Suitability of invertebrate data for assessing groundwater ecosystem health

Prepared for New Zealand's Biological Heritage National Science Challenge

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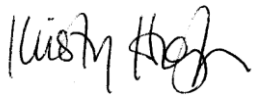	Reviewed by:	Kristy Hogsden
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Executive summary

The focus of New Zealand's Biological Heritage National Science Challenge Project 1.5 was improving knowledge of groundwater invertebrate biodiversity, specifically the abundance and distributions of species. As part of this project, the suitability of the resulting invertebrate biodiversity data was assessed for developing indices of groundwater ecosystem health, similar to indices widely used for measuring and monitoring ecosystem health in surface water environments. Sixty-five wells for sampling stygofauna across two islands, four regions and eight aquifers (catchments) were identified with help from regional councils. These wells differed in construction, location within the catchment, surrounding land-use intensity and several other factors. A combination of methods was used to sample stygofauna and water chemistry, and the River Environment Classification linked each well to a river reach for assessing other important environmental variables, such as climate and geology.

Eight of the 19 environmental variables were significantly correlated with at least one other. Further, there were significant differences in some environmental variables between some regions (e.g., soil types, land cover, etc.) and between some catchments within regions. (e.g., pH, dissolved oxygen, etc.). These cross-correlations and catchment differences, plus our inability to sample unimpacted groundwater, meant that cause and effect relationships could not be determined.

The small numbers of stygofauna obtained from 51 wells were morphologically identified, as far as practical. Taxa richness (diversity) was positively correlated with dissolved oxygen concentration and water temperature, and negatively correlated with well depth. No other significant relationship was found between richness and any environmental variable.

Stygofaunal community composition differed markedly between wells within most catchments and was significantly correlated with conductivity, dissolved oxygen, well diameter, latitude and several attributes of the associated up-stream river catchment (i.e., phosphorus content of rock/soils, pasture cover, alluvium cover, sediment particle size). Community composition was negatively correlated with water conductivity. Cross-correlations between variables, the absence of samples from unimpacted wells and the small numbers of stygofauna from most wells meant that changes in community composition could not reliably be attributed to land-use effects.

Successful CO1 DNA sequences for 211 amphipod, isopod and copepod specimens were assigned to barcode index numbers (BINs, which strongly correlate with species) within BOLD's (Barcode of Life Database) system. These DNA analyses resolved many more taxa (59 amphipods, isopods and copepods) than our morphological identifications. Up to 14 species (BINs) of these groups were found at a single well. Several species were restricted to single wells or locations (41%), and most were confined to single catchments (87%). Species common to more than one catchment were only found within Canterbury.

Data from this project revealed the nature of data necessary for resolving and quantifying any land-use effects on groundwater ecosystem invertebrate communities. Our data must be supplemented by more data from unimpacted locations, more comprehensive sampling of the fauna at most locations, and data from more locations. Thus, development of a stygofauna-based index of groundwater ecosystem health requires better stygofauna sampling methods, improved identifications (morphological identification using existing tools proved unreliable), a system for naming and re-recognising taxa so that they can be discussed and investigated, and that the physiological responses of key taxa to important land-use effects are known. Future research to develop such indices must also take account of this project's major finding that most groundwater amphipod and isopod species have highly restricted geographic ranges.

1 Background

When first devised, Project 1.5 of the New Zealand's Biological Heritage National Science Challenge (hereafter BioHeritage Challenge) was focussed on improving knowledge of groundwater invertebrate biodiversity, specifically the numbers and distributions of species. The primary reason for this was that current taxonomic investigations indicated a fauna comprised of either a smaller number of widely distributed taxa, or a larger number of morphologically-cryptic taxa that were less widely distributed. Resolving this question was very important for determining other research priorities (including biodiversity description and inventory, ecosystem functioning, etc.) and the likely resources required to progress these priorities.

In the process of finalising the proposal and contracting for Project 1.5, the research team was encouraged to make a preliminary investigation of the feasibility of using the project's groundwater invertebrate data for developing indices of groundwater ecosystem health, similar to indices widely used for measuring and monitoring ecosystem health in surface waters. This report presents our findings of this second part of the work.

1.1 What makes a useful ecosystem health index?

Surface water macroinvertebrates are used globally as indicators of human impacts to river ecosystems (Stark 1985, Armanini et al. 2011, Timm et al. 2011). Macroinvertebrate taxa often differ in their sensitivity to environmental stressors, such as organic pollution or increased stream-bed sedimentation. Macroinvertebrates are often better indicators of longer-term trends in ecosystem health than water chemistry or microbes because their life-histories (months to years) capture and integrate the effects of prior adverse environmental events (e.g., flood events, chemical spills, etc.). Macroinvertebrate metrics that combine and summarise the sensitivities (e.g., presence or abundance) of taxa to land-use effects can be used to quantify human impacts at specific locations. For example, the Macroinvertebrate Community Index (MCI) was developed as a metric of water quality in stony streams (Stark 1985) and is widely used in New Zealand to monitor and assess the health of surface freshwater bodies.

Invertebrate-based ecosystem health indices that are effective management tools:

- are based on species or taxa that occur across a wide range or entire geographic range of interest (e.g., most of New Zealand);
- are based on well-understood, stressor-response relationships, so that there is an obvious remedial pathway if the index value is low or declines;
- respond to single and multiple stressors; and
- can be generated relatively easily and at low cost to allow widespread and/or frequent monitoring over several years.

Ideally, groundwater invertebrates (or stygofauna) could be used in much the same way as indicators of human impacts, such as nutrient enrichment or water abstraction, on groundwater ecosystems.

Groundwater ecosystems (GEs) have been poorly studied world-wide, historically, relative to surface water systems, in part due to the difficulty of collecting samples (e.g., Gibert et al. 1994, Scarsbrook et al. 2003). Given the almost total lack of information on the diversity, structure and functioning of GEs compared with their surface water counterparts, development of ecosystem health metrics

similar to the MCI is likely to be challenging. However, recent developments of more cost-effective methods to both sample and identify components of GEs (e.g., molecular tools), combined with increasing pressures on the groundwater resources, mean that an assessment of our ability to develop invertebrate-based indicators of GE health is timely.

1.2 Report scope

As part of Project 1.5: "Indicators of groundwater biodiversity and ecosystem health", NIWA was contracted to provide the BioHeritage Challenge with a summary report detailing:

1. attempts to assess preliminary indices of GE health and total groundwater biodiversity,
2. any difficulties encountered,
3. any relevant findings, and
4. any recommendations for future work on this topic.

This report summarises our preliminary investigation of the potential for groundwater invertebrates (stygofauna) to be used as indicators of GE health, based on the data collected as part of Project 1.5. It does not discuss biodiversity patterns in detail. Further analysis and details of the biodiversity patterns identified in invertebrate data are in a draft paper (in preparation) for Project 1.5.

An aligned, multi-year project aimed at developing indices of GE health, focused principally on microbes has been prepared by ESR, a key partner (see separate report).

2 Collection location and methods

2.1 Sampling locations

To determine spatial scales of stygofauna biodiversity, based on amphipods and isopods (the larger-sized taxa within New Zealand's stygofauna), sampling was stratified by island (South and North), by region and by catchment (as surrogates for aquifers[1]). Sixty-two wells[2] were sampled in four regions: Southland, Canterbury, Nelson and Hawkes Bay. Four catchments were sampled within Canterbury, two in Nelson (Tasman District), and one catchment each in Hawkes Bay and Southland (Table 2-1; Figure 2-1). We used existing wells for all sampling.

The original design also sought to stratify sampling by human impact, using location within a catchment as a measure of relative impact (i.e., headwater locations were assumed to be less impacted than lowland locations). However, there were very few bores or wells within upper parts of catchments where human impacts were presumed lowest.

Table 2-1: Numbers of wells sampled in eight catchments across four regions in New Zealand.

Region	Catchment	Number of wells sampled
Southland (2 wells)	Mataura	2
Canterbury (35 wells)	Orari	9
	Selwyn	14
	Waimakariri	2
	Ashley	10
Nelson (19 wells)	Waimea	12
	Motueka	7
Hawkes Bay (9 wells)	Tukituki	9
TOTAL	8	65

Candidate wells for sampling were identified in discussions with local groundwater monitoring agencies (regional and district councils), using their databases of regional groundwater wells. However, locating suitable wells for stygofauna sampling was challenging. There are few dedicated research or monitoring wells that are suitable for stygofaunal sampling. Most other wells are in active use, making them unavailable for deploying the nets, bailers and hoses with packers usually required for capturing stygofauna. Active wells are usually sealed at the head (above ground) and have intake pipes (dip-pipes) permanently installed within them. Significant effort by well specialists would be required to open the wells and, once opened, the risk of sampling gear becoming

[1] Aquifer boundaries are poorly known. Hence, we used catchments (based on the River Environments Classification V2) as surrogates for the shallow aquifers in all regions.

[2] We use the terms well and bore as synonyms for simplicity, but recognise that some workers distinguish wells (established by excavating) from bores (drilled and, therefore, usually deeper).

entangled with dip-pipes, in-well pumps, etc. is very high. Together, these factors greatly reduced the numbers of wells available for sampling for this investigation.

Differences between wells are also important to note. Those sampled in this study ranged in diameter from 50 to 1300 mm. They also differed in lining (casing) material. Casing may be concrete (mostly <5 m deep), steel or PVC. Almost all wells are designed to keep out everything except water. Water enters most concrete wells by flowing up through the open bottom. It enters some steel- and PVC-cased wells in the same way, but many are variously screened or slotted (e.g., have saw-cuts (1-2 mm wide) or perforations in the casing, usually within the aquifer's saturated zone) or incorporate screened sections, which may comprise stainless steel mesh (meshes up to c. 3 mm across). Details of slotting or screening are often not available, so wells were usually evaluated and used without knowing this important attribute.

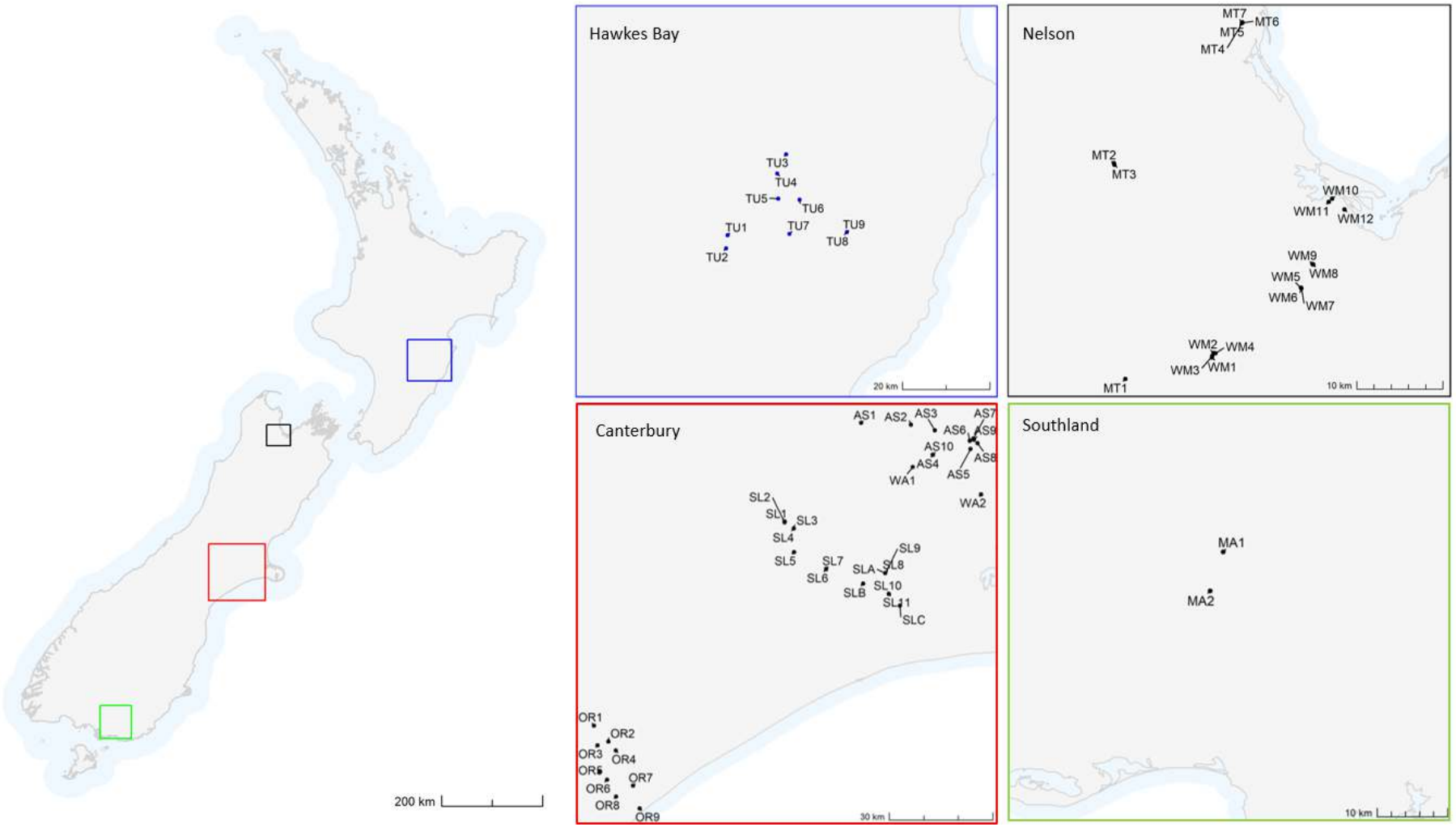

Figure 2-1: Location of sampled wells within the four regions. Wells identified by a two-letter code for catchment (TU, Tukituki; MT, Motueka; WM, Waimea; AS, Ashley; SL, Selwyn; OR, Orari; MA, Mataura) with sampled wells within the catchment numbered from highest elevation (1) to lowest elevation (n). Specimens from wells SLA, SLB and SLC in Canterbury, sampled prior to this study, were included to supplement genetic data.

