BioSense
ENVIRONMENTAL RESEARCH

Kauri dieback disease surveillance of Watercare's proposed replacement water treatment plant site at Waima Catchment

By BioSense Limited
For Watercare
Date November 2020

Preface

This report has been prepared by BioSense Limited for Watercare. No liability or responsibility is accepted by BioSense Limited or its employees for any use of or reliance upon this report by any third party.

Confidential – for Watercare use only.

For all enquiries contact
lee@biosense.co.nz

Contents

Executive summary 4
1. Who we are 5
2. Background 6
3. The problem 7
4. Overview of the solution 8
5. Approach and methodology 9
5.1 Grid based search to identify risk 10
5.2 Stratified sampling 10
5.3 Kauri rootzone sampling 10
5.4 Watercourse sampling 11
5.5 Track sampling 12
5.6 Animal disturbance sampling 12
5.7 Sample processing and testing 12
6. Results 13
6.1 *Phytophthora agathidicida* and *P. cinnamomi* detections 13
6.2 Stratified sampling 14
6.3 Kauri rootzone sampling 14
6.4 Watercourse sampling 14
6.5 Track sampling 15
6.6 Animal disturbance sampling 15
6.7 Positive and negative control samples 15
6.8 Detection buffering 15
7. Conclusions 16
8. References 16
9. Appendix 19
Appendix 1 – Selected bibliography of this team's kauri dieback related outputs 19
Appendix 2 – Selected bibliography of this team's kauri dieback related outputs 21
Appendix 3 – A map of stratified sampling locations 22
Appendix 4 – A map of the kauri rootzone sampling locations 23
Appendix 5 – A map of watercourse sampling locations 24
Appendix 6 – A map of the track sampling locations 25
Appendix 7 – A map of the animal disturbance sampling locations 26
Appendix 8 – A map of all sampling locations 27
Appendix 9 - A map of the kauri health survey and complete site detection results 28
Appendix 10 - A map of the stratified sampling detection results 29
Appendix 11 – A map of kauri and kauri dieback status 30
Appendix 12 - A map of the kauri rootzone sampling detection results 31
Appendix 13 – A map of the watercourse sampling detection results 32
Appendix 14 – A map of the track sampling detection results 33
Appendix 15 - A map of the animal disturbance sampling detection results 34
Appendix 16 - A map of *Phytophthora agathidicida* and *Phytophthora cinnamomi* detections with a 30 m buffer 35

Executive summary

BioSense was requested by Watercare to develop and implement a field-testing protocol to assess the presence and distribution of *Phytophthora agathidicida,* the kauri dieback causing pathogen, within the proposed construction footprint, the wider Project Site, and within a surrounding buffer area.

The objective of this work was to develop and implement sampling protocol to test for:

1. The disease status of kauri trees within and surrounding the proposed works footprint.
2. The presence and distribution of phytophthora in soil within the Project Site and a surrounding buffer.
3. The presence and distribution of phytophthora in soil within the Project Site and a surrounding buffer.

A sampling protocol was directed to address the following prescription:

- The buffer is to be of sufficient extent to encompass interconnected root systems of vegetation within and surrounding the Project Site, so the risk that these networks may as a pathway move phytophthora into or out of the site can be assessed.
- Watercourses are identified as a key potential vector for the spread of kauri dieback disease.
- Sampling will include all sites of human and feral animal disturbance.
- Sample collection and analysis methods will seek to maximise detection probability within practical limits, i.e., the scale and pattern of sampling is to give the best chance that kauri dieback-causing pathogens would be detected if present within the construction footprint and wider Project Site.

A field survey confirmed the presence of kauri dieback symptoms within the Project Site, and surrounding buffer within Clarks Bush.

Analysis of soil samples detected the presence of *Phytophthora agathidicida* and *Phytophthora cinnamomi* in soil taken from the Project Site, and adjoining buffer area within Clarks Bush.

Analysis of water samples detected the presence of *Phytophthora agathidicida* and *Phytophthora cinnamomi* in water taken from the Waituna stream tributary network within the Project Site, and adjoining buffer area within Clarks Bush.

