eDNA as a holistic measure of pastoral landscape effects on taonga species

Funded by Rural Professionals Fund – Our Land and Water National Science Challenge

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Summary

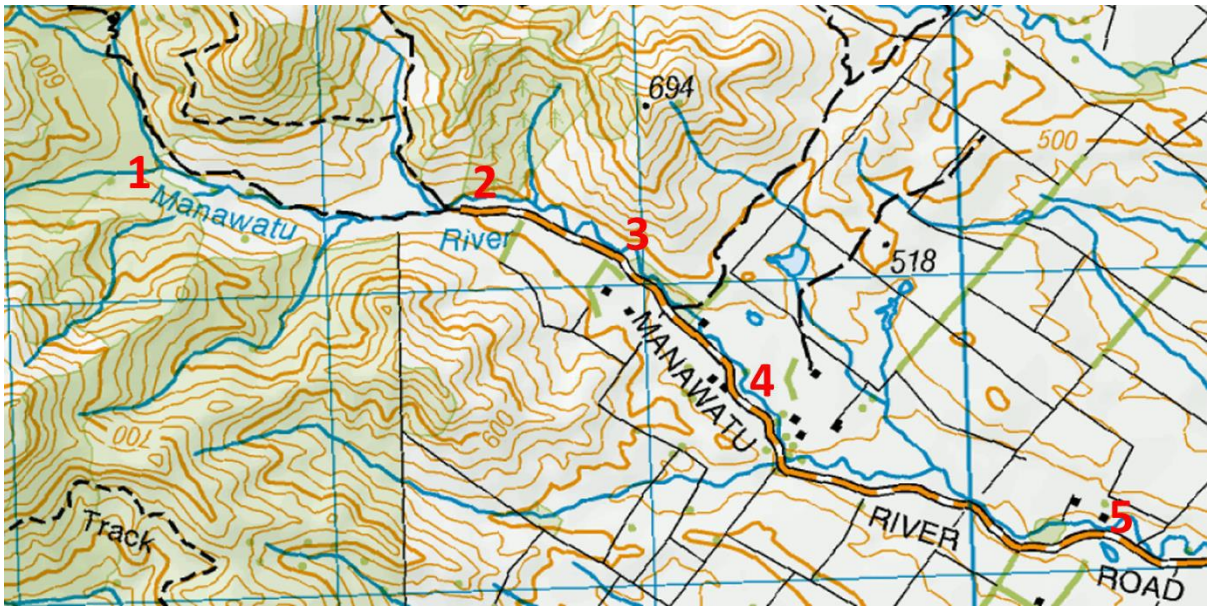
Our objective was to increase commitment to action in catchments by demonstrating a new approach of assessing freshwater quality for the agricultural sector. This was done by using tiny traces of genetic material, or environmental DNA (eDNA) as novel indicators which provide a living context for understanding the ecological health of waterways. Currently freshwater quality is measured with key indicators such as faecal indicator bacteria (*E. coli*), nitrates, phosphates and other physico-chemical parameters for Regional Councils to identify potential health risks to humans. For communities however, such indicators are vague and somewhat abstract.

Our case study site area was the upper reaches of the Manawatū River encompassing native bush, a hill country sheep and beef farm, and a dairy farm where freshwater samples were obtained from five separate sites for eDNA analysis. Freshwater sites were sampled for eDNA detection on four occasions December 2022 and February 2023, before Cyclone Gabrielle, and April 2023 and June 2023, after Cyclone Gabrielle. The project used six separate freshwater samples collected from each site on each sample occasion, to maximise eDNA detections for aquatic communities and terrestrial biodiversity, and used a new eDNA based ecological health index score, the taxon-independent community index (TICI) incorporating information across the tree of life.

On all four sampling visits TICI ratings varied from >125 (pristine) in water samples from the bush site down to 110 to 120 (excellent) at downstream sites impacted by pastoral farming. Taonga species identified using eDNA included whio/blue duck, ruru/morepork, kōtare/kingfisher, tuna, kaharore bully, dwarf galaxias and kōura. Conventional water quality assessment attributes including *E. coli* and physico-chemical parameters including conductivity, total nitrogen, nitrate, phosphorus, and turbidity were also measured and compared to eDNA reads. Using *E. coli*, physico-chemical data, eDNA reads from invasive/pastoral species (possum, deer, cattle and sheep) and local knowledge, likely inferences were made on contamination sources and potential mitigations.

As well as the Drysdale whānau, AgResearch staff and Pūhoro STEMM Charitable Trust staff and summer interns, mana whenua from three hapu environmental groups including Taiao Ora Contracting, Mauri Oho, and Rangitane Wai Warriors received training on eDNA sampling and interpretation of results. The eDNA kits were small and easy to use as a system to monitor catchment biodiversity and were viewed as an exciting addition to conventional *E. coli* microbial water quality assessments and SHMAK monitoring.