2.1.1 Water chemistry

Once pumping from the well was completed and before plankton net samples were collected, two 250 ml water samples were collected from the pumped water in acid-washed bottles (glass for organic carbon, PVC for other nutrients) for determining nutrient concentrations. These water samples were stored on ice immediately and kept in the lab at -20 °C until analysed. A further 5 L of water was gently pumped into a clean plastic container and dissolved oxygen concentration measured using a recently calibrated dissolved oxygen meter (TPS WP-82). After pumping was completed, conductivity (µS/cm), water temperature and pH were measured in situ using a recently calibrated[3], water quality instrument (TPS WP-81).

All equipment (nets, bailer, lines, pumps, pipes, packers) were washed thoroughly between bores to avoid transferring any specimens between bores and locations. The cleaned sampling equipment was air dried over several days before sampling bores in different aquifers.

2.2 Environmental conditions

The intensity and effects of land use on surface waters can be difficult to measure, and more so for groundwaters, because aquifer boundaries and flow paths are largely unknown at meaningful scales. Thus, we used data for the nearest surface stream as measures of key independent variables (e.g., nutrients) and of land-use effects on groundwater at each sampled bore. We did this by linking each sampled bore to its nearest large river reach (determined manually using best judgement for each well) within the River Environment Classification (REC) geodatabase (Snelder et al. 2010b) and allocating rank data for each reach from this database to its associated bore (see Table 3-1). The mean phosphorus content of the upstream regolith also was extracted from the REC database. Land cover data (proportions of each reach's for the upstream catchment of each reach (i.e., proportions assigned to alluvium, pasture, urban, exotic and natural landcover classes) were extracted from the Land Cover Database-3 (LCDB3).

Following Larned et al. (2004,2016) and Snelder et al. (2010a)'s approaches for surface-water quality analyses, each bore was classified, as follows:

- Dominant (by area) land use: pastoral (P; largest proportion or >25%), exotic forest (EF), urban (U; largest proportion or >15%), or natural (N; includes indigenous forest, tussock, scrub, bare-land (as in Larned et al. 2004a, 2016, Snelder et al. 2010a).
- REC climate category: warm extremely wet (WX), warm wet (WW), warm dry (WD), cool extremely wet (CX), cool wet (CW), and cool dry (CD), based on mean annual air temperature and precipitation.
- Source of flow: lowland (L), hill (H), lake-fed (Lk), mountain (M) or glacial (GM)).
- Upstream rock type (spatially-dominant): alluvium (Al), hard sedimentary (HS), soft-sedimentary (SS).
- Predominant (by area) upstream soil particle size: clay/silt (1), sand, gravel, coarse gravel or boulders-massive (5) (as in Milne et al. 1995, Leathwick et al. 2003, Larned et al. 2017).

[3] Instrument calibrations, undertaken by an experienced environmental instrumentation technician before and after each sampling event, were to within ±0.5% for conductivity, ±0.1 units for pH, ±0.2 °C for temperature and ± 0.2 mg/L for dissolved oxygen concentration.

2.3 Water quality sample processing

Water samples were processed at NIWA Hamilton and Hill Laboratories (Christchurch and Hamilton) for different analytes. Where the same analyte from the same bore sample was processed at multiple labs, we combined the results using the following approach:

- If all concentrations were above detection limit, we took the median value.
- If one concentration was below detection and one above, we kept the value above the detection limit.
- If all readings were below the detection limit, we used the lowest detection limit as the value.

When wells occurred in close proximity (<100 m) to each other, water chemistry samples were not always collected for all wells. In these cases, we assigned the water quality results from one well to nearby wells:

- Lab water quality data from WWD 2178 were used for WWD 2175, WWD 2177 and WWD 2180 as the bores are within 100 m of each other.
- Lab water quality data from WWD 20502 were used for WWD 20503 and WWD 20504 as the bores are within c. 20 m of each other.

The analytes measured for most samples were: dissolved organic carbon (DOC), dissolved reactive phosphorus (DRP), nitrite nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammoniacal nitrogen (NH_4–N), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP).

2.4 Invertebrate sample processing

2.4.1 Total stygofauna (identified morphologically)

The contents of each invertebrate sample bag were concentrated on a 250 µm sieve and washed in subsamples into a Bogorov tray with 100% ethanol. Each subsample was examined under a Leitz stereomicroscope (>40 x magnification), and individual invertebrates placed into separate, labelled glass vials for each major taxon from each sample. After sorting, capped vials of stygofauna were stored in the dark at -20 °C.

Samples collected using different sampling methods were combined to provide one pooled collection for each bore. Terrestrial taxa (e.g., Collembola and centipedes) from the ground surface or bore walls were discarded. All crustaceans in each vial were identified to the lowest practical taxonomic level using existing literature (Scarsbrook et al. 2003) and our unpublished guides to the NZ stygofauna. We avoided dissecting specimens during identification (e.g., higher magnification examination of appendages is required for resolving most genera and species morphologically) to ensure reasonably intact specimens and sufficient tissues were available for DNA extraction. Identification to low taxonomic levels (genus or species) proved challenging because most specimens were very small (e.g., some adult amphipods were <2 mm long; many adult copepods were <0.5 mm long), morphologically cryptic and because existing guides for identifying New Zealand's stygofauna were inadequate.

2.4.2 Genetic analyses (amphipods, isopods, copepods)

Individual amphipods, isopods and copepods were placed in separate, labelled glass vials filled with 100% ethanol prior to DNA extraction. CO1 nucleotide sequences for all specimens were prepared by

Guelph University, (Canada), following specimen cataloguing, photographic cataloguing, digestion and extraction by the University of Waikato. Post extraction, residual cuticular material for each specimen was returned to its labelled vial and deposited with the NIWA Invertebrate Collection (NIC; National Institute of Water and Atmospheric Research Ltd, Wellington) as museum vouchers available for further morphological study, as funds allow.

3 Summary of data

3.1 Environmental data

Well location and elevation, and date, time and method of sampling were recorded for each bore. Several environmental variables were collected for most wells (Table 3-1). Spot measurements of conductivity, water temperature, dissolved oxygen and pH were not available for eight wells due to meter failures. Dissolved organic carbon measurements were available for 47 of the 65 wells, and nutrient samples from 48 wells were analysed for DRP, NO_2-N, NO_3-N, NH_4-N, TDN and TDP. Water quality data were not available for 17 wells because some were sampled for other purposes, or because samples were damaged (bottles broke; freezer failures, lost in transit). Additional water samples for c. 20 wells were analysed for alkalinity, chloride, dissolved calcium, magnesium and sodium (results not shown here).

Bore attributes (e.g., diameter, available for all except one bore), casing material (all except four bores), screen depth and screen type (all except five bores) were extracted from council records (where available) or recorded in the field. Well depth was measured in the field, although council records were used (18 wells) where field measurements were not possible (i.e., depth probe too short or impeded by machinery). This combined approach resulted in well depths for all but three wells.

Each bore was linked to a surface water catchment and one REC river reach using best judgement (i.e., priority was given to nearby large river segments). Key parameters for each river reach were extracted from the REC and applied to the relevant well (see Section 2.3 and Table 3-1) as indicators of factors potentially affecting conditions and GE health within the aquifer at each well.

Available indicators of potential human impacts on GEs included the proportion of the upstream assigned surface water catchment in pasture, spot nitrate, DRP, DOC, TDN and TDP concentrations and conductivity. We note that surface waters within a catchment probably do not share a common geochemistry with their underlying groundwaters because of differences in their hydrologies (notably sources, rates of flow) and exposure to biogeochemical agents (e.g., bedrock, microbes, photosynthesis, land-surface activities, etc.).

3.2 Total stygofauna

Groundwater invertebrates were collected from 51 of the 65 wells (Figure 3-1). Two of the most widespread taxonomic groups were copepods (42 wells) and amphipods (29 wells). Twenty-five taxa across a range of taxonomic levels were distinguished morphologically. These morphological identifications were mostly relatively coarse (i.e., generally family or higher; identifications of only six genera considered robust) due to very poor taxonomic knowledge of this fauna, the inadequate identification tools and the morphologically-cryptic nature of this fauna. Better resolved identifications by specialist taxonomists were impractical at this time.

3.3 Amphipods, isopods, copepods (genetic sequencing)

Sequencing of the CO1 gene was attempted for 368 individual specimens. Of these, 211 returned useable sequences. Unique genetic sequences (haplotypes) were assigned a Barcode Index Number (BIN) by the Bar Code of Life Project using the algorithm developed by (Ratnasingham and Hebert 2013). Clusters of BINs generated using BOLD's BIN System algorithms "show high concordance with species" across "a broad set of taxonomic groups" (Milton et al. 2013): 10). Sequencing was successful for all 139 amphipods collected from 26 wells, 45 copepods from seven wells, and 27 isopods from 11 wells (Figure

3-2). Fifty-eight BINs were identified (all with > 90% bootstrap support), 36 of which were amphipod taxa, 13 copepods and 9 isopods.

Table 3-1: Summary of environmental data available for the 65 sampled bores. For categorical variables, the number of sites in each category is listed in the median column.

Parameter	Description	Bores with missing data (%)	Median	Range
Region	Region that the bores were located in	0	See Table 2-1 for bore regions	
Catchment	Bores were assigned to surface water catchment names	0	See Table 2-1 for bore catchments	
Latitude	Latitudinal location of the bore	0		
Longitude	Longitudinal location of the bore	0		
Bore depth (m)	Depth of bore in metres	0	9.15	2.73 - 38.7
Water column depth (m)	Depth of water within the bore	2 (3%)	6.4	0.8 – 37.9
Bore diameter (mm)	Diameter of the bore	1 (1.5%)	142.5	51 – 1200
Casing material	Material of the bore casing	4 (6%)	Steel: 34 bores PVC: 16 bores Concrete: 11 bores	NA
Conductivity (µS/cm)	Specific conductance spot measurement	7 (11%)	138	1.2 - 1014
Water temperature (°C)	Water temperature spot measurement	7 (11%)	13.2	9 – 15.3
pH	Scale of water acidity or basicity (0-14) spot measurement	7 (11%)	6.8	5.3 – 11.8
Dissolved oxygen (ppM)	Amount of dissolved oxygen spot measurement	7 (11%)	4.53	0.4 – 8.6
DOC (g/m^3)	Dissolved organic carbon spot measurement	15 (24%)	2.1	0.2 – 23.6
DRP (mg/ m^3)	Dissolved reactive phosphorus spot measurement	14 (23%)	0.00298	0.001 – 0.055
NH4-N (mg/ m^3)	Ammoniacal nitrogen spot measurement	14 (23%)	11.5	2.0 – 774
NO3-N (mg/ m^3)	Nitrate nitrogen spot measurement	14 (23%)	1900	1.0 – 11000
TDN (mg/ m^3)	Total dissolved nitrogen spot measurement	14 (23%)	2.035	0.023 – 10.9
TDP (mg/ m^3)	Total dissolved phosphorus spot measurement	14 (23%)	0.003	0.001 – 0.06