1. Who we are

At BioSense we seek to understand more about our environment to protect it for the future. To do this, we provide surveillance, management, treatment, and eradication options to combat invasive pests and pathogens and we are committed to doing this in a collaborative, co-created manner. We believe that collaboration is essential for successful and durable outcomes and we work with Iwi, community-groups, Universities, Crown Research Institutes, and other experts to develop the best qualified team, for the protection of our environment and the well-being of Aotearoa. We are also guided by research and use this to constantly improve and develop best practice.

An area of focus for the BioSense team members to date has been the management of kauri dieback and many of the team have been involved in leading the research to mitigate the impact and management of kauri dieback since 2010. We have also developed best practice in surveillance and have designed, conducted, analysed, and reported on all kauri dieback surveillance on land across Auckland between 2010 and 2018, and Waipoua forest since 2010. This work includes investigating more than 2,000 sites of potential kauri dieback, conducting health assessments on more than 75,000 kauri, and collecting more than 4,500 diagnostic samples for analysis across Northland, Auckland, Waikato, and Bay of Plenty.

We have also been involved in research into potential treatment tools for kauri dieback since 2010, most notably the research and development of the phosphite tool. BioSense designed and conducted the first large-scale application of phosphite as a treatment for kauri dieback with more than 15,000 kauri treated and assessed to enable long-term impact monitoring by 2020, with treatment of a further 2,000 planned for 2021. Members of BioSense are also involved in designing and managing a community-based social science project aimed at engaging the community to care for kauri and manage kauri dieback, with a range of potential tools being assessed.

A selected bibliography of kauri dieback research outputs is listed in appendix 1 and a list of kauri dieback surveillance project implemented by this team are listed in appendix 2.

2. Background

Watercare operates water supply dams within the Waitākere Ranges, including the Upper and Lower Huia Dams and the Upper and Lower Nihotupu Dams. The Huia Water Treatment Plant (WTP) located in Waima (named for the source of the water) treats the water from these dams before it is distributed via the water transmission network.

Watercare seeks to construct a new WTP to replace the which is nearing the end of its operational life. As part of this project Watercare is also proposing to construct two treated water reservoirs on the Project Site to increase treated water storage capacity.

Watercare proposes to construct the replacement WTP at a site on the corner of Manuka Road and Woodlands Park Road, directly across from the existing Huia WTP site. A new 25ML treated water reservoir will be located on the northern side of Woodlands Park Road (Reservoir 1), with another 25ML reservoir (Reservoir 2) subsequently constructed on the existing Huia WTP site once the existing plant has been decommissioned.

While the Project Site is within Watercare land designated for water supply purposes (water treatment plants and associated structures) in the Auckland Unitary Plan, it is also identified as part of an extensive Significant Ecological Area (SEA_T_5539) that essentially encompasses the entire Waitakere Ranges. Native forest and scrub cover 3.5 ha of the total 4.3 ha construction footprint.

The construction footprint encompasses secondary vegetation communities (kanuka and mahoe-dominated) of varying age and condition. Old-growth kauri and podocarp forest remnants are present immediately adjacent to the site.

The Project Site is in the headwaters of two Waituna Stream tributaries, including Armstrong Stream to the west and Yorke Stream to the east. Little Muddy Creek estuary is the receiving environment for the site.

The Project Site adjoins Clarks Bush, a public reserve containing several very large kauri trees, and for many years the Watercare land was essentially managed as part of the reserve. A walking track through the reserve intersects the proposed construction footprint for the WTP.

Kauri forest interspersed with residential development dominates the broad ridgelines immediately southward of the Project Site. Kauri dieback (causal agent *Phytophthora agathidicida*, PA) is a chronic, currently incurable disease affecting kauri trees of all ages (Waipara et al., 2013), and possibly also affecting other native plant species. Kauri dieback disease is caused by a soil and water borne primary pathogen of New Zealand kauri (*Agathis australis*). The above-ground symptoms of kauri dieback infection include yellowing of the leaves, thinning of the canopy and lesions on the lower stem which often encircle the base and produce copious amounts of resin (kauri gum).

The disease is known to be present throughout some, but not all, kauri areas within the Auckland region and surveys suggest that the extent of symptomatic trees in the region has substantially increased in recent years. However, with the "disease" expression lagging behind fine-root infection, not all healthy-looking forest can be assumed to be free of PA, due to the latent-phase of the disease process.

