

# Assessing the empirical evidence for wet-adapted fauna in wetlands

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*Manaaki Whenua – Landcare Research*

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## 1 Introduction

A recent decision of the New Zealand Court of Appeal (Page v Greater Wellington Regional Council [2024] NZCA 51) highlights the need to assess whether wet-adapted fauna are present in purported wetlands, where an area is the subject of criminal proceedings, given that the term 'wetland' is defined in the Resource Management Act 1991 (RMA) as follows:

**wetland** includes permanently or intermittently wet areas, shallow water, and land water margins that support a natural ecosystem of plants and animals that are adapted to wet conditions. (RMA 1991, section 2(1) **wetland**)

Recent work (Ministry for the Environment [MfE] 2024) suggests that wet-adapted fauna are likely to be ubiquitous in wetlands, including non-permanently inundated wetlands. That MfE report noted that wet-adapted fauna included meiofauna such as nematodes (very small invertebrates that pass through a 0.5 mm sieve), and earthworms.

## 2 Background

To test the empirical evidence for the reasoning in Ministry for the Environment (2024), Manaaki Whenua – Landcare Research (MWLR) was asked to arrange for nematode and earthworm sampling by regional councils, and assess the resulting samples for the presence of nematodes and earthworms.

## 3 Objectives

- Assess the frequency of nematode presence in wetlands.
- Assess the frequency of earthworm presence in wetlands.

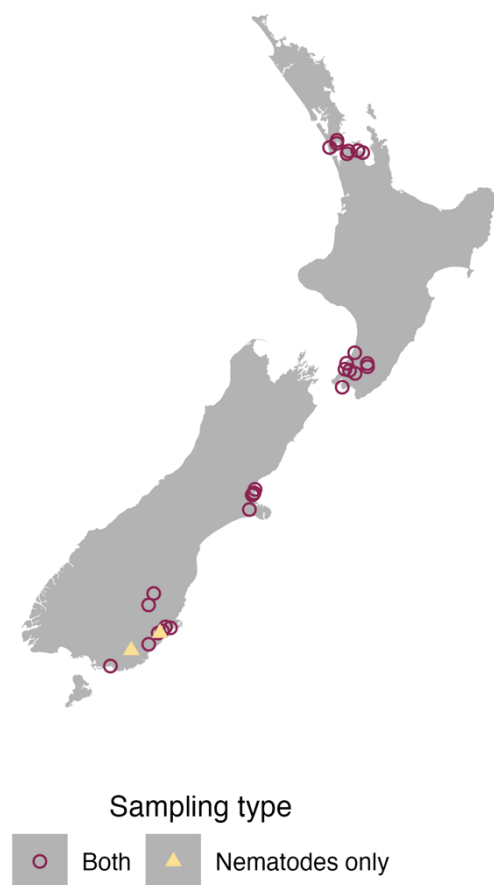
## 4 Methods

Manaaki Whenua – Landcare Research developed sampling protocols for regional councils. These protocols had a reduced intensity of sampling compared to what is usually expected/needed to confirm the presence or absence of wet-adapted fauna in any given wetland. This reduction in intensity was unique to this study, where regional councils were providing assistance in kind, and there was a ceiling on the total number of samples that could be processed by Manaaki Whenua – Landcare Research due to contractual scope. Our protocols are attached as Appendix 1 and Appendix 2.

We asked five regional councils (Auckland Council, Greater Wellington Regional Council, Environment Canterbury, Otago Regional Council, and Environment Southland) to take three subsamples across each wetland and combine this into one sample for analysis. We decided to report results at the wetland scale. We asked for samples to be received by 28 February 2025. However, where time allowed some samples received after this date were processed. In several cases, fewer than three samples per wetland were obtained.

In discussions with the regional councils, it was agreed that the focus of sampling would be wetlands that are commonly the source of enforcement action (e.g. drier, marsh-type wetlands). It was also agreed that only inland freshwater (non-saline) wetlands would be sampled.

The names and locations for each sampled wetland are given in Appendix 3. Samples from one of the wetlands MWLR was provided with were sampled from an area that had been previously delineated as wetland. It was identified that at the time of fauna sampling, the area would no longer satisfy the wetland delineation protocols due to land use change (Jean Jack, Environment Canterbury, pers comm, 19 June 2025). As such, the two samples from this area were excluded from our results. Figure 1 is a map of the sample locations that were included in our results.