Parameter	Description	Bores with missing data (%)	Median	Range
Climate	REC climate class (Snelder et al. 2010a), Snelder et al. (2010b)	0	Cool-wet: 30 Cool-dry: 29 Warm-dry: 6	
Source-of-flow	REC source of flow class (Snelder et al. 2010a), Snelder et al. (2010b)	0	Mountain: 1 Hill: 23 Lowland: 41	
Geology	REC geology class (Snelder et al. 2010a)	0	Hard sedimentary: 7 Soft sedimentary: 19 Alluvial: 39	
usAlluvium	Proportion of the assigned surface catchment area occupied by alluvium land resource inventory	0	0.38	0 – 1
usPhos	Mean assigned surface catchment phosphorous content of regolith	0	3	1.1 – 4.0
PropPasture	Proportion of assigned surface catchment in pasture landcover classes from LCDB3	0	0.63	0.14 – 1
PropUrban	Proportion of assigned surface catchment in urban landcover classes from LCDB3	0	0.001	0 – 0.48
PropExotic	Proportion of assigned surface catchment in exotic landcover classes from LCDB3	0	0.11	0 – 0.59
PropNatural	Proportion of assigned surface catchment in natural landcover classes from LCDB3	0	0.13	0 – 0.66

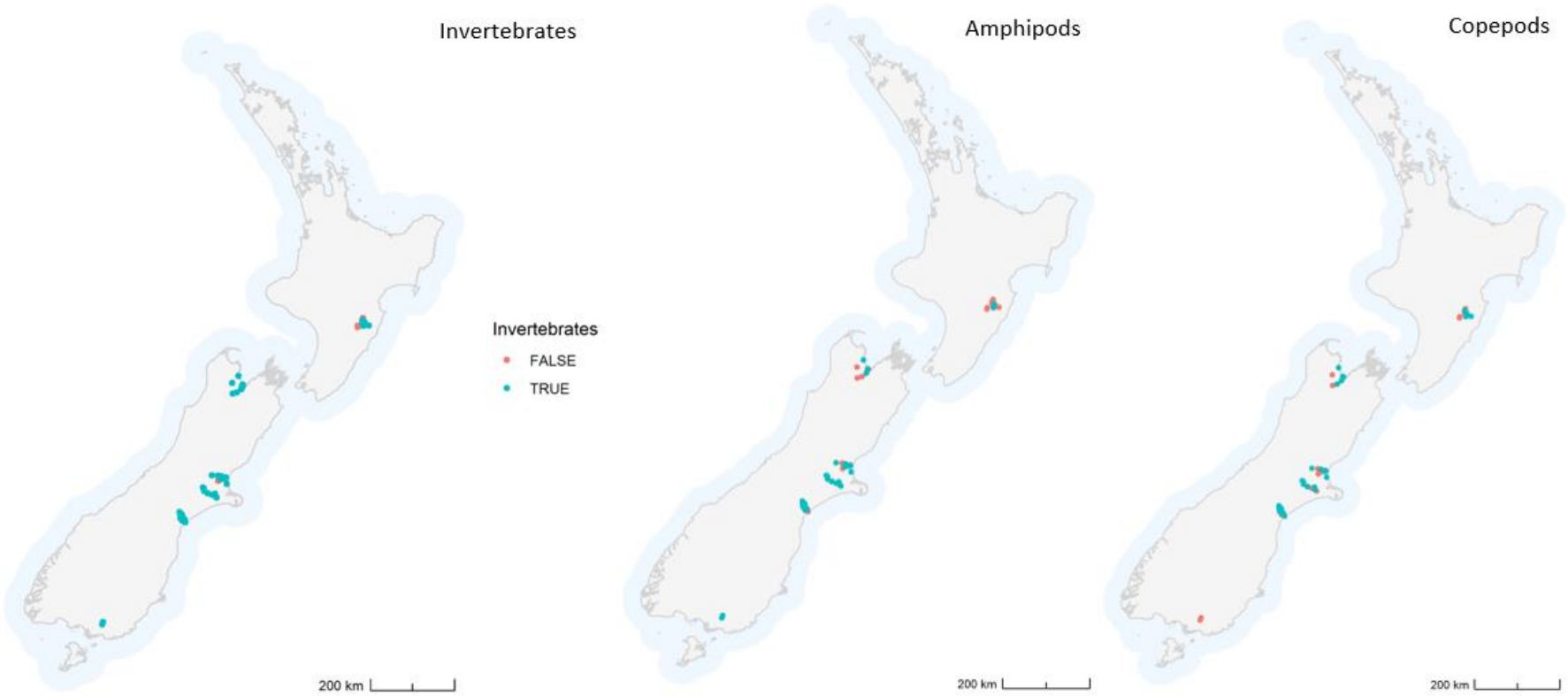

Figure 3-1: Location of 65 sampled wells colour coded by taxa presence/absence. Wells where invertebrates were present are blue. Red indicates wells where no invertebrates were collected. Maps of taxa presence are shown for all invertebrates, amphipods and copepods.

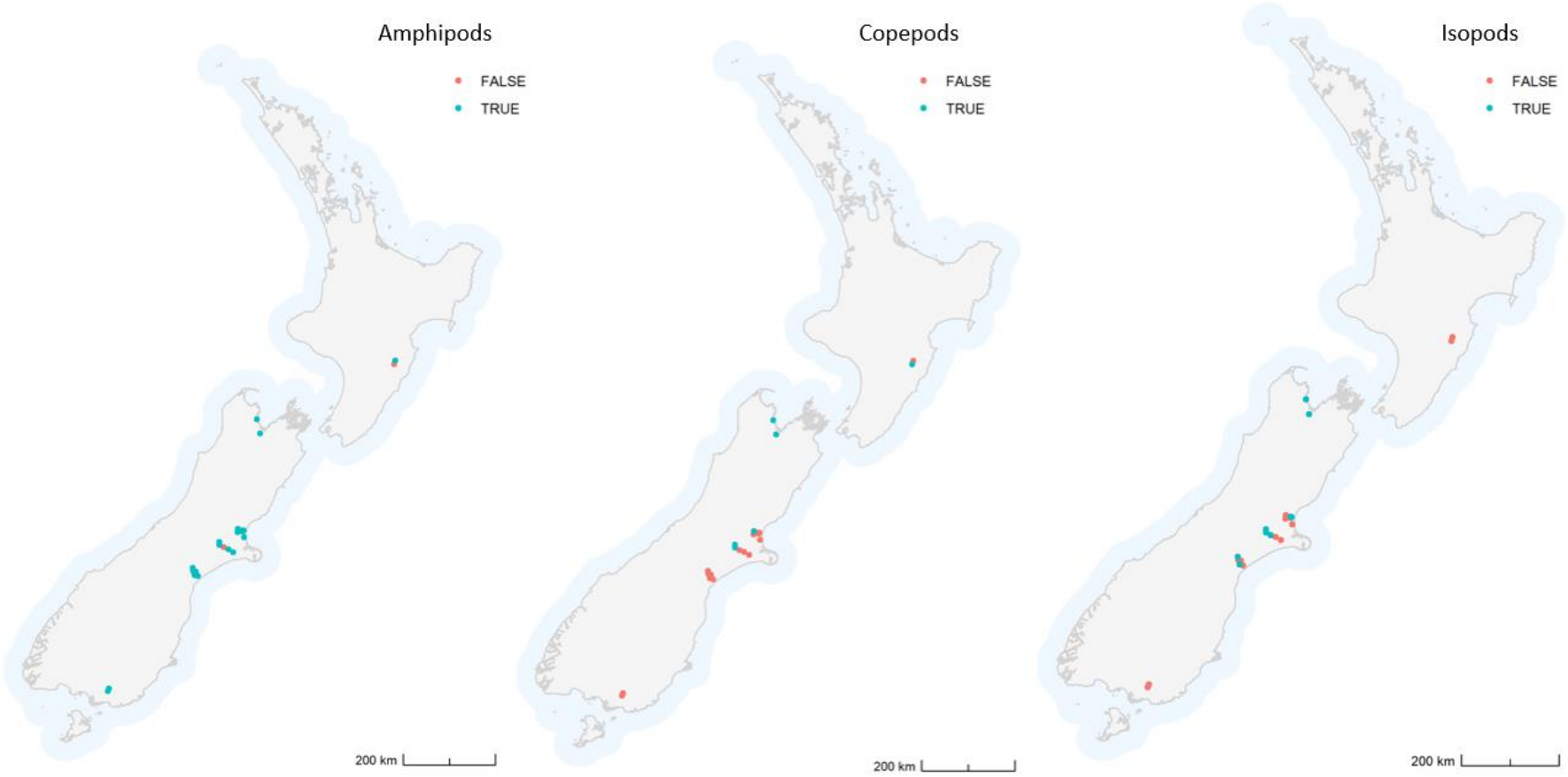

Figure 3-2: Locations where genetic data for amphipods, copepods and isopods were available. Blue indicates at least one individual was successfully sequenced. Red indicates either the taxon was not present, was not sent for sequencing or was not successfully sequenced.

4 Results and statistical analyses

Details of statistical analyses are included within each section below.

4.1 Environmental parameters: regional and catchment differences

Several environmental parameters were significantly correlated with others (Table 4-1). For example, pairwise Pearson correlations indicated that groundwaters with warmer temperatures tended to have higher conductivities and lower pH (Table 4-1). Likewise, locations further north (lower latitudes) tended to have more upstream alluvium, a higher proportion of pastoral landcover, a lower proportion of exotic landcover and rock upstream within the catchment contained more phosphorus (Table 4-1).

Table 4-1: Correlations between environmental parameters across the 65 sampled bores. Refer to Table 3-1 for parameter descriptions. Significant ($p < 0.001$) pairwise Pearson correlations only shown, with direction (positive, +; negative, -). Data transformed, where necessary, to ensure normality and homoscedasticity for correlation and further statistical analyses.

Parameter	Transformation for analysis	No. bores missing data	Correlated with:
Catchment		0	
Region		0	
Conductivity (µS/cm)		7	Temperature (+)
Water temperature (°C)		7	Conductivity (+) pH (-)
pH		7	Temperature (+)
Dissolved oxygen (ppM)		7	
DOC (g/m^3)		12	
DRP (mg/ m^3)	Log_{10}	11	
NO3-N (mg/ m^3)	Log_{10}	11	
Bore diameter (mm)		3	
Well depth (m)		3	
Climate		0	
Source-of-flow		0	
usAlluvium		0	Latitude (-) usPhos (+)
usParticle		0	

Parameter	Transformation for analysis	No. bores missing data	Correlated with:
PropPasture		0	PropExotic (-) usPhos (+) Latitude (-) usParticle (-) usAlluvium (+)
PropExotic		0	usPhos (-) Latitude (+) PropPasture (-)
usPhos		0	Latitude (-) PropPasture (+) PropExotic (-) Alluvium (+)
Latitude		0	Alluvium (-) propExotic (+) Phosphorus (-) PropPasture (-)

Upstream pasture cover within a well's assigned surface water catchment (PropPasture) was higher in Canterbury, Southland and Hawkes Bay regions than in Nelson (Figure 4-1, one-way ANOVA: $F_{3,58}$ =15.4, p <0.001; Tukey HSD post hoc tests). The two Nelson catchments (Waimea, WM and Mouteka, MT) had less upstream pastoral land cover than catchments in the other regions (Figure 4-1, one-way ANOVA: $F_{6,55}$ =9.9, p <0.001).

Spot water temperatures were higher in the Nelson region than in Canterbury (Figure 4-2, one-way ANOVA: $F_{2,52}$ =7.8, p = 0.001). At the catchment scale, Canterbury's Selwyn (SL) was cooler than the nearby Ashley catchment (AS) and the Hawkes Bay and Nelson catchments (Figure 4-2, one-way ANOVA: $F_{5,49}$ =6.6, p <0.001).

While pH did not differ significantly between regions, it was significantly lower in the Selwyn catchment (SL) bores than in wells in any other catchment (Figure 4-3, one-way ANOVA: $F_{5,49}$ =19.9, p < 0.001).

Well diameters were larger in Nelson than Canterbury and Hawkes Bay (Figure 4-4, one-way ANOVA: $F_{3,55}$ =12.5, p < 0.001). They were significantly larger also in Nelson's Waimea catchment (WM) than all other catchments, except in Motueka (MT; the second Nelson catchment) (Figure 4-4, one-way ANOVA: $F_{6,52}$ =6.6, p < 0.001).