Kauri dieback has a wide distribution, both on a local and regional scale and is found on private, and public land. In Auckland it is most prevalent in the Waitākere Ranges Regional Park, with nearly 19% of the dense kauri forest within the regional park found to be infected with kauri dieback, and nearly 5% possibly infected. There is also kauri dieback present on local parks and private land throughout West Auckland, private land on the Awhitu Peninsula, and in North Auckland, local parks on the North Shore, and on public land in Albany, Okura, Pakiri, and Aotea and Hauturu.

However, some substantial tracts of kauri ecosystems remain non-symptomatic and spread prevention and containment are top management priorities.

A total of six phytophthora species are known to occur in the kauri forest: *P. agathidicida*, *P. chlamydospora*, *P. cinnamomi*, *P. kernoviae*, *P. multivora*, and *P. nicotianae* (Scott and Williams, 2014). All are considered exotic except for *P. kernovieae* (Studholme et al. 2016).

3. The problem

The proposed works footprint for both the replacement WTP and Reservoir 1 extend into an area of mature kauri trees, and stands of kauri are present nearby, both within the Project Site and in adjacent residential and reserve land to the south. No mature kauri had been reported within the works footprint itself. Kauri seedlings and saplings had been found within the WTP footprint, in the vicinity of large trees near the southern boundary. Systematic assessment of kauri presence in the forest directly adjacent to the project site had not been conducted but saplings had been observed and mature kauri have been mapped within the forest.

Symptoms consistent with kauri dieback (collar rot and gummosis) had been observed on a single large kauri tree within a mature kauri forest stand on the northern side of Woodlands Park Road (north-western corner of the Project Site). Auckland Council recorded a tree with kauri dieback symptoms in a similar location in 2009, and this may be the same tree. Auckland Council has recently identified a symptomatic tree near the northern Project Site boundary (on the escarpment above Exhibition Drive Walk) via aerial surveillance, and a typical "gummy" lower-trunk lesion was noted on a tree adjacent to the existing WTP in December 2019.

No laboratory testing had previously been undertaken within the project site to ascertain the presence of *Phytophthora agathidicida* or other phytophthora implicated in kauri decline, but the BioSense team has worked extensively in the Waitakere Ranges and Titirangi area (Bellgard et al. 2014; Hill et al. 2016) and shown that *P.agathidicida*, *P. cinnamomi*, *P. multivora*, *P. kernoviae*, and *P. chlamydospora* are all present in the surrounding area (Bellgard et al. 2013).

Spread of kauri dieback from the site into the surrounding catchment and beyond has been identified as a potentially risk of the proposed WTP development, due to the extent and volume of earthworks required, and the substantial quantity of soil to be transported and disposed of offsite.

4. Overview of the solution

BioSense was requested to develop and implement a field-testing protocol to assess the presence and distribution of *Phytophthora agathidicida,* the kauri dieback causing pathogen within the proposed construction footprint, the wider Project Site, and within a surrounding buffer area.

The objective of this work was to develop and implement sampling protocol to test for:

1 The disease status of kauri trees within and surrounding the proposed works footprint.
2 The presence and distribution of phytophthora in soil within the Project Site and a surrounding buffer.
3 The presence and distribution of phytophthora in soil within the Project Site and a surrounding buffer.

A sampling protocol was directed to address the following prescription:

- The buffer is to be of sufficient extent to encompass interconnected root systems of vegetation within and surrounding the Project Site, so the risk that these networks may as a pathway move phytophthora into or out of the site can be assessed.
- Watercourses are identified as a key potential vector for the spread of kauri dieback disease.
- Sampling will include all sites of human and feral animal disturbance.
- Sample collection and analysis methods will seek to maximise detection probability within practical limits, i.e., the scale and pattern of sampling is to give the best chance that kauri dieback-causing pathogens would be detected if present within the construction footprint and wider Project Site.

Sampling was also requested to test for *Phytophthora cinnamomi* presence due its known co-occurrence and impact upon kauri (Podger and Newhook 1971). *Phytophthora multivora* was suggested as a pathogen to test for but a LAMP-based diagnostic does not exist for this species of *Phytophthora*.

The methodology was developed and submitted to Watercare. It was peer reviewed by Dr Nick Waipara and Jack Craw (on behalf of Titirangi Residents Association), David Havell (Department of Conservation), and Dr Murray Fea (Auckland Council) before being agreed to by Watercare and implemented by BioSense.