**Figure 1. Sampling locations; samples were sourced from five regional councils (the regions of Auckland, Wellington, Canterbury, Otago, and Southland). Sampling type – whether both nematodes and earthworms were sampled, or just nematodes, are shown as per the colour and shape legend.**

## 4.1 Earthworm processing and identification

All collected earthworms were preserved in 80% ethanol. Specimens were then dissected, if necessary, and observed with a Zeiss Stemi 2000-C microscope. Identification was made using the keys of Simms and Gerard (1986) and K. Lee (1959) to identify individual specimens to relative taxonomic unit (RTU) because many New Zealand earthworms cannot be reliably identified to a described species. Immature specimens with undeveloped features were classed as 'unknowns'. Identification time was limited to a maximum of one hour per wetland to identify all collected specimens. The naming convention we used follows the New Zealand Organisms Register (as at May 2025; see <https://www.nzor.org.nz/>).

## 4.2 Nematode extraction and identification

Nematodes were obtained from 43 samples using the tray method described by Whitehead and Hemming (1965). For each sample, 300 g of soil was used for extraction. After 48 hours of extraction, a 20 µm sieve was used to collect approximately 15–20 mL of nematode suspension, which was retained in tubes. One mL of nematode suspension was then transferred to a counting slide for scoring using a compound microscope at 20–200× magnification (Nikon Eclipse 90i, Japan). Each sample was counted twice to remove any errors. Nematodes were identified using the compound microscope at 20–200× magnification. Occasionally, a few nematodes were mounted in 20 µL of water on a glass slide and examined at 1000× magnification to assist with identification.

## 5 Results

Table 1 presents a summary of fauna presence and absence by wetland, including the region in which the wetland occurs. Numbers of individuals for earthworms and nematodes are presented in Table 2. Detailed results to as finer taxonomic resolution as possible are available from Manaaki Whenua - Landcare Research's datastore: <https://doi.org/10.7931/vr8d-4y08>.

Of the 30 wetlands sampled for earthworms, all contained earthworms, and of the 32 wetlands sampled for nematodes, all contained nematodes (Table 1). Earthworm samples were collected but not received on time to be included for Waipori Boot Swamp or Hazeldale Fens in Otago but nematodes from these sites were received and counted.

**Table 1. Presence or absence of earthworms and nematodes in soil samples taken from wetlands across New Zealand. Wetlands marked as 'N/A' had no soil sample taken for nematode or earthworm extraction**

<b>Wetland</b>	<b>Region<sup>a</sup></b>	<b>Earthworms present</b>	<b>Nematodes present</b>
<b>Awarua</b>	Southland	Yes	Yes
<b>Upper Taieri Wetlands Complex – Maniotato Basin</b>	Otago	Yes	Yes
<b>Otokia Swamp</b>	Otago	Yes	Yes
<b>Waipori/Waiholo Wetland Complex</b>	Otago	Yes	Yes
<b>McKays Triangle Wetland</b>	Otago	Yes	Yes
<b>Kaikorai Lagoon Swamp</b>	Otago	Yes	Yes
<b>Upper Taieri Wetlands Complex – Styx Basin</b>	Otago	Yes	Yes
<b>– Lake Tuakitoto Wetland</b>	Otago	Yes	Yes
<b>Waipori Boot Swamp</b>	Otago	N/A	Yes
<b>Hazeldale Fens</b>	Otago	N/A	Yes
<b>Tūtaepatu Lagoon</b>	Canterbury	Yes	Yes
<b>Poynters Wetland</b>	Canterbury	Yes	Yes
<b>Te Ruakaakaa</b>	Canterbury	Yes	Yes
<b>Kainga Wetland</b>	Canterbury	Yes	Yes
<b>Dickeys Road Wetland</b>	Canterbury	Yes	Yes
<b>Ahuriri Wetland A</b>	Canterbury	Yes	Yes
<b>Swampy Gully</b>	Wellington	Yes	Yes
<b>McGhies Wetland</b>	Wellington	Yes	Yes
<b>Baring Head</b>	Wellington	Yes	Yes
<b>Poplar Ave</b>	Wellington	Yes	Yes
<b>Ladel Bend</b>	Wellington	Yes	Yes
<b>Fensham Reserve</b>	Wellington	Yes	Yes
<b>Duntulm Farm Oxbow</b>	Wellington	Yes	Yes
<b>O Te Pua</b>	Wellington	Yes	Yes
<b>Waitawa – coastal</b>	Auckland	Yes	Yes
<b>Waitawa – inland</b>	Auckland	Yes	Yes
<b>Tapapakanga Wetland</b>	Auckland	Yes	Yes
<b>Bronwylan Drive wetland</b>	Auckland	Yes	Yes
<b>Kerrs Rd</b>	Auckland	Yes	Yes
<b>Lake Wainamu_G26</b>	Auckland	Yes	Yes
<b>Luckens Reserve_K24A</b>	Auckland	Yes	Yes
<b>Kowhai_K27</b>	Auckland	Yes	Yes