The proportion of the upstream catchment with alluvium was lower in Nelson and Southland than in Canterbury (Figure 4-5, one-way ANOVA: $F_{3,58}$ =10.0, p < 0.001). Upstream alluvium was lower in the Motueka (MT) than in any of the three Canterbury catchments (AS, OR, SL; Figure 4-5, one-way ANOVA: $F_{6,55}$ =7.2, p < 0.001).

The upstream regolith phosphorus concentration was lower in Nelson and Hawkes Bay than in Canterbury and Southland regions (Figure 4-6, one-way ANOVA: $F_{3,58}$ =77.4, p < 0.001). At the catchment scale, upstream phosphorus was lower in the Motueka catchment (MT) than in the three

Canterbury catchments (AS, OR, SL) and the Southland catchment (MA); (Figure 4-6, one-way ANOVA: $F_{6,55}$ =42.7., p < 0.001). The upstream regolith phosphorus in the Orari (OR) catchment was lower than that in all regions, except the Selwyn catchment (SL).

Upstream particle size was smaller in Southland and Hawkes Bay regions than in Nelson (Figure 4-7, one-way ANOVA: $F_{3,58}$ =9.9, p < 0.001). At the catchment scale, upstream particle size was larger in the Motueka catchment (MT) than in other catchments, and particle size was smaller in the Mataura catchment (MT)than any of the other catchments, except for Hawkes Bay (TU; Figure 4-7, one-way ANOVA: $F_{6,55}$ =6.6, p < 0.001).

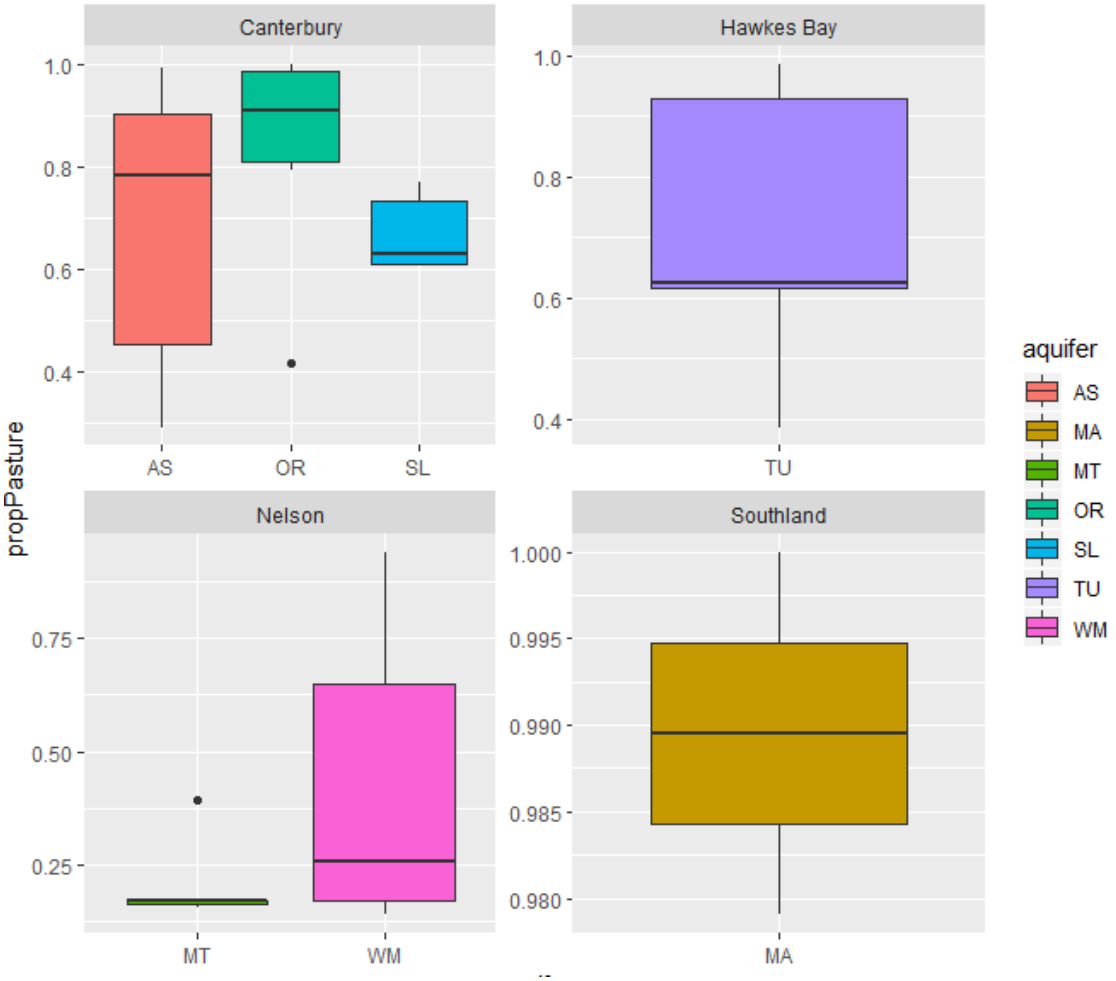

Figure 4-1: Box and whisker plots of the proportion of pasture upstream for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura. Horizontal line: median; box: 25th and 75th percentiles, whisker: extends to largest value no further than 1.5 times the distance between 25th and 75th percentiles; solid points, outliers.

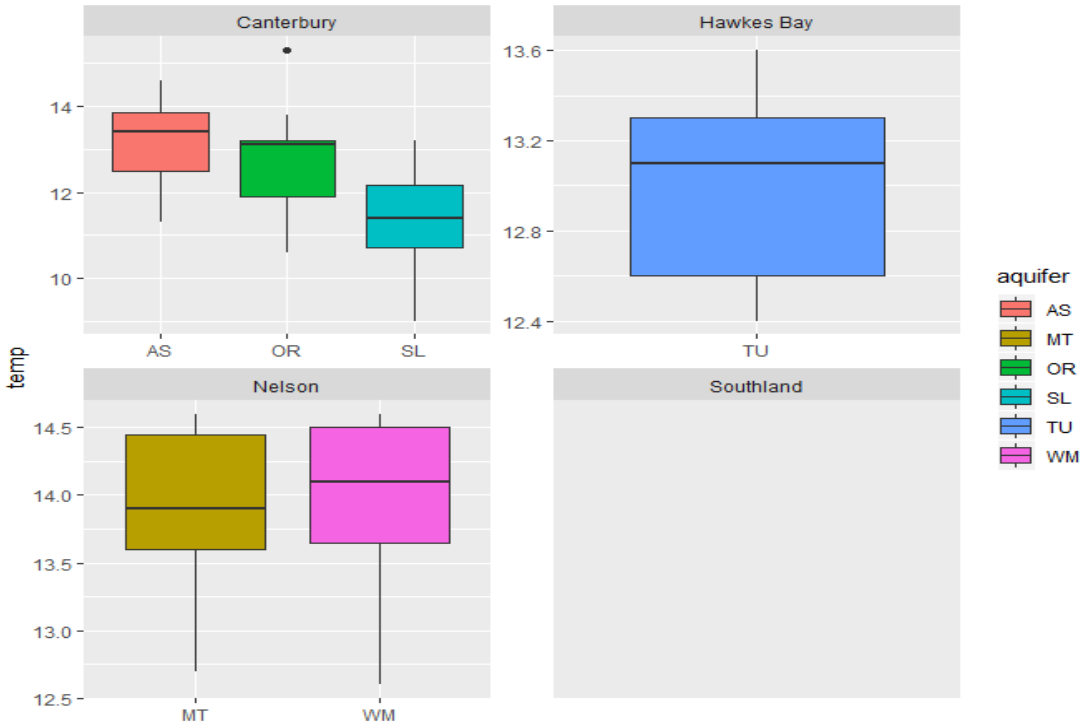

Figure 4-2: Box and whisker plots of the groundwater spot water temperatures (°C) for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura. No data were available for Southland.

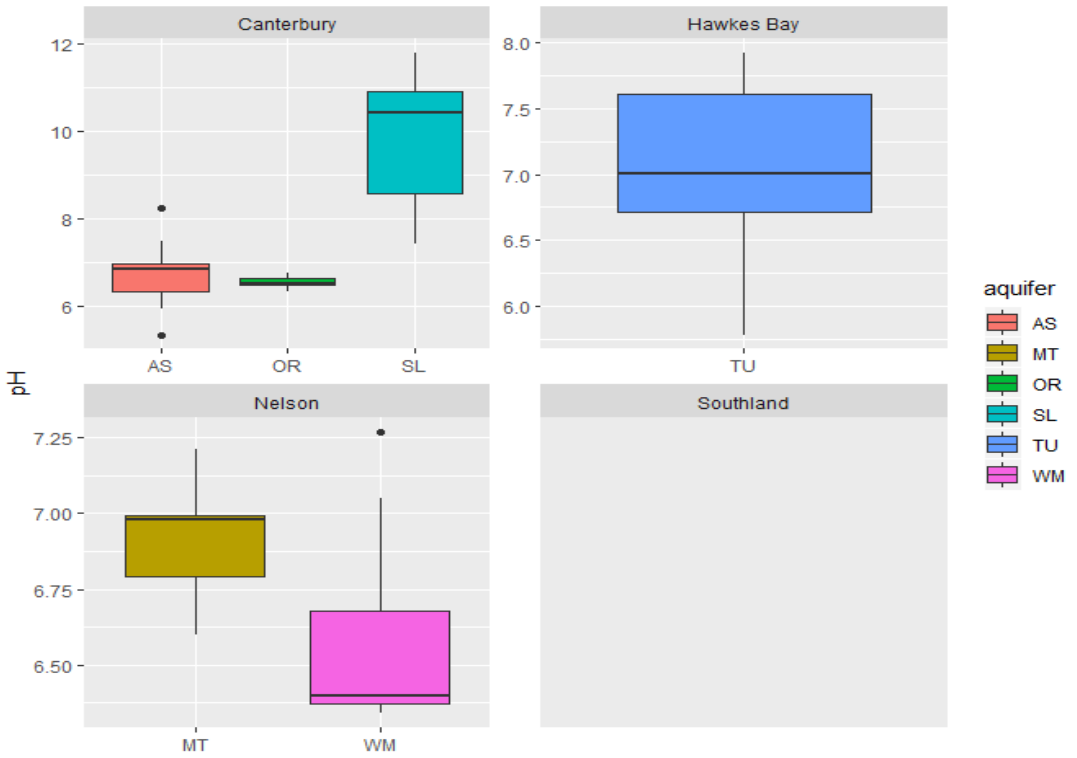

Figure 4-3: Box and whisker plots of the groundwater pH for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura. No data were available for Southland.

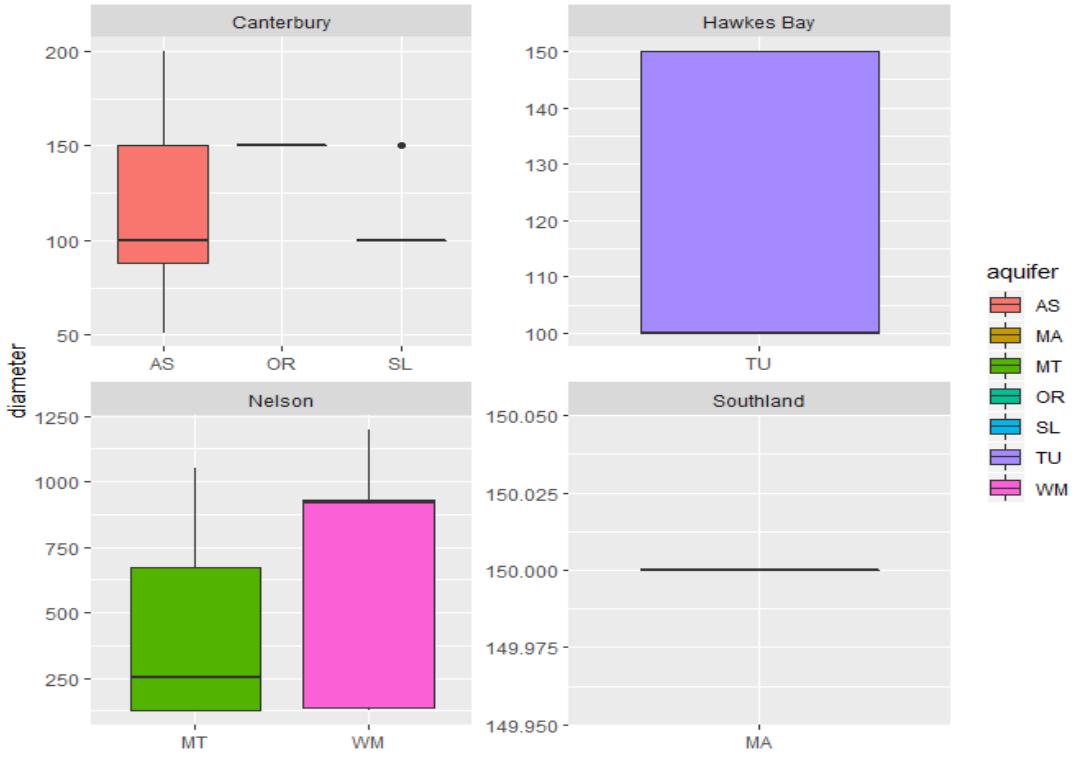

Figure 4-4: Box and whisker plots of the bore diameter (mm) for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura.

