a range of 3.9 pH units) of 154 wetlands surveyed throughout the country, where the median variability was only 0.6 pH units. The large variability within the Bullock Creek wetland most likely reflects the underlying geology within the wetland: low pH limestone intersecting with higher pH quartz-bearing rocks. Habitat conditions also varied between the waterways, with the stream site in particular differing from the wetland drains in terms of having slightly wider channels and deeper water than the drains, larger streambed sizes, and less benthic organic matter.

TABLE 4. SUMMARY OF PHYSICO-CHEMICAL CONDITIONS MEASURED IN THE THREE WATERWAY TYPES SAMPLED IN THE BULLOCK CREEK WETLAND DURING THE INTERANNUAL VARIATION STUDY.

The mean and range (min–max) is given for each variable. Means with different superscript letters are significantly different from each other (Tukeys post-hoc tests, $P < 0.05$).

VARIABLE	STREAM SITE (W5)	UNVEGETATED SITES (N1, W3, W4, E2)	VEGETATED SITES (E1, E3, SE, NE, N2)
pH	7.1[a]	5.8[b]	6.8[a]
	(6.8-7.5)	(5.2-7.4)	(5.7-8.0)
Conductivity (mS/cm)	0.130[a]	0.047[b]	0.157[a]
	(0.110-0.150)	(0.015-0.085)	(0.030-0.300)
Dissolved O_2 (mg/L)	10.1[a]	5.9[b]	9.4[a]
	(9.5-10.3)	(3.4-9.4)	(7.7-12.7)
Temperature (°C)	10.1[a,b]	12.1[b]	10.3[a]
	(8.9-11.7)	(9.0-16.5)	(7.7-13.6)
Width (m)	1.97[a]	1.22[b]	1.78[a,b]
	(1.12-2.70)	(0.25-3.1)	(0.65-3.4)
Water depth (m)	0.40[a]	0.25[b]	0.29[b]
	(0.30-0.48)	(0.06-0.80)	(0.09-0.56)
Depth of organic matter (m)	0.01[a]	0.23[b]	0.69[c]
	(0-0.05)	(0.0-0.44)	(0.35-0.98)
Bank height (m)	2.42[a]	1.07[b]	0.86[b]
	(2.2-2.58)	(0.2-1.4)	(0.25-1.50)
% macrophyte cover	20%	0%	60%
			(25%-90%)
Substrate size (mm)			
D_{16}	0.1	0.032	0.064
D_{50}	0.7	0.075	1.2
D_{84}	4.2	0.28	2.6
% organic matter	0.9[a]	11.3[b]	19.9[b]
	(0.7-1.3)	(2.6-26.1)	(1.4-41.3)

4.2.2 Seasonal variation

Over the 15-month period of the seasonal study, measured water quality parameters always remained distinctive between the two wetlands sampled. Water pH was always higher at Mahinapua than at Shearer, and although this changed over time, it did not follow any seasonal patterns. Water levels in both wetlands varied over time, and reflected the unpredictable rainfall patterns in the area. Variation was higher at Mahinapua than at Shearer, but at no time did any of the sampling sites dry. Spot water temperature was higher at Shearer (Fig. 8). Conductivity was low (< 100 µS/cm) at both wetlands, but was usually slightly higher at Mahinapua (except in February 2006). Such distinctive water chemistry signatures presumably reflect the different hydrological source of water in each wetland: rainfall-dominated hydrology at Shearer and lake floodplain hydrology at Mahinapua (Johnson & Gerbeaux 2004). We expect such differences in water quality to persist over time and to result in consistently distinctive invertebrate communities within each wetland, as was found in the interannual study at Bullock Creek (section 4.2.1).