<sup>a</sup> Full geographic locations for named wetlands are given in Appendix 3 of this report.

**Table 2. Numbers of earthworms and nematodes recovered from each wetland sample or samples**

Wetland	Region <sup>a</sup>	Earthworms / wetland sample	Nematodes / 300 g soil
<b>Awarua</b>	Southland	7	1,360
<b>Upper Taieri Wetlands Complex – Maniotato Basin</b>	Otago	52	1,062
<b>Otokia Swamp</b>	Otago	13	201
<b>Waipori/Waihola Wetland Complex</b>	Otago	4	9
<b>McKays Triangle Wetland</b>	Otago	13	5,522
<b>Kaikorai Lagoon Swamp</b>	Otago	36	845
<b>Upper Taieri Wetlands Complex – Styx Basin</b>	Otago	60	355
<b>Lake Tuakitoto Wetland</b>	Otago	16	2,694
<b>Waipori Boot Swamp</b>	Otago	N/A	1,440
<b>Hazeldale Fens</b>	Otago	N/A	2,507
<b>Tūtaepatu Lagoon</b>	Canterbury	9	1,090
<b>Poynters Wetland</b>	Canterbury	3	470
<b>Te Ruakaakaa</b>	Canterbury	27	1,115
<b>Kainga Wetland</b>	Canterbury	30	7
<b>Dickeys Road Wetland</b>	Canterbury	31	153
<b>Ahuriri Wetland A</b>	Canterbury	2	1,540
<b>Swampy Gully</b>	Wellington	61	63
<b>McGhies Wetland</b>	Wellington	22	306
<b>Baring Head</b>	Wellington	16	1,578
<b>Poplar Ave</b>	Wellington	4	48
<b>Ladel Bend</b>	Wellington	4	600
<b>Fensham Reserve</b>	Wellington	91	20
<b>O Te Pua</b>	Wellington	51	58
<b>Duntulm Farm Oxbow</b>	Wellington	9	30
<b>Waitawa – coastal</b>	Auckland	4	80
<b>Waitawa – inland</b>	Auckland	9	100
<b>Tapapakanga Wetland</b>	Auckland	6	200
<b>Bronwylian Drive wetland</b>	Auckland	19	100
<b>Kerrs Rd</b>	Auckland	13	2,480
<b>Lake Wainamu_G26</b>	Auckland	8	1,310
<b>Luckens Reserve_K24A</b>	Auckland	17	608
<b>Kowhai_K27</b>	Auckland	5	3,428

<sup>a</sup> Full geographic locations for named wetlands are given in Appendix 3 of this report.

## 6 Conclusions

Despite limited sampling, nematodes and earthworms were detected to be present in all wetlands sampled.

Detection of both earthworms and nematodes in the soil requires a considerable amount of labour as well as specialist capability in the identification of these invertebrates. A more efficient method that could be developed for future application in New Zealand wetlands is use of environmental DNA (eDNA).

An eDNA approach would detect the presence of nematode and earthworm DNA in soil samples taken from wetlands. Indeed, eDNA is now routinely used to detect and identify both earthworms (e.g. Lilja et al. 2023) and nematodes (e.g. Kawanobe et al. 2021) in soil. However, some background research would need to be undertaken to ensure that the presence of nematode and earthworm DNA is indicative of those organisms being present, and not just the result of DNA being transported into the wetland environment from elsewhere, for example, run-off from terrestrial habitats (Prosser & Hedgpeth 2018; Valentin et al. 2021). Other research would need to be undertaken to optimise polymerase chain reaction (PCR) primers, as the commonly used animal primers tend not to work well on nematodes (Ren et al. 2024). As with any eDNA study there is also the potential for some species to not anneal well to the available primers– so the primers may need optimising for local diversity. Finally, attention needs to be paid to spatial sampling and the replication of eDNA subsamples, to ensure fine-grained spatial variation in nematode and earthworm distribution does not compromise the analysis (Hermans et al. 2022).