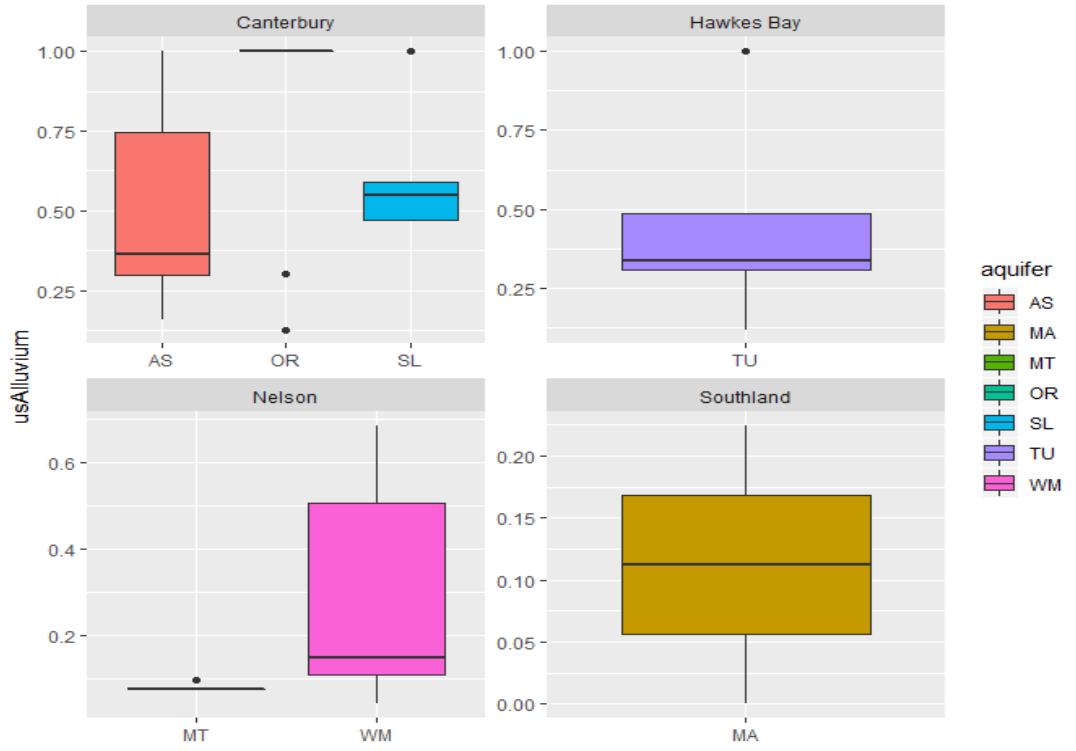

Figure 4-5: Box and whisker plots of the proportion of upstream alluvial land for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura.

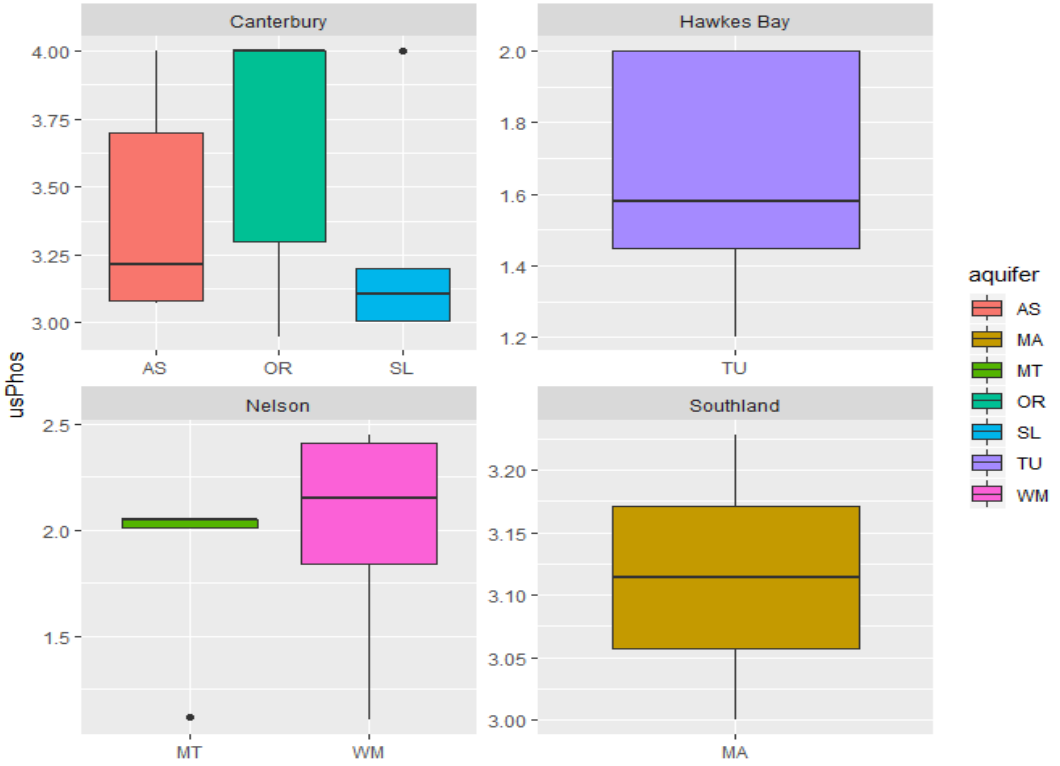

Figure 4-6: Box and whisker plots of upstream regolith phosphorus content for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura.

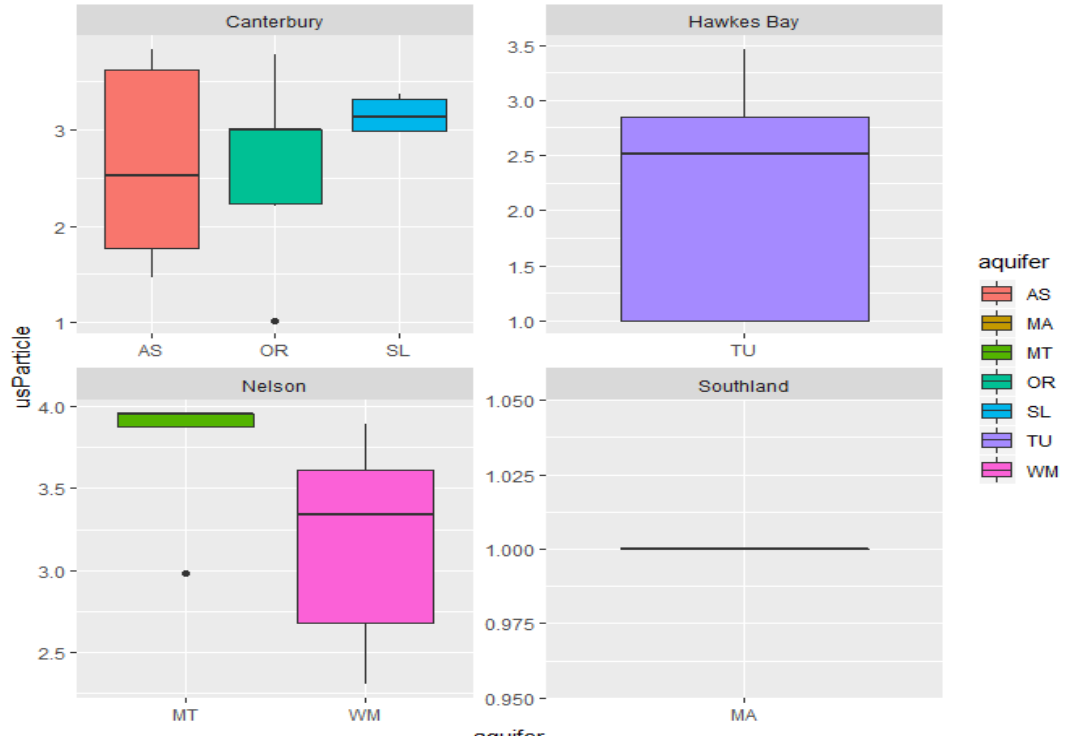

Figure 4-7: Box and whisker plots of the upstream particle size for wells in each aquifer (surface water catchment) and region. AS = Ashley, OR = Orari, SL = Selwyn, TU = Tukituki, MT = Motueka, WM= Waimea, MA = Mataura.

4.2 Stygofaunal community composition

Fifty-one of the 65 sampled bores contained groundwater invertebrates. Twenty-five taxa were distinguished morphologically with reasonable certainty. Six taxa were identified to genus, five taxa to family, four taxa to order and the rest to sub-class or higher taxonomic levels (class or phylum).

Fifteen wells contained only one taxon. The highest taxonomic richness per well was 14 taxa. Five taxa were found in Nelson and Southland/Canterbury but not in Hawkes Bay. No taxa were shared between Hawkes Bay and Southland/Canterbury or Nelson, but not found in the other South Island region (Figure 4-8).

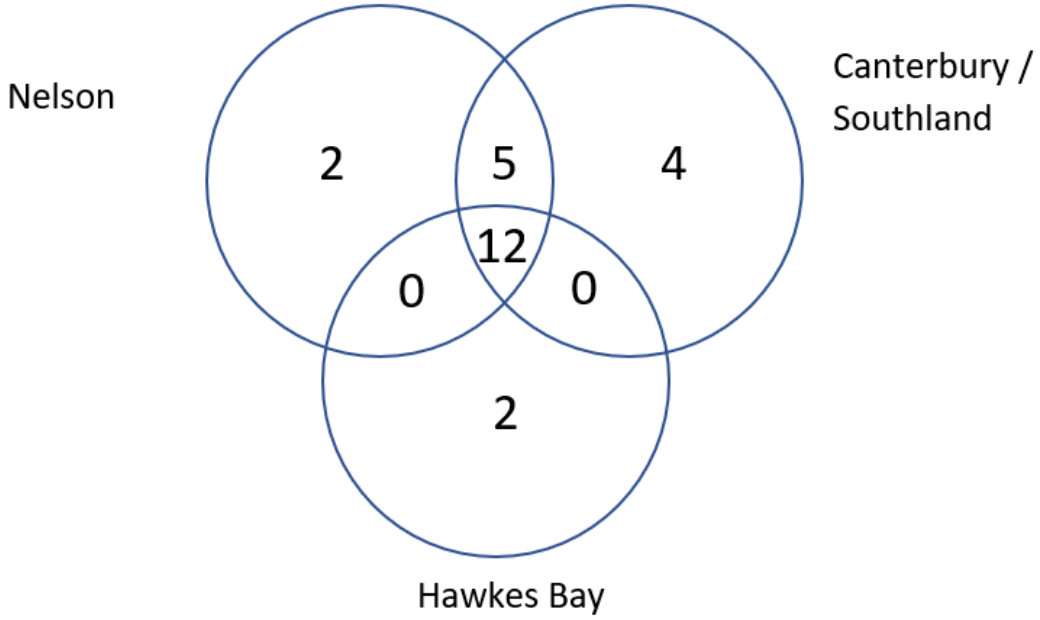

Figure 4-8: Venn diagram showing the number of morphologically identified taxa that were common to single (2, 4), pairs of (0, 5) and all (12) regions. All taxa shared by Nelson and Hawkes Bay were also found in Canterbury. Likewise, all taxa found in Canterbury/Southland and Hawkes Bay occurred in Nelson.

The most common taxonomic groups were Acarina (present in 39 bores), cyclopoid copepods (36 bores), harpacticoid copepods (24 bores) and amphipods belonging to the Family Paraleptamphopidae.