BioSense recognises the importance of mana whenua as kaitiaki of the ngahere. It was highlighted that the surveillance was being conducted within the rohe of Te Kawerau ā Maki and acknowledged that Te Kawerau ā Maki had been informed of the surveillance project during consultation on the HTP development and were supportive of the surveillance being conducted.

5. Approach and methodology

A core concept to this investigation was to examine the presence/absence and distribution of kauri pathogens within and surrounding the Project Site and to provide levels of confidence in the data delivered.

The national Kauri Dieback Programme has not yet bench-marked the effectiveness or efficiency of the conventional soil bioassay protocol developed in 2010. Consequently, we do not yet have an estimate of the frequency of isolation of *P. agathidicida* which can be used to carry out a power analysis and/or deliver confidence of freedom of disease for this study. Therefore, The investigation could not produce statistical significance of the results generated to provide confidence via power analysis. The design has been based solely on current knowledge and expert opinion.

The surveillance and sampling design took a multi-layered approach based on identified risk factors. The approach utilised the concept of layering of risk factors to create higher levels of sampling within higher risk areas. The risk factors/layers of sampling targeted were:

- Grid based survey of risk – survey of the entire site in a 25 m x 25 m grid pattern to identify Kauri dieback related risk factors. No samples taken but data generated to inform future sampling
- Stratified sampling - uniform sampling on a 25 m x 25 m grid pattern across the entire site
- Kauri rootzone sampling – kauri health survey and soil sample from all kauri across the site
- Watercourse sampling – a sample at points where a watercourse intersects a project site boundary, construction boundary and buffer zone boundary and 25 m (linear) spacings along the watercourse.
- Track sampling – a sample at points where a track intersects a project site boundary, construction boundary and buffer zone boundary and 25 m (linear) spacings along the track
- Animal disturbance sampling – a sample at the point of disturbance. If the disturbance was longer than 25 m then samples were also taken every 25 m along the disturbance.

A site visit informed development of the buffer zone surrounding the project site and took into consideration, slope, vegetation, and physical factors such as the soil type. This was compared with the activity which has and will be occurring on site and the known biology and movement of the phytophthoras being investigated. A buffer zone of 100 m around the project site boundary was applied and this was then clipped to remove private property and hard surfaces, effectively limiting the buffer zone area to south eastern section of the site, extending into Clarks Bush Reserve. This is to address the brief of investigating the presence of phytophthora in the adjacent reserve but rather than investigating the whole reserve the design focused on intensive sampling in the risk area associated with the proposed Watercare works. Inclusion of private properties was considered when designing the buffer however it was determined that extending sampling in to private properties could be logistically difficult and would also be sampling where the risks of an potential future phytophthora introduction is beyond the control of Watercare with no way of identifying if any new introductions to those areas were due to the construction work or simply by the private landowners.

5.1 Grid based search to identify risk

Data supplied by Watercare gave indication of risk factors and allowed preliminary planning and display of the option. However, systematic surveillance of kauri through the buffer area and in-depth identification of animal disturbance across the entire site had not been conducted. A systematic grid-based search of kauri, watercourses and human and animal disturbance was conducted to add to data already supplied. This was used to identify points of additional sampling at any new locations of risk which are identified.

5.2 Stratified sampling

Phytophthora cinnamomi is known to be associated with non-kauri vegetation and knowledge about prevalence of *Phytophthora agathidicida* in non-kauri areas is unknown but expected. Bellgard et al. (2014) found PA in 19% of asymptomatic trees, compared with 60% of symptomatic trees. Nonetheless, all those samples were taken within a stand with many unhealthy kauri in proximity (< 50 m linear distance) to asymptomatic tree samples; hence these results cannot be extrapolated to stands of entirely healthy trees. Knowing how widespread *P. agathidicida* is in asymptomatic forests would have potentially profound impacts on the management strategies. For example, if *P. agathidicida* is widespread in a non-symptomatic state, then management of spread would be a lower priority and suggest a greater focus on precipitating causes of forest dieback (Black and Dickie 2016).