This project utilised eDNA technologies to provide a holistic view of aquatic and associated terrestrial ecosystem changes as the river passes through different land-uses. Our case study area was a culturally significant site including Ngāmoko whare (https://www.manawaturiver.co.nz/activities/tu-te-manawa/) and the upper reaches of the Manawatū River encompassing native bush, a hill country sheep and beef farm, and a dairy farm where freshwater samples were obtained from five separate sites (separated by 3.5km) for eDNA analysis (Figure 1).



1. Native bush reserve
2. Ngāmoko whare/information kiosk within hill-country sheep and beef farm
3. Site where Manawatū River enters Te Miro Farm
4. School's freshwater quality wānanga site
5. Site where Manawatū River exits Te Miro Farm

Figure 1. Spatial representation of Manawatu River samples sites for eDNA investigation.

Using eDNA detections, each of the freshwater sites was sampled on four occasions (December 2022, and February, April and June 2023, to understand the ecosystem changes as the river travels through contrasting landscapes. Additional water samples were collected for measurements of *E. coli* (MPN, most probable number per 100ml water; Colilert analysis undertaken at AgResearch, Hopkirk Research Institute, Palmerston North) and physico-chemical attributes including conductivity, total nitrogen, ammonia, nitrite, nitrate, dissolved oxygen, dissolved reactive phosphorus, pH and turbidity undertaken by a diagnostic laboratory (Central Environment Laboratories, Palmerston North).

This project was a partnership between Te Miro Farm, Taiao Ora Contracting, WilderlabNZ Ltd., AgResearch and the broader community encompassing local schools/kūra and other dairy farming whānau, stemming from the extensive riparian planting and water quality monitoring undertaken at Te Miro Farm. By partnering directly with Wilderlab, such methods enabled early detection of positive change by local farmers and provided communities with a means for further reconnection with the environment.

Overall taxon-independent community index (TICI) ecological health index scores (www.wilderlab.co.nz/tici) consistently decreased from Site 1 to Site 5 on all four visits (Figure 2), but

consistently received a pristine/excellent TICI rating. TICI scores also showed increased variation according to distance downstream i.e. TICI scores from Site 1 showed the least variation and the TICI scores from Site 5 showed the most variation. Rainfall was measured using the closest Horizons monitoring site (1.7km from Te Miro farm) at the Manawatū at Apiti Track station (https://envirodata.horizons.govt.nz/?siteName=Manawatu%20at%20Apiti%20Track GPS 40° 02' 41.82 S, 176° 08' 33.05 E). Increasing rainfall over the preceding 48 hours prior to eDNA sampling appeared to reduce TICI scores due to potential dilution of eDNA with increased river loadings. eDNA instructions recommend sampling during low rainfall periods (ideally December to May inclusive in NZ) with streams being at or near base flow, not discoloured, and no more than 10mm of rain in the past 24 hours.

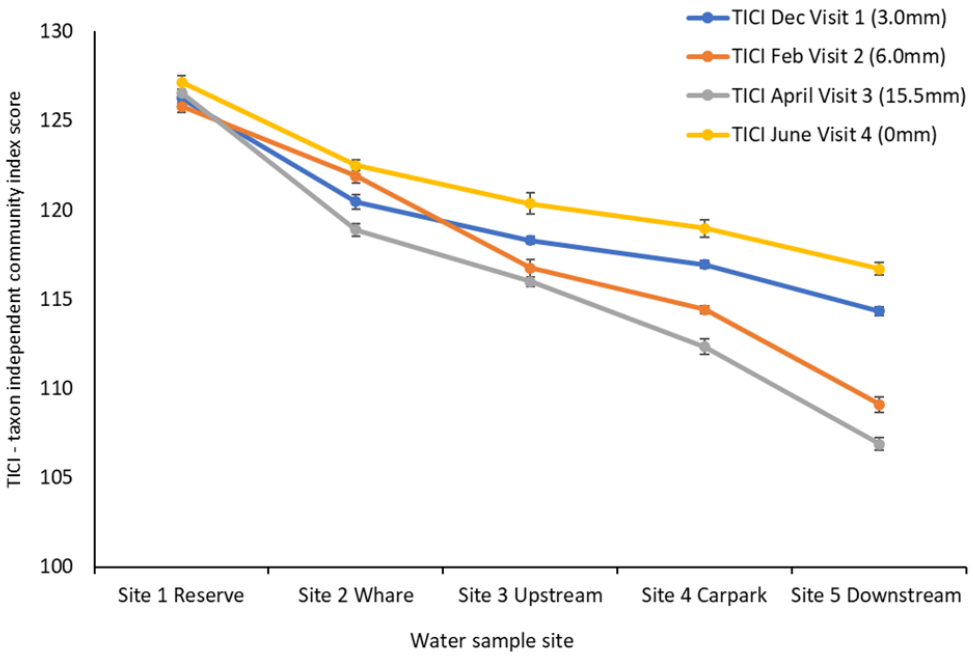


Figure 2. Taxon independent classification index (TICI) metrics for the Te Miro Farm water samples sites (with associated rainfall in the preceding 48 hours).Error bars represent SEM.