4.3 Environmental effects on community composition

Pairwise Spearman correlations were used to investigate the relationship between taxonomic composition (taxa richness, total abundance, number of amphipod individuals, number of copepod individuals, and the commonly found amphipod Family Paraleptamphopidae). Although Acarina were common, they were not included in the analyses because specimens of non-aquatic taxa often contaminated samples (detailed examination required to identify these) and were considered unlikely to provide useful information at the level of taxonomic resolution achieved here. Wells that contained more individuals tended to contain more taxa, especially of the common groups (amphipods, copepods and Paraleptamphopidae; Figure 4-9).

Taxa richness was used as an indicator of these changes in community composition and Spearman correlations were used to investigate relationships between this parameter and continuous environmental parameters, including some of the potential indicators of human impacts (e.g., up-stream pasture, spot nitrate, DRP, TDN and TDP concentrations and conductivity). One-way ANOVAs were used to test the significance of differences in taxa richness with region, catchment and climate variables.

More taxa (and often more individuals; Figure 4-9) were found in wells with higher dissolved oxygen ($R = 0.50$, $p < 0.001$), and cooler temperatures ($R = -0.36$, $p = 0.007$). Fewer taxa were found in deeper wells ($R = -0.29$, $p = 0.02$). There were no significant correlations of taxa richness with any of the other environmental parameters listed in Table 3-1.

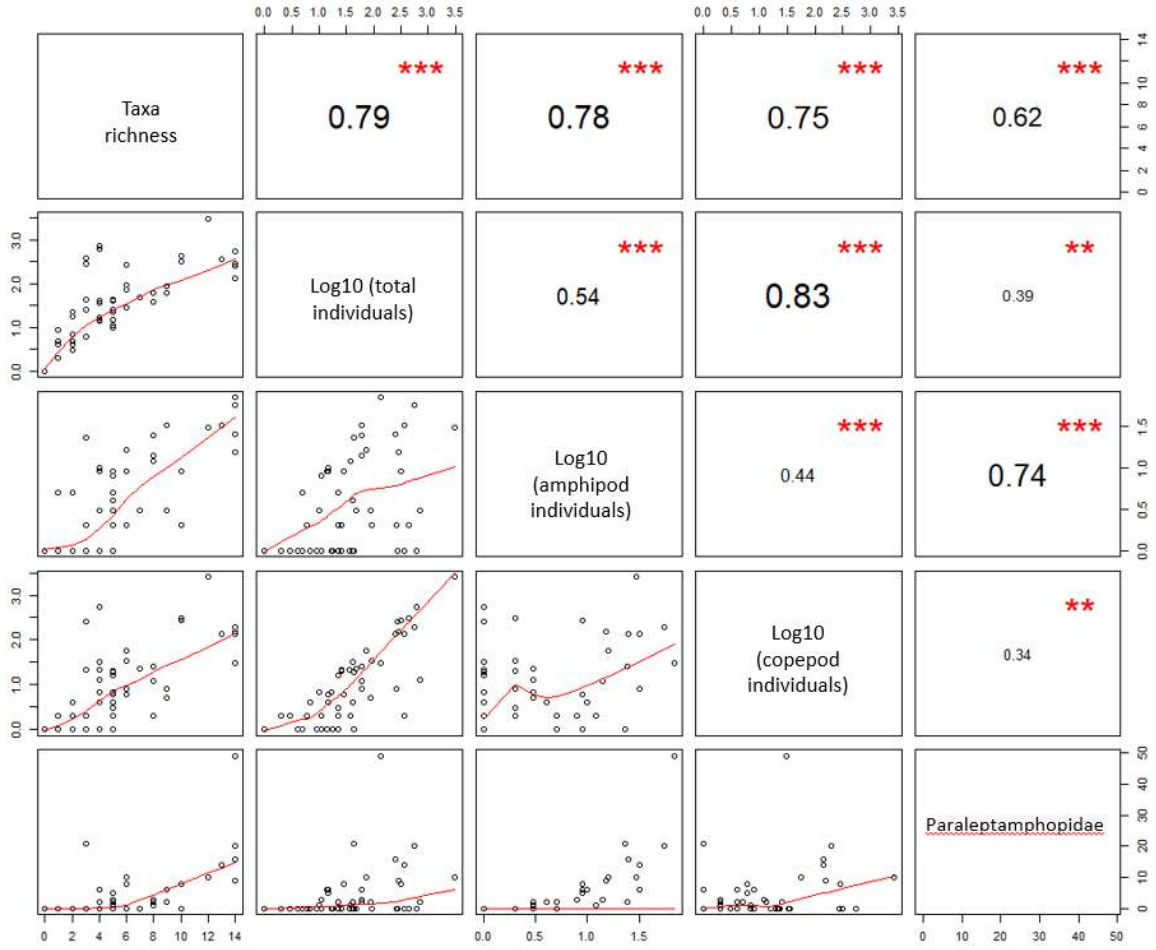

Figure 4-9: Pairwise correlation matrix of invertebrate richness and abundance for all stygofauna, amphipods, copepods, paraleptamphopid amphipods. The top panels show the pairwise Pearson correlation coefficients. Asterisks indicate probability (p) values: *** <0.001, ** <0.001, * <0.01.

Fewer taxa were found in deeper wells ($R = -0.29$, $p = 0.02$). There were no significant correlations between taxa richness and any of the environmental parameters (Table 3-1).

We used non-metric multidimensional scaling (unconstrained NMDS, Bray-Curtis rank differences similarity using Wisconsin square root transformed abundances) ordination was used to investigate

patterns in community composition[4] between the bores. The NMDS of community composition in the 51 wells had a stress level of 0.17, corresponding to an acceptable level (<0.20; (Lefcheck 2012)).

Ordination of wells based on their stygofaunal communities (Figure 4-10) showed differences in communities within each catchment (except Mataura where only two wells were sampled). With the exception of the Mataura wells, communities strongly overlapped between catchments on both dimensions, indicating that stygofaunal communities may differ as much or more within catchments, as between catchments.

Differences in community compositions between regions and catchments, and correlations of community composition with environmental parameters in Table 3-1 were tested using the Vegan R package's ENVFIT function with 999 permutations. Environmental parameters were overlaid on the community in two different analyses:

1. All environmental parameters in Table 3-1 (n = 36 bores, some missing because all environmental data were not available for all bores).

Community composition was correlated significantly with conductivity (R^2 = 0.22, p = 0.01), dissolved oxygen (R^2 = 0.16, p = 0.05), usPhos (R^2 = 0.24, p = 0.01) and latitude (R^2 = 0.24, p = 0.01). There were no significant correlations between community composition and any laboratory-measured dissolved nutrient concentration (DRP, NO_3-N and DOC) or other environmental parameters. These correlations were also detected in the second analysis below.

2. Environmental parameters excluding dissolved nutrients (n = 42 wells; nutrient concentrations missing for up to 12 wells).

Stygofaunal community compositions within wells were significantly correlated with ten environmental variables (wells with missing dissolved nutrient data excluded analysis by analysis). These ten variables included conductivity (R^2 = 0.18, p = 0.03), dissolved oxygen (R^2 = 0.16, p = 0.04), well diameter (R^2 = 0.18, p = 0.04), usAlluvium (R^2 = 0.15, p = 0.04), usParticle (R^2 = 0.16, p = 0.05), propPasture (R^2 = 0.20, p = 0.01), usPhos (R^2 = 0.25, p = 0.006) and latitude (R^2 = 0.28, p = 0.002; Figure 4-11). The other two variables significantly correlated with community composition were region (R^2 = 0.12, p = 0.03; Figure 4-10 and Figure 4-11) and source of catchment surface-water flow (i.e., Hill-fed, Lowland or Mountain; R^2 = 0.08, p = 0.05).

Overlaying these significant correlations on the NMDS ordination of well stygofaunas (Figure 4-11) indicated a complex gradient from top left to bottom right within ordination space that involves six variables: (latitude, proportion of upstream catchment in pasture, phosphorus content of upstream regolith, proportion of upstream catchment as alluvium, upstream particle size, well diameter). These variables are variously correlated with each other, so are indicative, rather than defining specific environmental conditions. The remaining two environmental variables, dissolved oxygen concentration and conductivity, were not correlated, but appear to have opposing effects on stygofaunal communities in this study (Figure 4-11).

A further NMDS located stygofaunal taxa in ordination space near sites of their highest abundance (Figure 4-12). Most taxa appear somewhat aligned along the top left to lower right diagonal. These include two poorly resolved taxa (i.e., taxonomic identification was poor due to very incomplete knowledge of them: Amphipoda indet., Paraleptamphopidae) that were grouped tightly with two

[4] Identifications used here are very coarse and may mask some differences in actual community compositions (i.e., community compositions may be apparent only when genus or species level identifications are available).

other taxa (Phreatogammaridae, Cruregens spp.) near the centre of the NMDS. Another poorly resolved taxon (Paraleptamphopus spp.) was separated from these, but was located along the same diagonal. Six environmental variables are associated with this diagonal (latitude, proportion of upstream catchment in pasture, phosphorus content of upstream regolith, proportion of upstream catchment as alluvium, upstream particle size, well diameter). Other taxa likely to include multiple species (e.g., Ostracoda, Nematoda, Acarina) were located more distant from this diagonal, suggesting either that their taxonomic grouping masked any dominant environmental effect, or that their abundance was influenced by a different factor or combination of factors.

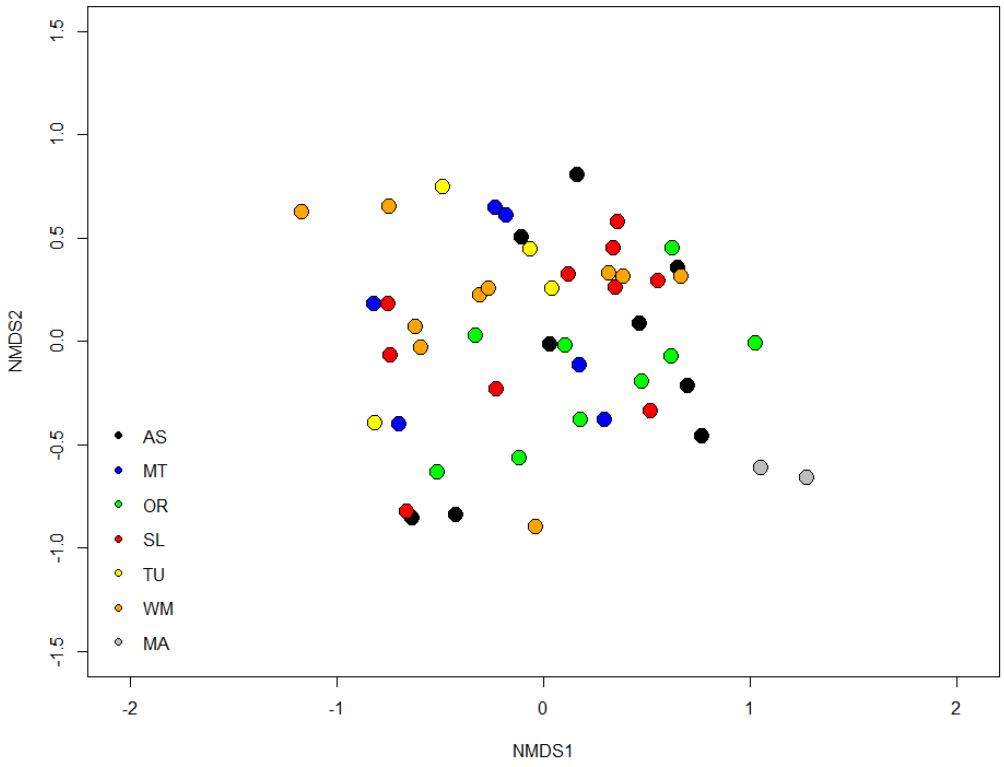

Figure 4-10: Non-metric multi-dimensional scaling ordination of stygofauna communities in wells (colour coded by catchment: TU, Tukituki; MT, Motueka; WM, Waimea; AS, Ashley; SL, Selwyn; OR, Orari; MA, Mataura). Points closer together have more similar stygofaunal communities than points further apart.