## 7 Recommendations

Confidently confirming the presence or absence of wet-adapted fauna in a wetland requires more sampling than undertaken in this project. We make two recommendations to MfE.

- For regional councils or others seeking to confirm nematode or earthworm presence in wetlands, we recommend an approach to future monitoring of five plots (5 m x 5 m) being randomly selected at each wetland. From each plot, 10 soil cores will be randomly collected and then combined to form a composite sample (approximately 500–1000 g) for nematode sampling, and three 25 × 25 × 20 cm deep earthworm pits excavated, substrate searched in the field, and all earthworms collected and preserved, at the site scale.
- We also recommend developing an eDNA tool for the robust detection of nematodes and earthworms in New Zealand wetlands.

## 8 Acknowledgements

This work would not have been possible without landowner permissions, and we thank those involved for allowing access. We also acknowledge the sampling efforts of regional councils: Darin Sutherland (Environment Southland); Georgianne Griffiths, Miriam Ludbrook, and Dani Guinto (Auckland Council); Helen White, Finn Michalak, Owen Spearpoint, Megan Banks, and Krsto Lukic (Greater Wellington Regional Council); Jean Jack, Mark Parker, and Miles Burford (Environment

Canterbury) and Kate McCombs (Urbeco for Environment Canterbury); Sami Khan (Otago Regional Council), Gretchen Brownstein, and James Arbuckle (Manaaki Whenua for Otago Regional Council). Becky Parmenter (Manaaki Whenua) is acknowledged for project support. Helen O’Leary and Carrie Innes (Manaaki Whenua) are thanked for contributing editing and formatting services, respectively.

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## Appendix 1 – Earthworm sampling protocols

### Protocol for sampling earthworms in wetlands for MWLR project 4818

Sampling the earthworms requires techniques that prevent the rapid decomposition of samples that can often occur when they are not stored in ethanol. Methods presented here allow for sampling of earthworms aligned to wetland sampling locations.

#### *1. Sampling location selection*

- **Determine representative areas** of the wetland for sampling. Sampling sites should be aligned with any wetland delineation protocol sampling (Ministry for the Environment 2022) to harmonise results, if wetland delineation is being undertaken.
- Within a wetland, earthworms will tend to be in **moist but not saturated areas**, and these areas will be most productive for sampling.
- Earthworms often have clustered distribution patterns, so it is suggested that several pits be examined and then combined in this project.

#### *2. Earthworm sampling*

Equipment required (refer to Figure A1.1):

- garden spade (on a 20 cm wide spade blade a vertical line can be added 5 cm in from one side and a horizontal line added 20 cm from the blade tip to assist in creating a 25 × 25 × 20 cm pit)
- a minimum of 5 x 130 mL pottles of 80% Ethanol (with labels) per sample pit
- bottle of c. 1 L of 30% ethanol solution
- blue tarpaulin (small), alternatively a large blue 'fish-bin' may be used in very wet locations; the colour blue provides a high contrast against the frequent pink of lumbricid earthworms
- bright headlamp
- gloves
- 30 cm ruler
- pencil and pottle label (with coordinates, date, site name, collector)
- 2 x 2 to 5 L container (e.g. ice-cream container or small bucket)
- small funnel (for returning 30% ethanol to bottle)
- large serrated 'bread-knife' or hand shears.



**Figure A1.1. Layout of a soil sample ready to be sorted and showing some of the equipment needed.**

Three plots will be randomly selected at each wetland. The three plots will be sampled as per the process below, and combined into one aggregate sample.

The sampling process is described in the bullets below.