Major faecal sources from cattle, sheep, deer and possums were compared for each visit and water sample site (Table 1). The eDNA results from Visit 1 (December 2022) indicated high reads of deer (3442) and possums (789) from the bush site (Site 1), increasing eDNA sequence reads from sheep (2435) through the upland sheep paddocks at Site 2, possums (14432) at Site 3, with lower counts of the four species from Sites 4 and 5. *E. coli* counts were low at the bush site (Site 1, 7.4 per 100ml water) increasing through to the downstream Te Miro Farm site (Site 5, 435.2 per 100ml water) where dairy cattle had recently been grazed close to the river.

The second sampling trip, immediately before the Cyclone Gabrielle weather event was characterized by very high cattle eDNA reads (32844) and high *E. coli* counts (86,640 per 100ml water) from Site 3. Notably these observations were aligned with an increased turbidity from water samples taken at this site and was thought to be incursion of beef cattle upstream from a neighbouring sheep and beef farm. Both the *E. coli* and cattle eDNA read counts were 'diluted' at the two sites (Site 3 and Site 4) downstream. High possum eDNA reads (4433) were noted from Site 1, deer from Site 2 (8331) and Site 4 (5742).

By April 2023 when the third water sampling trip was completed, all sheep had been removed from paddocks between Site 1 and Site 2 leading to a large reduction in sheep eDNA reads. Cattle eDNA reads were once again highest at Site 3 and then decreased through Te Miro Farm through the Site 5. Similarly, sheep eDNA reads remained low with higher cattle eDNA counts in June 2023. Possum eDNA reads decreased from Site 1 to Site 5, and relatively high deer eDNA reads (574) were recorded from Site 2 where they were known to frequent the pine plantation next to the sample site.

Visit 1 - Dec 2022	Site 1	Site 2	Site 3	Site 4	Site5
Cattle	6	622	106	122	337
Sheep	0	2435	312	127	34
Deer	3442	246	313	139	98
Possums	789	620	1442	266	275
E. coli MPN per 100ml	7	131	111	194	435

Visit 2 - Feb 2023	Site 1	Site 2	Site 3	Site 4	Site5
Cattle	7	31	32844	1705	517
Sheep	0	119	8	223	14
Deer	377	8331	645	5743	963
Possums	4433	903	492	547	129
E. coli MPN per 100ml	157	345	86640	1046	345

Visit 3 - April 2023	Site 1	Site 2	Site 3	Site 4	Site5
Cattle	1	41	6139	1606	450
Sheep	0	3	22	101	39
Deer	176	194	57	65	19
Possums	519	201	78	61	39
E. coli MPN per 100ml	18	16	47	69	56

Visit 4 - June 2023	Site 1	Site 2	Site 3	Site 4	Site5
Cattle	10	1124	764	1954	574
Sheep	0	35	250	53	11
Deer	49	574	144	49	57
Possums	854	445	143	288	23
E. coli MPN per 100ml	3	43	25	20	12

Table 1. eDNA reads from major faecal sources and E. coli counts (faecal indicator bacteria).

Kaharore bully, dwarf galaxias and longfin eel were the main fish species identified from this region of the Manawatū River (Table 2). Interestingly eDNA reads indicated the kaharore bullies increased downstream from Site 2 to Site 5 and were absent from the bush reserve site (Site 1) suggesting that there may be a fish barrier preventing these small fish moving further upstream between Site 2 and Site 1. The non-migratory dwarf galaxias were most common in the upland sites (Site 1 and Site 2). Like the kaharore bullies, the longfin eel and brown trout were more common from downstream sites. eDNA reads from shortfin eel and rainbow trout were more patchy. Kōura eDNA counts were highest at the downstream sites with a large spike from the carpark site (Site 4) during the last visit

(June 2023). Notably, post-Cyclone Gabrielle kōura eDNA counts from Sites 1 to 3 were comparatively low to pre-cyclone counts and read counts further downstream. Whether due to habitat change or lifecycle/breeding activity is unknown.