Testing of host-range of *P. agathidicida* is currently underway however recent research has confirmed that non-kauri areas adjacent to kauri forest, such as *Pinus radiata* forest and grass pasture can also harbour and host the kauri dieback pathogen (Lewis et al. 2019). Therefore, systematic sampling of kauri and non-kauri areas is essential to determine *Phytophthora* presence and distribution across the area.

A grid-based sampling regime of the entire site was implemented. Sampling occurred on a 25 m by 25 m grid pattern across the construction site, project site and buffer area with 250 g of soil/root being taken from each point across the grid. The design excluded areas of hard surface such as concrete drives and buildings.

A map of the stratified sampling locations is shown in appendix 3.

5.3 Kauri rootzone sampling

All kauri within the construction footprint, project site and buffer area were physically tagged, GPS location recorded and assessed for kauri dieback symptoms. The sampling followed the national 'Kauri Dieback Soil Sampling Guide' guidelines with the exception that all kauri were sampled rather than three trees per stand.

Eight points around a tree were sampled: four inner points and four beneath the outer edge of the canopy, which are then bulked into one single soil sample with a minimum mass of 250 g, relating to that kauri rootzone.

A map of the kauri rootzone sampling locations is shown in appendix 4.

5.4 Watercourse sampling

Phytophthora agathidicida is a soil-borne water mould and we know its movement is linked to soil physiological and hydrological factors. While intense investigation of the risk associated with watercourses and *Phytophthora agathidicida* has not been conducted, watercourses have been confirmed as a vector of *Phytophthora* and other allied genera such as *Pythium* and *Nothophytophthora* (O'Hanlon et al. 2016; Bellgard et al. 2017).

Because of the variation in the types, depth, and permanency of the watercourses in and around the Project Site, a nested sampling approach was employed, utilising the traditional "stream-baiting" approach in areas of flowing water, as well opportunistic "grab-samples" from non-permanent, ephemeral pools, and "*soil sampling*" from points along the watercourse where there is no flowing or standing water.

Stream baiting from areas of flowing water
For the monitoring of perennial streams, traditional stream baiting was conducted using the sampling technique described by Randall (2011). Plant leaves (cedar and pine needles) were placed in plastic "bait-cassettes" and placed in the stream course for two-weeks. At each sampling location, there were two sampling cassettes, pre-loaded with fifteen cedar and pine needles, and linked together. The "bait-cassettes" were submerged in the stream at depth of 30 cm below the water surface and secured to one side of the bank. After two weeks the needles were removed from the cassettes, washed in water and frozen.

Grab-samples from "seasonal" streams and/or pools
In the absence of flowing streams, filtration of one litre "grab samples" of water was collected from each "pond". Filtration was validated as an effective method for detecting *P. ramorum* in streams in California where this pathogen previously had been recovered (Hwang et al. 2008). Filtration was found to be more effective and efficient than the "baiting method" for detection of diverse populations of *Phytophthora* species in forest streams.

Soil samples from areas of watercourse with no flowing or standing water
Watercourses still reflect a potential past and present risk pathway for the movement of phytophthora and so sampling still occurred in the absence of water. To maintain consistency in the approach, in areas where "*stream-baiting*" or "*grab sampling*" was not possible due to lack of water a soil sample was taken. A 250 g sample of the soil will be collected and assessed for phytophthora presence and identification.

A sample was taken at points where a watercourse intersects a project site boundary, construction boundary and buffer zone boundary. Additional samples were collected at 25 m (linear) spacings along the watercourse.

A map of the watercourse sampling locations is shown in appendix 5.

5.5 Track sampling

The phytophthora species being investigated spreads in soil and with soil if it is moved. Within known areas of phytophthora presence factors such as slope, host distribution and drainage are likely to be influencing the spread of phytophthora from the initial point of infection. These are factors which influence the rate of spread, vegetation infected and the overall size and shape of the affected area. However, evidence suggests that the highest risk vector for phytophthora movement into new distinct locations is soil disturbance associated with human activity.

Phytophthora agathidicida has been isolated from track soils as well as soil removed from tramping boots (Ian Horner *pers comm.* 2014; Pau'Uvale et al. 2011) and the risk associated with kauri dieback movement and track networks have been highlighted by reports produced by the Kauri Dieback Programme (Hill et al. 2012).

A 250 g soil sample was taken at points where a track intersects a project site boundary, construction boundary and buffer zone boundary. Additional samples were collected at 25 m (linear) spacings along the track.