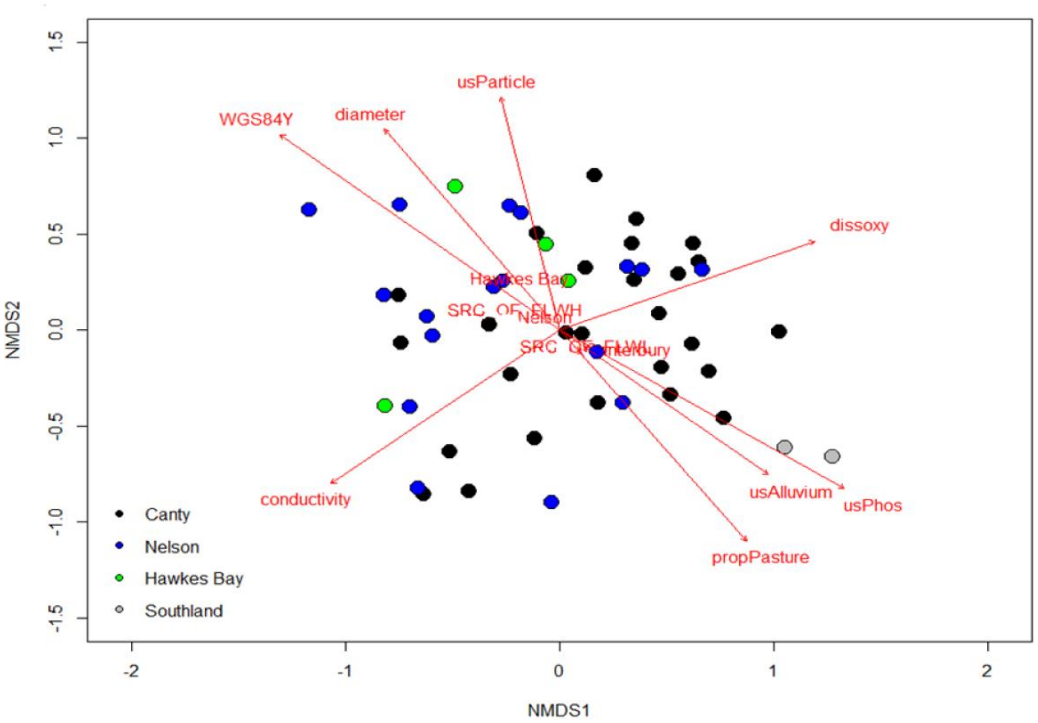

Figure 4-11: Non-metric multi-dimensional scaling ordination of stygofauna communities in wells (coloured by region) and significantly ($p < 0.05$) correlated environmental parameters (arrow directions show greatest change, length indicate correlation strength). WGS84Y: latitude.

Aggregation of the more resolved taxa along the ordination diagonal, compared with the more peripheral location of less resolved taxa, indicated that stygofaunal community composition differed between regions and is associated with gradients in environmental parameters, such as the local water chemistry (e.g., dissolved oxygen), well characteristics (well diameter), variables relating to the larger assigned surface water catchment (proportion alluvial material and natural phosphorus content in the rocks) and along a latitudinal gradient (latitude: WGS84Y).

The inter-correlations between many of these variables made disentangling environmental cause and ecological effect relationships difficult (see Section 5.1). For example, pastoral landcover in the upstream catchment was significantly related to differences in community composition between wells. However, this parameter was also correlated with latitude and catchment physical characteristics (e.g., upstream alluvium, upstream regolith phosphorus, etc.), as well as other variables. This makes attributing the change in community composition to land use type or other human activity difficult.

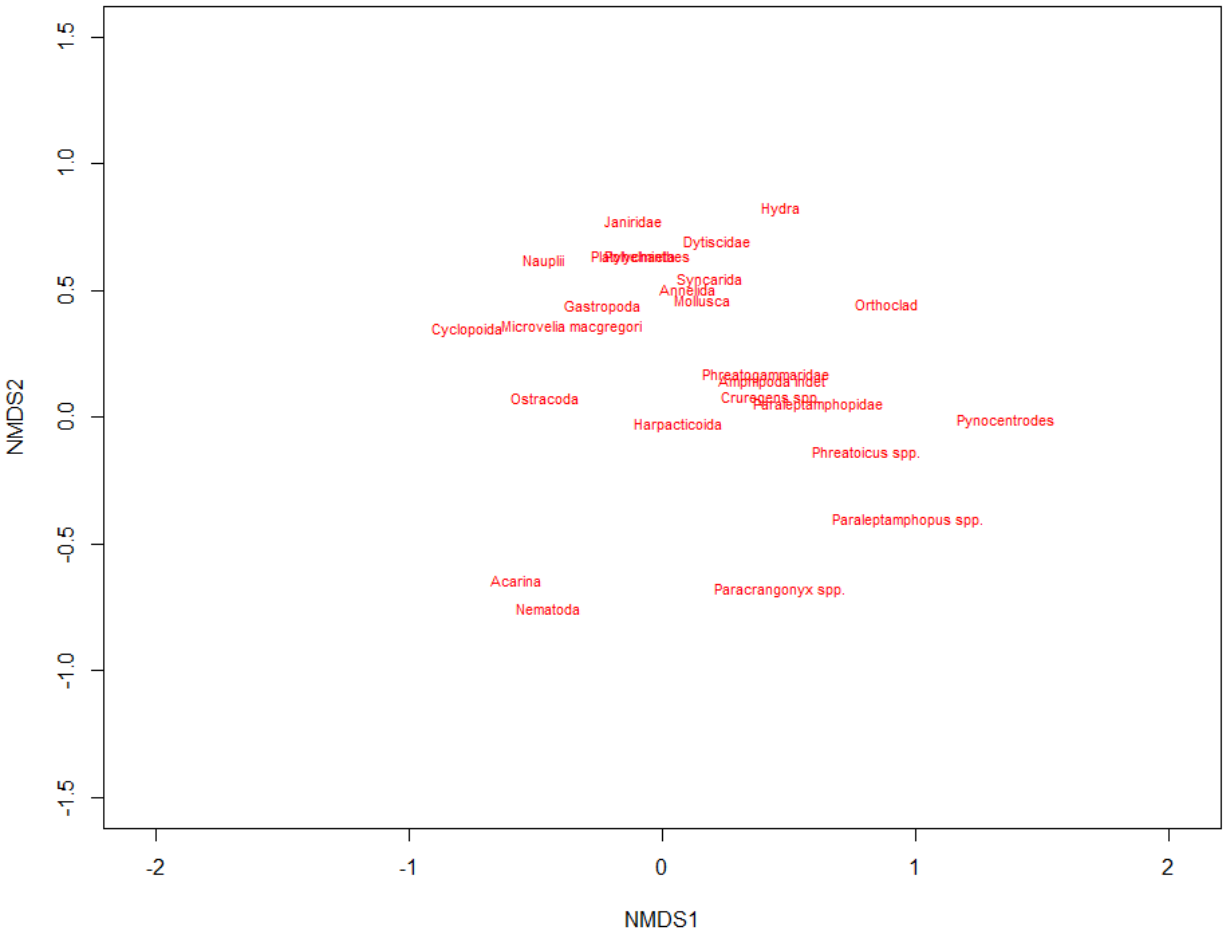

Figure 4-12: Non-metric multi-dimensional scaling ordination of the stygofauna for all wells, showing locations of taxa relative to each other. Locations of taxa indicate their highest abundances relative to the site locations present in Figures 4-10 and 4-11.

4.4 Amphipods, isopods, copepods: summary of genetic data

Genetic (CO1) data distinguished amphipod taxa across all four regions, isopods in two regions (Nelson, Canterbury) and copepods from three regions (Hawkes Bay, Nelson, Canterbury) (Figure 4-13). Of the total 59 taxa distinguished genetically, 24 taxa (41%) were found at only one well, and 46 (79%) were found at five or fewer locations. Similarly, there were 14 locations from which only one taxon was found, and 24 wells yielded five or fewer taxa. The highest taxa richness of (14) was found at just one location.

Trees or dendrograms using genetic data for amphipods (see Figure 4-14), isopods and copepods (not shown) reveal that most individual taxa were restricted to single catchments and even to individual wells or locations (Figure 4-14: specimens, coloured by catchment, are mostly clustered together within the tree). Taxa were common to different catchments only within the Canterbury Plains: three taxa were common to the Ashley and Selwyn rivers, and another two were shared between these and the Orari River (four of these shared records were single specimens, so require confirmation).

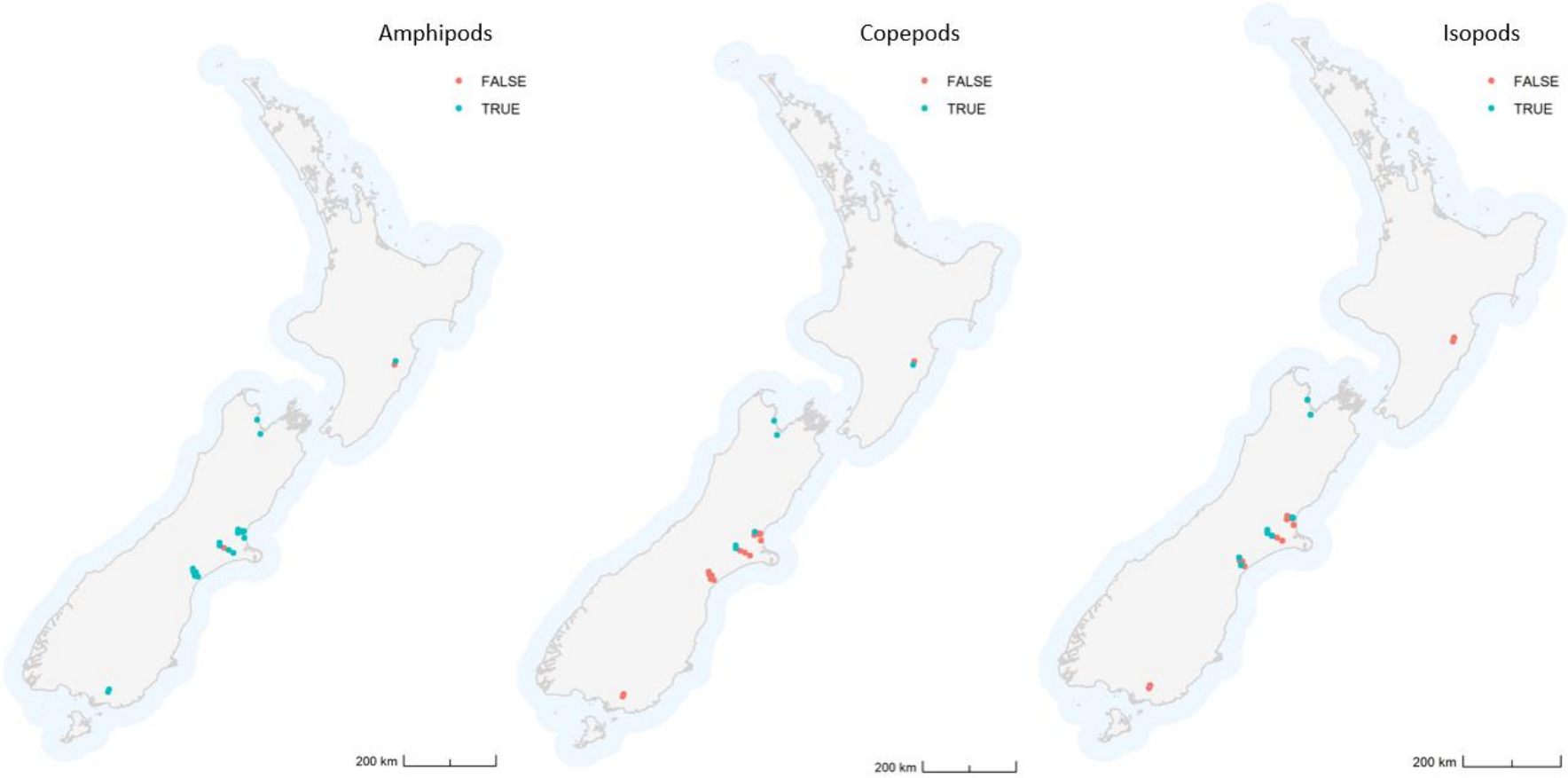

Figure 4-13: Locations where genetic data for amphipods, copepods and isopods were available. Blue indicates at least one individual was successfully sequenced. Red indicates either the taxon was not present, was not sent for sequencing or was not successfully sequenced.