- Cut away vegetation to just above the ground with the knife or shears. An area for the sorting may also need to have the vegetation removed.
- Excavate a 25 cm × 25 cm × 20 cm deep turf of soil and place on the blue tarpaulin.
- Sort through by hand, searching with a headlamp and initially placing any earthworms found into the 2–5 L sorting container.
- Specimens can then be 'relaxed' in 30% ethanol for 5 minutes. They may need to be washed in water first to have excess soil removed. This important for quality identifications.
- Specimens should then be placed into the 80% ethanol collecting pottles. Ensure correct sample label is completed in pencil and attached to each pottle.
- The volume of earthworm samples inside a pottle should be a third, or less, of the available volume. This means you may need more than 1 pottle per aggregate sample.
- The pottles should be filled to the top with 80% ethanol.
- All remaining excavated soil should be returned to the pit.

### *3. Transport and storage*

The specimen pottles are best transported in a small cool chilly bin/bag and packed to avoid unnecessary movement or sloshing of the preserving solution. They should be sent for identification as soon as practical. If they need to be stored for more than 24 hours then the 80% ethanol solution should be exchanged with fresh 80% ethanol.

### *4. Provide samples to Manaaki Whenua – Landcare Research*

Courier samples to:

Scott Bartlam  
Manaaki Whenua – Landcare Research  
Gate 10 Silverdale Road,  
University of Waikato,  
Hamilton 3216

[bartlams@landcareresearch.co.nz](mailto:bartlams@landcareresearch.co.nz)

+64 7 859 3733

### *5. Health, safety and the environment*

Ethanol is a Hazardous Substance, and appropriate precautions should be taken in both its transport and its use in sensitive environments. Please consult the appropriate Safety Data Sheet. All ethanol taken into a wetland should be removed from the wetland.

### *6. References*

Lee, K.E. 1959. The earthworm fauna of New Zealand. Bulletin 130. Wellington, New Zealand  
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The New Zealand Organisms Register (2011) NZOR. <https://nzor.org.nz> (accessed 16 May 2025.)

## Appendix 2 – Nematode sampling protocols

### Protocol for sampling for nematodes in wetlands for MWLR project 4818

Sampling nematodes in wetlands requires careful techniques to capture them from soil. This method presented here allows for the collection of a diverse range of nematodes, including free-living, plant-parasitic, and predatory species common in wetland soil.

#### *1. Sampling location selection*

- **Determine representative areas** of the wetland for sampling. Sampling sites should be aligned with any wetland delineation protocol sampling (Ministry for the Environment 2022) to harmonise results, if wetland delineation is being undertaken.
- **Soil and root zones** are typically the most productive for nematode sampling, particularly in and around aquatic plants and moist sediments. Avoid extremely dry or excessively wet soil.

#### *2. Sampling timing*

**Best time:** Nematodes are more active during moist conditions, so sampling after rain or irrigation can improve recovery.

#### *3. Soil sampling (for sediment-dwelling nematodes)*

Equipment needed:

- trowel
- 3 cm diameter soil auger, or corer
- screwdriver and hammer to assist in releasing soil cores from the corer
- sealable plastic bags to hold soil (1200-1500 g)
- chilly bin for storing the samples.

The sampling procedure is described in the bullets below.

- Where wetland delineation protocols are being undertaken, and multiple samples are being collected, we suggest sampling at each vegetation plot, adjacent to any soil sampling being undertaken. Where wetland delineation protocols are being undertaken and one or a restricted number of samples are being collected, we suggest selecting a subset of the vegetation delineation plot locations with reference to the factors discussed in Step 1 (sampling location selection).<sup>1</sup>
- Three plots (5 m × 5 m) will be randomly selected at each wetland.
- Insert the auger or corer into the soil to a depth of 10–20 cm.
- Randomly collect five soil cores (3 cm inner diameter) from each plot.

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<sup>1</sup> Where the purpose of the sampling is not for wetland delineation purposes but rather to characterise the wetland, we recommend a random sampling approach to collect soil samples, modified from the methods described by Wu et al. (2008).

- Combine the 15 soil cores to create a composite sample (c. 1,200–1,500 g).
- Place the soil in labelled, sealed plastic bags. Keep samples cool to avoid nematode degradation.

#### *4. Transport and storage*

Immediate analysis is important: For best results, samples should be transported to a lab as soon as possible. If storage is necessary, keep samples in a cool place (refrigerated at 4°C) but avoid freezing.