A map of the track sampling locations is shown in appendix 6.

5.6 Animal disturbance sampling

Feral pigs have been implicated in the spread of *Phytophthora cinnamomi* in the O'hia forests of Hawai'i (Kleijunas and Ko 1976). Krull et al. (2012) identified that pigs have the capacity to spread *Phytophthora cinnamomi* on their trotters. Therefore, signs of fresh animal disturbance were surveyed and geo-referenced. Instances human-mediated disturbance (e.g. farm infrastructure) were sampled, as these may represent a historical pathway for soil movements.

A 250 g soil sample was taken from the point of disturbance, if the disturbance was longer than 25 m (for instance a sites of illegal driving across the site viewed on the site visit) then samples were taken every 25 m along the disturbance.

A map of the animal disturbance sampling locations is shown in appendix 7.

5.7 Sample processing and testing

The traditional bioassay for kauri dieback detection involves flooding and baiting of soil samples followed by plating of plant tissue baits onto phytophthora selective media and visual identification of the pathogen. Recent research (Winkworth et al. 2020) suggests that competition among co-occurring oomycetes (e.g., *Phytophthora* and *Pythium* species) on the selective media can result in failure to detect *P. agathidicida*. Other *Phytophthora* have faster *in vitro* growth rates than *P. agathidicida* and will therefore tend to overgrow *P. agathidicida* making visual detection of this species difficult. To overcome this potential limitation of the conventional "bait-n-plate" methodology Winkworth et al. (2020) instead implemented an isothermal loop-mediated amplification (LAMP) assay for the detection of *P. agathidicida*. The LAMP bioassay makes use of the same baiting procedure as the traditional bioassay but instead of plating the plant tissue baits, total DNA (i.e., DNA from the plant bait and colonising microbes) is extracted from the baits and subjected to testing using the LAMP assay. Published and unpublished testing (Winkworth et al., 2020; Winkworth *pers comm*.) indicates that detection rates for *P. agathidicida*

are higher using the LAMP assay.

Soil samples
Baiting of soil samples was conducted under Physical Containment (PC) level 2 at Massey University, Palmerston North following a standard methodology for *P. agathidicida* (adapted form . The soils were first air-dried and then moist incubated for four days; the soils were then flooded with 500 ml of reverse osmosis (RO) water and five lupin four-day old lupin sprouts were floated on the water surface. After two days the lupin sprouts were removed; the radicals were removed using sterile technique, and immediately stored at -20°C.

DNA extractions and LAMP assays for *P. agathidicida* were carried out as described by Winkworth et al. (2020); for the present work tests were carried out on a Roche Lightcycler 480 II instrument rather than Diagenetix BioRanger devices as the former allow 96 samples to be tested simultaneously. Testing for *P. cinnamomi* was also conducted using a species-specific LAMP test (Winkworth et al., in prep). Each reaction set included multiple technical positive (i.e., DNA from known *P. agathidicida* or *P. cinnamomi* isolates) and negative (i.e., no DNA) controls. Additionally, sprouted lupin radicals, "baited" in RO water for two days were also included as "negative" controls.

Following baiting flooded soils were decontaminated using an MPI approved procedure (MPI ABTRT 2020).

Stream baits
Total DNA was extracted from frozen cedar and pine needle stream baits as described for soil tissue baits by Winkworth et al. (2020). Testing of bait DNA for *P. agathidicida* and *P. cinnamomi* followed the procedure described above for DNA from soil baits. Again technical positive and negative controls were included as well as DNA from cedar and pine needles not used for baiting.

Grab samples
Grab samples were vacuum filtered onto glass filters with 1.6 µm pore sizes; where necessary due to high sediment load two or three filters were used. DNA was extracted from up to one quarter of a filter using the Mackeray-Nagel Nucleospin Soil kit and Water DNA extraction kits. Testing of grab sample DNA for *P. agathidicida* and *P. cinnamomi* followed the procedure described above for DNA from baits. Again technical positive and negative controls were included.

6. Results

6.1 *Phytophthora agathidicida* and *P. cinnamomi* detections

The layered approach to sampling has led to a wide distribution of sampling locations across the entire site with a higher density of sampling in areas identified as higher risk of *Phytophthora agathidicida* presence. A map of all sampling locations is shown in appendix 8.