A total of 58 taxa were collected during the seasonal variation study. Mahinapua supported more taxa (50) than Shearer (38). Taxonomic richness varied over time in both wetlands, but in different ways: richness increased over time at Mahinapua, but was low each autumn (April–May) in Shearer (Fig. 9A). Relative abundances of six of the 11 most common taxa differed between wetlands (Table 5), with three taxa (Cyclopoida, Orthocladiinae and *Xanthocnemis*) being more common in the Mahinapua wetland, and three taxa (Hydroptilidae, Nematoda and *Tanytarsus*) more common in Shearer (Fig. 9B–L). Relative abundances of two taxa (Harpacticoida and Ilyocryptidae) displayed spring or summer maxima and autumn minima at both wetlands (Fig. 9B & C), while relative abundance of *Tanytarsus* peaked in autumn, and was lowest in spring at both wetlands (Fig. 9D). Relative abundances of Nematoda peaked in spring at Shearer Swamp only (Fig. 9E), while *Paroxyethira* had highest relative abundances in Autumn at Mahinapua (Fig. 9F). Relative abundances of six of the 11 taxa (Acarina, cyclopoid copepods, small unidentified hydroptilid caddisflies, orthoclad and tanypodinid midges, and *Xanthocnemis*) varied significantly ($P < 0.05$) over time, but without obvious seasonal patterns in either wetland (Fig. 9G–L).

Five taxa had significant wetland × time interaction terms (Table 5), suggesting that their relative abundances varied inconsistently over time between the two wetlands. Relative abundances of orthoclad midges, cyclopoid copepods and *Paroxyethira* varied over time at Mahinapua but not at Shearer, where they were found only rarely (Fig. 9F, H & J). In contrast,
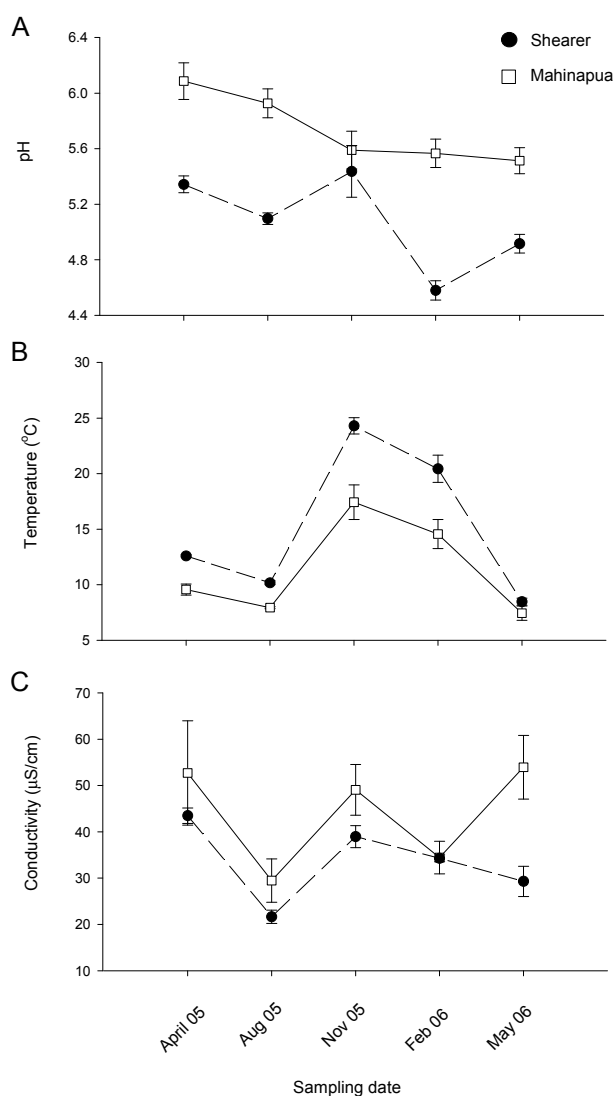

Figure 8. Seasonal differences in pH (A), temperature (B) and conductivity (C) in the Shearer (black circles) and Mahinapua (open squares) wetlands over the 15-month study period (mean ± 1 SEM, *n* = 3).