Visit 1 - Dec 2022	Site1	Site2	Site3	Site4	Site5
kaharore bully	0	3822	5307	7382	8192
dwarf galaxias	6140	3686	1912	1139	532
longfin eel	810	829	596	614	573
brown trout	0	5	19	79	186
shortfin eel	0	0	6	0	47
rainbow trout	0	0	1	18	16
kōura; crayfish	2388	3983	2082	4987	7282

Visit 2 - Feb 2023	Site1	Site2	Site3	Site4	Site5
kaharore bully	0	4336	7630	14626	19511
dwarf galaxias	4252	5158	2218	697	362
longfin eel	1958	956	105	1513	2549
brown trout	0	31	0	102	370
shortfin eel	0	12	0	6	48
rainbow trout	0	0	1	14	39
kōura; crayfish	5740	3185	4681	8352	9726

Visit 3 - April 2023	Site1	Site2	Site3	Site4	Site5
kaharore bully	0	1981	4673	12100	12142
dwarf galaxias	1631	1945	1983	894	423
longfin eel	235	474	191	514	464
brown trout	0	9	1	25	96
shortfin eel	0	18	0	18	5
rainbow trout	3	0	20	7	0
kōura; crayfish	463	881	729	1629	2717

Visit 4 - June 2023	Site1	Site2	Site3	Site4	Site5
kaharore bully	0	2186	9249	13054	11997
dwarf galaxias	1087	4226	2325	1673	31
longfin eel	392	2188	3066	2697	4631
brown trout	0	37	2	1095	1854
shortfin eel	0	0	64	0	62
rainbow trout	8	0	105	52	0
kōura; crayfish	0	518	0	16102	5884

Table 2. eDNA reads from freshwater fish and crayfish.

Invasive wildlife species such as Norway rat, black rat, hedgehog and pig were detected from eDNA reads on most sampling trips along with mice, and on one occasion an unidentified mustelid species (Table 3). eDNA counts from introduced and endemic bird species (Table 4) were generally low with

the highest counts in late spring likely due to nesting (mallard and paradise shelduck) and increased foraging activity. eDNA from whio/blue duck was detected from the upper three sites during visits 2, 3 and 4 suggesting some movement of birds from close-by catchments (Pohangina) to feed in the upper Manawatū River.

Visit 1 - Dec 2022	Site1	Site2	Site3	Site4	Site5
Norway Rat	0	12	53	38	89
Black Rat	75	18	0	8	0
Pig	0	0	38	2	21
Dog	0	0	11	0	0
Hedgehog	0	0	1	0	0
Rabbit	0	2	188	919	135

Visit 2 - Feb 2023	Site1	Site2	Site3	Site4	Site5
Norway Rat	0	2	0	102	79
Black Rat	0	6	0	3	10
Pig	0	0	0	4	3
Rabbit	0	0	0	0	5
Hedgehog	0	0	1	1	1
House mouse	0	0	0	2	0
Dog	0	0	0	0	1

Visit 3 - April 2023	Site1	Site2	Site3	Site4	Site5
Norway Rat	0	3	0	31	35
Pig	3	34	0	0	4
House mouse	0	0	2	5	23
Black Rat	3	11	0	0	0
Brown hare	0	0	0	0	4
Hedgehog	0	0	0	2	0

Visit 4 - June 2023	Site1	Site2	Site3	Site4	Site5
Black Rat	11	0	121	4	0
Dog	0	0	0	0	64
Pig	0	43	6	6	0
House mouse	0	0	0	43	8
Norway Rat	0	0	0	15	29
Weasels	0	0	0	0	63

Table 3. eDNA reads from invasive mammal species.

Visit 1 - Dec 2022	Site1	Site2	Site3	Site4	Site5
Blackbird	17	6	68	27	10
Chaffinch	31	21	110	18	23
Goldfinch	0	0	1	0	0
House sparrow	21	0	11	17	17
Magpie	0	78	0	0	0
Mallard duck	34	0	379	399	1431
Morepork; ruru	0	0	56	3	16
Paradise Shelduck	0	0	369	0	0
Pīwakawaka	0	36	5	0	0
Silvereye	2	0	0	10	0
Song thrush	4	3	36	145	22
Starling	0	39	11	33	16
Tui	0	0	13	0	0
Welcome swallow	0	0	0	23	0
Yellowhammer	48	0	0	7	0

Visit 2 - Feb 2023	Site1	Site2	Site3	Site4	Site5
Blackbird	0	13	0	5	1
Chaffinch	0	5	0	3	10
Dunnock	0	2	0	2	0
Grey warbler	0	2	0	0	0
Kereru	413	3	0	0	0
Magpie	0	5	0	29	0
Mallard duck	4	0	0	4	2
Pīwakawaka	0	2	0	1	0
Kōtare	7	8	0	0	10
Silvereye	0	13	0	15	5
Song thrush	6	0	0	8	13
Tui	0	2	0	22	29
Welcome swallow	0	0	1	2	0
Whio	1	4	0	0	0