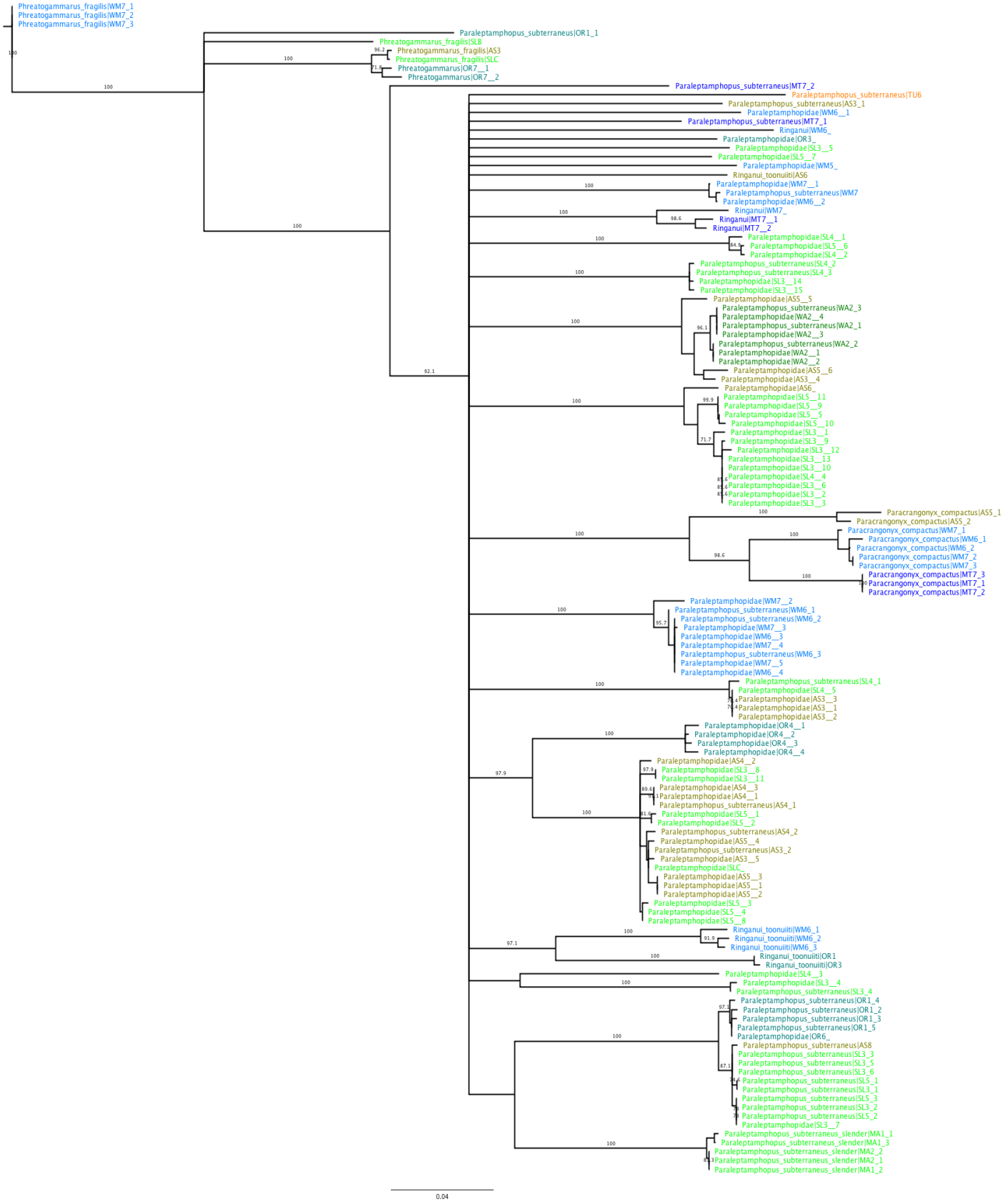

Figure 4-14: Example genetic tree showing sequenced amphipod individuals. Taxa coloured by catchment (except Mataura (MA) colour same as for Selwyn (SL)). Note, most branches comprise taxa from the same catchment (and often the same well).

5 Conclusions

The data from this study provide insights into the sampling and data needs for resolving and quantifying any effects of human activities on stygofaunal amphipods and isopods. Additional data are required for developing indices of GE health based on invertebrates because:

- Aquifers differed in physical and environmental characteristics both within and between regions and some environmental variables were correlated with others.
- Few of the wells sampled were located within relatively unimpacted environments, so that communities inhabiting unimpacted (reference or control) groundwaters could not be characterised. This problem is shared with surface water ecosystem health indices, because pristine lowland environments are rare or often do not exist in some regions.
- Our sampling obtained mostly small numbers of stygofauna, which were inadequate for robust statistical analyses of distribution patterns and detecting any potential land-use effects. Methods for sampling stygofaunas are very poorly developed, and repeat sampling appears essential for obtaining more reliable community composition data.

These factors made it impossible to disentangle potential cause and effect relationships between the land-use effects (i.e., potential ecosystem stressors, such as high nutrient concentrations) and stygofauna community composition with any certainty using data from this project. Much larger sample sizes (i.e., more wells) and more comprehensive samples of stygofauna from each well are essential for confirming and quantifying any such relationships. The observed differences in stygofaunal communities could be attributed to any one or more of 1) natural differences in physical conditions, 2) evolutionary change (genetic and/or morphological) due to geographic isolation, 3) inadequate sample sizes to adequately characterise community composition or 4) to the impacts of human stressors on groundwater ecosystems.

In consequence, we conclude that the two aims for the project required different sampling designs. Our sampling design prioritised understanding scales of biodiversity because we cannot use a fauna for monitoring purposes until we know its composition and diversity in some detail (i.e., we need to know what lives where so that we know what could be monitored in different places). Thus, the knowledge of stygofauna biodiversity obtained from this study is fundamentally important for designing sampling and research for developing any stygofauna-based indices for monitoring GE health.

The results did reveal strongly restricted distributions for stygofaunal amphipods, isopods and copepods (and probably most other groundwater crustaceans) in New Zealand, with most taxa apparently endemic to single aquifers or parts of aquifers. This restriction of taxa to single catchments (some possibly to regions) means that species of these invertebrates cannot be used for national or regional measures of GE health in the same ways that riverine species are used for surface water ecosystem health indices. This is because any species used or contributing to an indicators or measure of ecosystem health are relevant only within their geographic range. Conversely, any species or species-based measure of ecosystem health is useful only within the geographic range of those species.

Consequently, a single, New Zealand-wide index of GE health based on stygofauna currently seems unlikely, and region or aquifer-specific indices are likely to prove too costly to develop, validate and

implement. Stygofaunal community data identified (or aggregated) to higher taxonomic levels (i.e., genus or family rather than species) may prove more useful and will be explored as a follow-up to this project.

Developing an index of GE health based entirely on New Zealand's stygofauna seems premature for other reasons also. First, methods for collecting stygofauna are inadequate for consistently and reliably determining and monitoring community composition within an aquifer at any point. Stygobiology lacks the >150 years of methodological development available to most biological disciplines. Emerging developments in the use of eDNA hold promise for overcoming this issue.

Second, very few stygofaunal crustacean families, genera and species are known taxonomically (i.e., most are new to science), so that they lack names and, therefore, there are no adequate tools for reliably distinguishing them or consistently recognising them. The keys in Scarsbrook et al. (2003) appear very inadequate in light of this project's and unpublished, morphological taxonomic work. Resolving this issue is no simple matter. Most stygofaunal taxa are morphologically conservative, cryptic and frequently small; even experienced taxonomists find distinguishing many of the smaller, common taxa extremely difficult, even with time-consuming dissection and compound microscope examination of slide-mounted appendages. Developments in rapid genetic sequencing offer considerable promise for reducing or eliminating the need for morphological identifications, but their success depends on establishing a comprehensive library of DNA barcodes for re-recognising taxa.

Third, even with rapid, field-capable DNA barcode identifications, however, some form of classification or taxonomy assigning e identifiers or names to taxa appears essential because, without some type of name, we cannot discuss them. The conventional Linnaean taxonomic system could be used. An alternative system, such as BOLD's BINs, may also work, at least for assigning a name or number to resolved taxa. Presently, however, BINs lack a systematics component: BINs include no hierarchical phylogenetic content, so that consistent higher-level groupings (e.g., genera, families) are not readily accessible.

Fourth, the responses of any taxa used for assessing GE health to land-use (or other) effects that commonly impact GEs should be determined, ideally with dose-responses quantified for a range of conditions. As yet, there are few studies providing this type of information for any stygofauna globally (Fenwick et al. 2018), and none for any New Zealand stygofaunal species (Fenwick et al. 2018).

Thus, implementation of stygofauna-based measures of GE health seems challenging until improved methods for resolving stygofauna biodiversity and community composition are readily available. A GE health index based on a combination of abiotic and biotic variables has considerable merit (e.g., Korbel 2012, Korbel and Hose 2017), but we did not pursue such an approach here because resources were closely focussed on the investigation's primary objective of defining spatial and genetic scales of biodiversity.

6 Recommendations

A common criticism of invertebrate metrics is that they can be influenced by multiple natural and unnatural (i.e., land-use) factors that affect macroinvertebrate community composition (Boothroyd and Stark 2000). For example, the MCI was developed as an indicator of organic pollution, but it is also sensitive to changes in other river conditions, such as floods and extended periods of low flow (Boothroyd and Stark 2000), particularly in pristine waterways (Death et al. 2009). This response to multiple factors limits the diagnostic value of ecosystem health indices based on community composition (Chessman and McEvoy 1998). Ideally, stressor-specific metrics (e.g., Monk et al. 2006, Kairo et al. 2012) can be developed, but these require detailed experimental research results to quantify stressor-specific responses.

Despite this limitation, we believe that biodiversity-based indices do have merit for supporting groundwater management decisions. Currently, however, there is very little science directly applicable to developing an invertebrate-based MCI-equivalent for GEs in New Zealand. As a first step towards a GE health index, this BioHeritage Challenge project has revealed substantial local endemism within key crustacean groups, a factor that must be accommodated within any ecosystem health index developed for the whole country. Given this characteristic of New Zealand's stygofauna, the approach adopted by Korbel (2012), Korbel and Hose (2017) seems the most pragmatic option, at least in the medium-term. Although developed based on differences within a single very large catchment, these authors' groundwater health index (GHI) and weighted index (wGHI) require identifications at very high levels (crustaceans, oligochaetes, others) and putative assessments of stygoxenes from stygobites (using readily discernible morphological characteristics).

GE science lacks the body of exploratory biodiversity research, descriptive relationship investigation and quantitative, experimental cause-effect responses developed over decades that underpins most invertebrate ecosystem health indices used for addressing 21st century environmental management questions. Molecular approaches and other contemporary methods promise some alternative solutions, but substantial fundamental research focussed on facilitating research to address priority management issues appears essential. Thus, a dedicated, longer-term research programme aimed at building the key knowledge required to develop and implement a preliminary model-based, decision-support tool seems essential. The initial model will be preliminary, but capable of incremental refinement to address emerging GE management issues and approaches.

Genetic (eDNA) tools appear to be essential for building a robust GE health index based on stygofauna. Use of eDNA requires a comprehensive library of sequences accessible to all stakeholders (initiated during the current research project), determining eDNA decay rates within groundwaters, and establishing standard eDNA sampling, processing and analytical methods. The resultant data will better define endemism, biodiversity and community composition for different aquifer types and under natural to highly impacted conditions and will build on the results of this project. These data can then be interrogated to explore relationships between species, genera, families, communities, environmental factors and human impacts, and any cause-effect relationships can be established and quantified via experimentation. Ultimately, these results can be incorporated into the GE functioning model and a groundwater management decision support system for managing GEs and groundwater resources more sustainably.

Basic biological and ecological research of key species within each of the numerically and/or functionally dominant taxa will be required to underpin interpretations of changes in community compositions. Ecotoxicological research on the same or similar species is required for interpreting

and diagnosing GE changes and for guiding resource managers in setting appropriate limits for key attributes (e.g., water level change, DO, NO_3, DOC).

Alternative approaches to monitoring GE health, such as monitoring ecosystem functioning (e.g., organic material degradation), should also be evaluated. Such approaches may not detect changes in biodiversity that may have important consequences for ecosystem service delivery (e.g., loss of the ecosystem engineer, *Phreatoicus typicus*, may reduce bioturbation, leading to aquifer clogging and consequent reduced water quality (Boulton et al. 2008, Fenwick et al. 2018). However, they do provide measures of one process underlying GE service delivery.

Another approach is monitoring GE benthic (attached) microbial communities. Although methods for this are now reasonably well established (Close et al. 2019), diagnosing causes from observed effects remains elusive for microbes. The principal disadvantage of using microbes to monitor ecosystem health is that community composition does not necessarily integrate ambient environmental conditions over time due to the rapid responses of many microbes to short-term change (e.g., pulses of nutrients, changed dissolved oxygen, etc.), and persistence of others without being metabolically active (Stein et al. 2010). Thus, invertebrate-based measures of ecosystem health are generally more reliable than and preferred to equivalent microbial-based indices and we recommend supporting research to develop such measures.

7 Acknowledgements

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8 References

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