In total 996 samples were taken as part of the kauri dieback surveillance of the proposed water treatment plant site. Analysis detected *Phytophthora agathidicida* in 154 samples and *Phytophthora cinnamomi* in 128 samples (Table 1).

Table 1. Results of Phytophthora agathidicida and Phytophthora cinnamomi sampling across Watercare's proposed water treatment plant site and adjacent reserve.

Sampling layer	Number of samples	Number of *Phytophthora agathidicida* detections	Number of *Phytophthora cinnamomi* detections
Stratified sampling	273	26	5
Kauri rootzone sampling	461	83	108
Watercourse sampling	114	23	7
Track sampling	99	18	3
Animal disturbance sampling	49	4	5
Total	**996**	**154**	**128**

A map of the kauri health survey and complete site detection results is shown in appendix 9.

6.2 Stratified sampling

This generated 273 stratified samples across the site. Analysis detected *Phytophthora agathidicida* in 26 samples and *Phytophthora cinnamomi* in 5 samples.

A map of the stratified sampling detection results is shown in appendix 10.

6.3 Kauri rootzone sampling

In total 431 kauri sapling size and above were recorded during the surveillance:

- 190 were exhibiting no symptoms of kauri dieback.
- 184 were exhibiting ill-thrift
- 57 were exhibiting symptoms of kauri dieback

In addition, 15 kauri seedling clusters were recorded. No seedling clusters were exhibiting symptoms of kauri dieback.

Six young ricker kauri were recorded within the Extent of Works area.

A map of kauri and kauri dieback status shown in appendix 11.

Each of the individual kauri and seedling clusters were sampled resulting in 461 kauri rootzone samples. Analysis detected *Phytophthora agathidicida* in 83 samples and *Phytophthora cinnamomi* in 108 samples.

A map of the kauri rootzone sampling detection results is shown in appendix 12.

6.4 Watercourse sampling

The Project Site is in the headwaters of two Waituna Stream tributaries, including Armstrong Stream to the west and Yorke Stream to the east. Little Muddy Creek estuary is the receiving environment for the site. In addition to the streams a grid-based search of the area highlighted

an additional 70 areas of standing/pooling water or areas where previous water movements were obvious.

This generated 114 watercourse samples across the site. Analysis detected *Phytophthora agathidicida* in 23 samples and *Phytophthora cinnamomi* in 7 samples.

A map of the watercourse sampling detection results is shown in appendix 13.

6.5 Track sampling

Exhibition Drive Track and Clarkes Bush Track transect the site. The grid search also highlighted an additional informal track network which appeared to have semi-regular use.

This generated 99 track samples across the site. Analysis detected *Phytophthora agathidicida* in 18 samples and *Phytophthora cinnamomi* in 3 samples.

A map of the track sampling detection results is shown in appendix 14.

6.6 Animal disturbance sampling

The grid search discovered no signs of pig or large animal presence within the site. 49 instances of animal disturbance were recorded, and all of these were associated with humans, mainly associated with pest management activities.

This generated 49 animal disturbance samples across the site. Analysis detected *Phytophthora agathidicida* in 4 samples and *Phytophthora cinnamomi* in 5 samples.

A map of the animal disturbance sampling detection results is shown in appendix 15.

6.7 Positive and negative control samples

The investigation tested 6 'positive controls' using soil samples collected from a private property in the local area with previously confirmed area of *Phytophthora agathidicida*. Analysis detected *Phytophthora agathidicida* in all 6 samples and *Phytophthora cinnamomi* in 5 samples.

The investigation tested 2 'negative controls' using baits floated on water with no soil. Analysis did not detect *Phytophthora agathidicida* or *Phytophthora cinnamomi* in either of the samples.

6.8 Detection buffering

Although sampling for kauri dieback in non-kauri areas has not previously been conducted on this scale, it is accepted practice that for site management purposes such as the works proposed for the development of the water treatment plant, a 30 m buffer is applied to the point of *Phytophthora agathidicida* detection. A map of *Phytophthora agathidicida* and *Phytophthora cinnamomi* detections with a 30 m buffer is shown in appendix 16.

7. Conclusions

A field survey confirmed the presence of kauri dieback symptoms within the Project Site, and surrounding buffer within Clarks Bush.