#### *5. Provide samples to Manaaki Whenua – Landcare Research*

Courier samples to:

Zengqi Zhao  
 Manaaki Whenua – Landcare Research  
 231 Morrin Road  
 St Johns  
 Auckland 1072

zhaoz@landcareresearch.co.nz  
 +64 9 574 4109

#### *6. References*

Ministry for the Environment 2022. Wetland delineation protocols. ME 1713. Wellington, Ministry for the Environment. 14 p.

Wu HY, Li XX, Shi LB, Wang ZH, Ma FY 2008. Distribution of nematodes in wetland soils with different distance from the Bohai sea. Plant, Soil and Environment - UZPI (Czech Republic) 54: 359–366.

## Appendix 3 – Wetlands sampled for nematodes and earthworms

Wetland	Region	Coordinates (NZTM: Eastings, Northings)
<b>Awarua</b>	Southland	1274714.88 4834394.96
<b>Upper Taieri Wetlands Complex – Maniotato Basin</b>	Otago	1362852 4982881, 1363831 4982952, 1368207 4991568
<b>Otokia Swamp</b>	Otago	1383368 4907718, 1383249 4907574, 1383160 4907487
<b>Waipori/Waiholā Wetland Complex</b>	Otago	1374157 4903266
<b>McKays Triangle Wetland</b>	Otago	1389481 4916052, 1389485 4916109, 1389535 4915992
<b>Kaikorai Lagoon Swamp</b>	Otago	1399875 4913381, 1399862 4915378, 1399815 4913391
<b>Upper Taieri Wetlands Complex – Styx Basin</b>	Otago	1355250 4962965, 1351562 4959564, 1356018 4963233
<b>Lake Tuakitoto Wetland</b>	Otago	1355027 4879699, 1354176 4879705, 1354331 4880197
<b>Waipori Boot Swamp</b>	Otago	1378061 4903240, 1378060 4903252, 1378069 4903260
<b>Hazeldale Fens</b>	Otago	1317873 4867339, 1318109 4867267, 1318255 4867253
<b>Tūtaepatu Lagoon</b>	Canterbury	1575974.065 5202664.372, 1576184.343 5203271.482, 1576177.397 5203171.507
<b>Poynters Wetland</b>	Canterbury	1573363.866 5194956.119, 1573408.603 5195111.777, 1573482.6 5195264.155
<b>Te Ruakaakaa</b>	Canterbury	1574056.624 5195494.115, 1574016.318 5195445.094, 1574013.94 5195431.758
<b>Kainga Wetland</b>	Canterbury	1572360.108 5192678.724, 1572411.965 5192865.514, 1572453.048 5192915.658
<b>Dickeys Road Wetland</b>	Canterbury	1570203.645 5191538.968, 1570229.703 5191503.544, 1570203.974 5191464.56
<b>Ahuriri Wetland A</b>	Canterbury	1564293.779 5160922.512, 1564371.371 5160877.389, 1564291.178 5160956.928
<b>Swampy Gully</b>	Wellington	1763454 5453765
<b>McGhies Wetland</b>	Wellington	1773144 5451187
<b>Baring Head</b>	Wellington	1757758 5416112
<b>Poplar Ave</b>	Wellington	1766837 5466235
<b>Ladel Bend</b>	Wellington	1784505 5444897
<b>Fensham Reserve</b>	Wellington	1810484 5458870
<b>O Te Pua</b>	Wellington	1783821 5487948
<b>Duntulm Farm Oxbow</b>	Wellington	1809999 5465400
<b>Waitawa coastal</b>	Auckland	1790886 5909728, 1790934 5909778, 1790865 5909774
<b>Waitawa inland</b>	Auckland	1790171 5909565, 1790174 5909543, 1790164 5909532
<b>Tapapakanga Wetland</b>	Auckland	1800471 5905563, 1800474 5905557, 1800465 5905551
<b>Bronwylan Drive wetland</b>	Auckland	1770593 5908773, 1770590 5908790, 1770633 5908785
<b>Kerrs Rd</b>	Auckland	1767935 5903104, 1767927 5903108, 1767844 5903139
<b>Lake Wainamu_G26</b>	Auckland	1731218 5916088, 1731578 5916226, 1731555 5916218
<b>Luckens Reserve_K24A</b>	Auckland	1746304 5924664, 1746412 5924727, 1746420 5924866
<b>Kowhai_K27</b>	Auckland	1746601 5912226, 1747026 5936895, 1747154 5944309