Visit 3 - April 2023	Site1	Site2	Site3	Site4	Site5
Blackbird	0	0	0	14	0
Chaffinch	0	0	0	13	0
Grey warbler	15	0	0	0	0
Mallard duck	1	0	0	2	2
Pīwakawaka	0	5	0	0	0
Pūkeko	0	0	0	0	3
Song thrush	0	0	0	0	12
Starling	0	0	0	15	4
Tui	3	0	0	0	0
Whio	10	0	0	0	0

Visit 4 - June 2023	Site1	Site2	Site3	Site4	Site5
Blackbird	42	1	0	0	0
Chaffinch	0	0	0	0	71
Kōtare	0	0	0	4	0
Mallard duck	0	0	0	0	30
Morepork; ruru	0	59	0	0	0
Song thrush	0	21	0	0	62
Starling	0	607	98	0	238
Welcome swallow	0	0	0	0	27
Whio	0	7	3	0	0

Table 4. eDNA reads from endemic and introduced bird species.

Endemic plant species were readily detected through eDNA analysis (Table 5) including native beeches, putaputawētā, miro (rare), *Weinmannia recemosa* (kāmahi) and tutu commonly from the more open samples sites (Site 2 to Site 5).

Visit 1 - Dec 2022	Site1	Site2	Site3	Site4	Site5
Coprosma	376	465	613	628	742
Hangehange	106	42	109	47	26
Kaikomako	4	21	22	5	3
Māhoe	104	547	392	688	618
Mānuka	3	59	79	20	18
Miro	1	2	1	4	1
Harakeke	0	0	1	0	0
NZ tree fern	2	0	0	0	0
Putaputawētā	441	451	484	325	198
Southern beeches	7160	3046	2736	1576	845
Kōtukutuku	0	10	42	21	13
Tutu	0	731	1884	1438	1325
Kāmahi	269	69	90	40	60
Makomako	9	90	185	215	146

Visit 2 - Feb 2023	Site1	Site2	Site3	Site4	Site5
Coprosma	745	679	357	641	228
Hangehange	346	134	36	83	22
Kaikomako	15	49	10	11	0
Māhoe	866	333	125	193	79
Mānuka	20	76	41	38	10
Miro	5	5	1	4	0
Harakeke	0	0	5	8	0
NZ tree fern	10	6	0	0	0
Putaputawētā	1312	772	351	369	129
Southern beeches	2197	764	186	178	78
Kōtukutuku	0	14	28	38	9
Tutu	22	1695	1358	2718	813
Kāmahi	392	119	34	64	12
Makomako	56	163	63	131	43

Visit 3 - April 2023	Site1	Site2	Site3	Site4	Site5
Coprosma	707	627	634	441	199
Hangehange	307	165	87	57	44
Kaikomako	53	53	38	6	4
Māhoe	649	166	116	117	53
Mānuka	20	48	36	14	5
Miro	41	6	0	0	1
Harakeke	0	0	12	12	4
NZ tree fern	3	4	0	0	0
Putaputawētā	956	881	300	145	84
Southern beeches	624	276	132	22	16
Kōtukutuku	4	13	2	0	9
Tutu	0	1371	1804	1686	1081
Kāmahi	272	89	52	25	12
Makomako	34	178	50	61	59

Visit 4 - June 2023	Site1	Site2	Site3	Site4	Site5
Coprosma	950	1181	1091	772	334
Hangehange	213	74	134	51	25
Kaikomako	75	43	37	3	9
Māhoe	810	183	90	186	43
Mānuka	28	108	114	26	8
Miro	35	0	6	0	0
Harakeke	0	1	8	2	2
NZ tree fern	29	0	5	2	0
Putaputawētā	2214	1107	636	140	77
Southern beeches	1312	363	227	76	55
Kōtukutuku	0	5	15	5	10
Tutu	19	2141	3183	3286	1324
Kāmahi	497	107	146	34	29
Makomako	120	106	157	68	38

Table 5. eDNA reads from endemic plant species.

Overall physic-chemical water quality attributes provided some insight into increasing pastoral activity and use of pasture supplements/fertilisers downstream (Figure 3). Total nitrogen increases were largely attributable to nitrates; nitrite and ammonia detections were often below detection levels, as was dissolved reactive phosphorus. Generally turbidity increased in association with loss of canopy cover and increased pastureland, however the 3.5km stream length encompassing the five water sampling sites and close proximity to the source of the Manawatū River resulted in only brief increases in turbidity through stormwater sediment run-off and a rapid return to base flows.

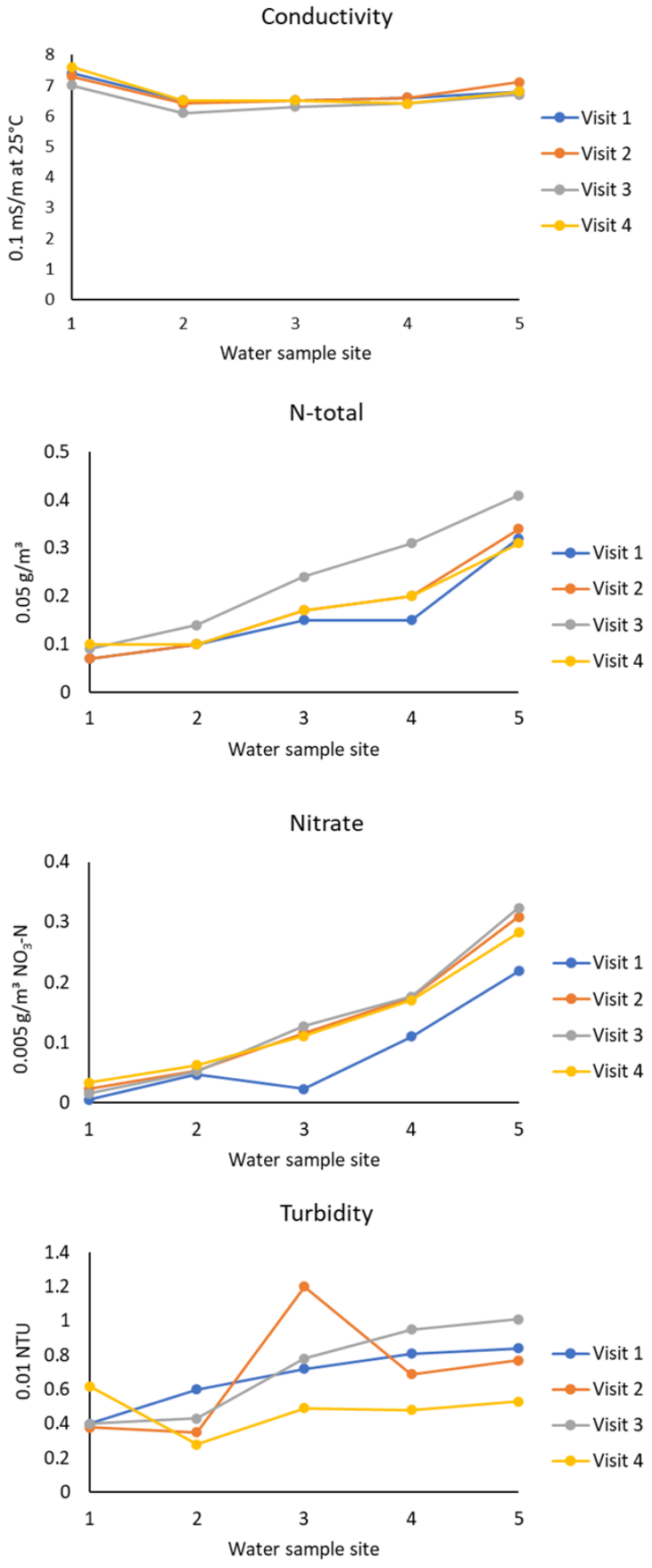


Figure 3. Data from physico-chemical water quality attributes.

The eDNA data were analysed more broadly to understand the number of taxa (e.g. the hierarchical divisions of a species from Kingdom to subspecies) identified from each site over the four visits (Figure 4). On average the native bush site (Site 1) had the fewest different taxa with overall numbers increasing through to site 5. These further taxa may represent those associated with pastoral activities; furture work will examine the overall taxa diversity across the visits and sites using for example, principal component analysis or non-metric multidimensional scaling. It was noteworthy that the significant contamination events at Site 3 in February 2023 (Visit 2) associated with high cattle eDNA reads, high *E. coli* counts and increased turbidity was also associated with a noticeable drop in total taxa at this site suggesting that contaminants may have overwhelmed background biodiversity.

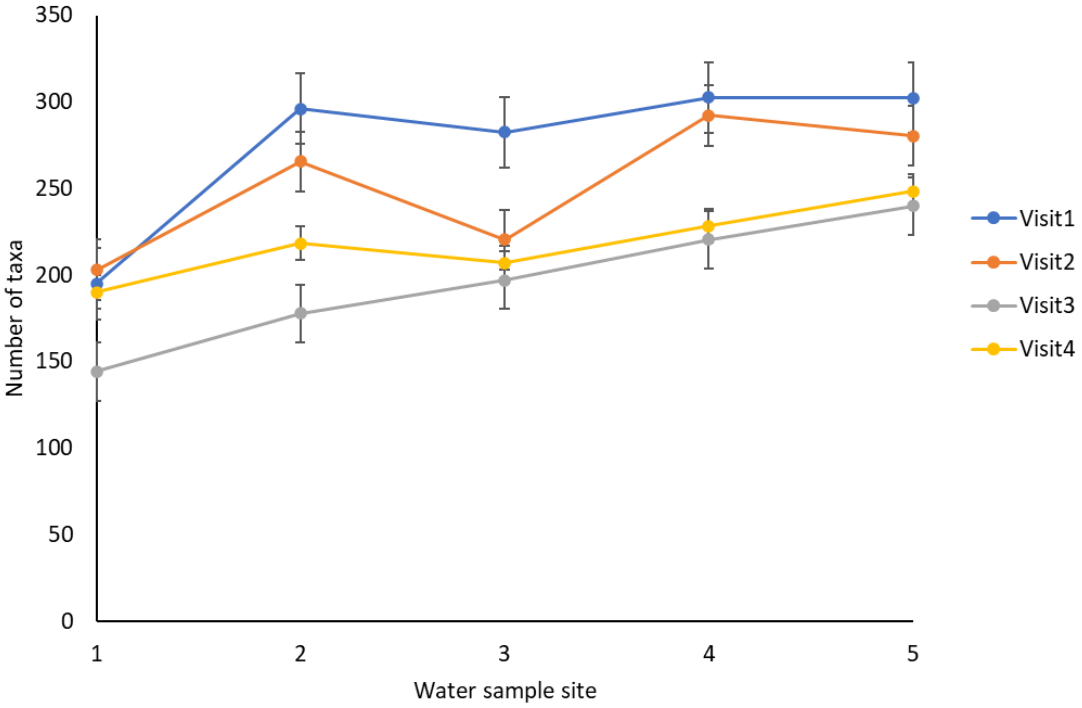


Figure 4. Number of taxa (at least one sequence read) identified from water sampling sites.

By identifying eDNA signatures from common species results provided a more practical overview of freshwater quality enabling us to tell many positive stories in the catchment alongside indicators from pest species and livestock. Such methods provided options to have honest conversations with farmers as it enabled them to see where wildlife and farmed animals are contributing to eDNA and speaks to all, empowering communities to see the full picture of catchment ecosystems. Identification of fish, bird and plant species along the awa also considers taonga species encompassing a mātauranga Māori perspective and aligns with key indicators currently used by Regional Councils.

Results from the eDNA analysis have been widely socialised during community events undertaken at Te Miro Farm – e.g. riparian community planting day Sunday 11 June 2023 attended by about 25 supporters including farmers, teaches, school children, Regional Council (Horizons) staff and local conservation workers. The water sampling also provided an opportunity for kaihautū and rangatahi

from Pūhoro STEMM Charitable Trust to participate in the kaupapa and experience new technologies which align with mātauranga Maori and cultural health measurements of ecosystem health. Once complete, this report and links to the eDNA results and Wilderlab website will be made available to the local community through Facebook posts and through permanent posterboards highlighting the tree of life (Figure 5) associated with different sample sites, and visits. The posterboards situated in the native plant nursery will provide an educational focus for students from Norsewood and Districts' School during open days at Te Miro Farm undertaken most terms.

Outputs from this work included features in the Dairy Exporter magazine in January 2023 (https://nzfarmlife.co.nz/what-lives-in-the-river/ and https://nzfarmlife.co.nz/paradise-by-the-awa/) and REX Rural podcasts (https://open.spotify.com/episode/30D2R5omuFzwJMfRGEHgrT?si=XI6rXRe_SGW-TMMG4agi6w and https://open.spotify.com/episode/12juzAPg25fZCog4j6o42f?si=MUqjYqyDRKS2KNcz_2VOrQ

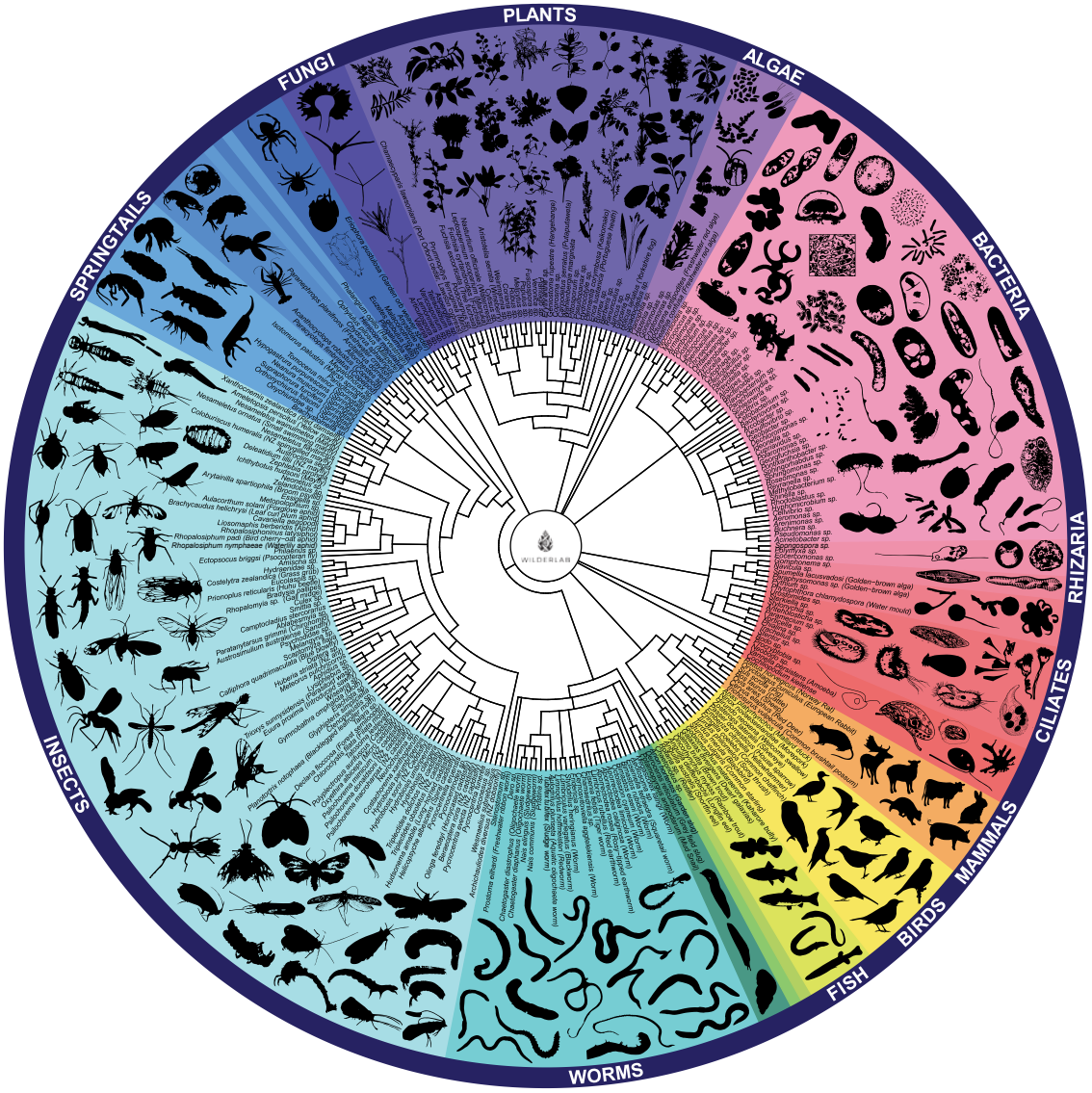


Figure 5. Tree of life generated from eDNA data Visit 1, Site 5.

Future work – a workshop to be held at the Wilderlab facilities (Miramar, Wellington) is scheduled for 21 July. This workshop will provide an opportunity for community members and members of the hapu environmental groups to observe the processes which occur when the filters are received by Wilderlab and an idea of the technologies required to generate the eDNA results and tree of life data. It will also be an opportunity to come together to celebrate the project and identify opportunities to continue and extend the project. Already a proposal has been submitted by Te Miro Farm, Taiao Ora Contracting, Pūhoro STEMM Charitable Trust, Wilderlab and AgResearch for a Horizons Freshwater Community Grant to continue this kaupapa requesting financial support to continue the community project with water sampling and associated eDNA, *E. coli* and physico-chemical measurements through to the end of 2023. These further tests would allow a full year of analysis to occur providing indications of ecosystem resilience across different land-uses before and after heavy rainfall events such as that caused by Cyclone Gabrielle (Figure 5).

Figure 5. Study site in full flood associated with Cyclone Gabrielle heavy rainfall event 14 February 2023.

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WilderlabNZ weblinks to results from this study.

Visit1

Site1 - Wilderlab Batch Report (amazonaws.com)

Site2 - Wilderlab Batch Report (amazonaws.com)

Site3 - Wilderlab Batch Report (amazonaws.com)

Site4 - Wilderlab Batch Report (amazonaws.com)

Site5 - Wilderlab Batch Report (amazonaws.com)

Visit2

Site1 - Wilderlab Batch Report (amazonaws.com)

Site2 - Wilderlab Batch Report (amazonaws.com)

Site3 - Wilderlab Batch Report (amazonaws.com)

Site4 - Wilderlab Batch Report (amazonaws.com)

Site5 - Wilderlab Batch Report (amazonaws.com)

Visit3

Site1 - Wilderlab Batch Report (amazonaws.com)

Site2 - Wilderlab Batch Report (amazonaws.com)

Site3 - Wilderlab Batch Report (amazonaws.com)

Site4 - Wilderlab Batch Report (amazonaws.com)

Site5 - Wilderlab Batch Report (amazonaws.com)

Visit4

Site1 - Wilderlab Batch Report (amazonaws.com)

Site2 - Wilderlab Batch Report (amazonaws.com)

Site3 - Wilderlab Batch Report (amazonaws.com)

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