Analysis of soil samples detected the presence of *Phytophthora agathidicida* and *Phytophthora cinnamomi* in soil taken from the Project Site, and adjoining buffer area within Clarks Bush.

Analysis of water samples detected the presence of *Phytophthora agathidicida* and *Phytophthora cinnamomi* in water taken from the Waituna stream tributary network within the Project Site, and adjoining buffer area within Clarks Bush.

8. References

Bellgard, S.E., Weir, B.S., Pennycook, S.R., Paderes E.P., Than, D.J., Anand, N. and Beever, R.E.† 2014. Specialist research into the biology, pathology, ecology, and detection of PTA. Landcare Report.

Bellgard, S.E., Probst, C.M., Parry, D., Jacobs, R., Waipara, N.W., Kelly, L., Ratcliffe, S., Weir, B.S, Pattison, N. 2017. "Unlocking a Nation of Curious Minds": a science participatory platform for the next generation of *Phytophthora* scientists. SciPlant Conference, Brisbane, September 2017.

Black, A., Dickie, I. 2016. Independent review of the state of kauri dieback knowledge. Report for Chair of Kauri Dieback Leadership Team. Bio-Protection Research Centre, Lincoln University.

Bradshaw, R.E., Bellgard, S.E., Black, A., Burns, B.R., Gerth, M.L., McDougal, R.L., Scott, P.M., Waipara, N.W., Weir, B.S., Williams, N.M., Winkworth, R.C., Ashcroft, T., Bradley, E.L., Horner, I.J. 2019. *Phytophthora agathidicida*: Research progress, cultural perspectives and knowledge gaps in the control and management of kauri dieback in New Zealand. Plant Pathology – Special Edition. Doi: 10.1111/ppa.13104

Dance, M, Newhook F.J., and Cole J.S. 1975. Bioassay of Phytophthora spp. in soil. Plant Disease Reporter 59(6), 523-527.

Davison, E.M. and Tay, F.C.S. 2005. How many soil samples are needed to show that Phytophthora is absent from sites in the south-west of Western Australia? Australasian Plant Pathology 34, 292-297.

Dunstan WA, Rudman T, Shearer BL, Moore NA, Paap T, Calver MC, Armistead R, Dobrowolski MP, Morrison B, Howard K, O'Gara E, Crane C, Dell B, O[1]Brien P, McComb JA, Hardy GEStJ (2008) Research into natural and induced resistance in Australian native vegetation of Phytophthora cinnamomi and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia. Eradication of Phytophthora cinnamomi from spot infections in native plant communities in Western Australia and Tasmania. Prepared by the Centre for Phytophthora Science and Management for the Australian Government Department of the Environment, Water, Heritage, and the Arts

Hill, L.M.W., Davis, A., Waipara, N.W. 2012. An investigation into the distribution and spread of kauri dieback in the Waitakere Ranges. Auckland Council Technical Surveillance Report 80pp.

Hwang, J., Oak, S.W., Jeffers, S.N. 2008. Detecting Phytophthora ramorum and Other Species of Phytophthora in Streams in Natural Ecosystems Using Baiting and Filtration Methods. Proceedings of the Sudden Oak Death Third Science Symposium.

Jones, S.R., Carly, S., and Harrison, M. 2003. A introduction to power and sample size estimation. Emergency Medical Journal 20(5):453-8. doi: 10.1136/emj.20.5.453.

Kliejunas, J. T. and Ko, W. H. 1976. Dispersal of Phytophthora cinnamomi on the island of Hawaii. Phytopathology 66: 457-460

Krull, C., Waipara, N.W., Choquenot, D., Burns, B., Gormley, A., and Stanley, M. 2012. Absence of evidence is not evidence of absence: Feral pigs as vectors of soil-borne pathogens. Austral Ecology doi:10.1111/j.1442-9993.2012.02444.x.

Lewis, K., Black, A., Condron, L.M., Waipara, N.W., Scott, P., Williams, N., and O'Callaghan, M. 2019. Land-use changes influence the sporulation and survival of *Phytophthora agathidicida* , a lethal pathogen of New Zealand kauri (*Agathis australis*). Forest Pathology https://doi.org/10.1111/efp.12502.

MPI ABTRT 2020. Approved Biosecurity Treatments, Ministry of Primary Industries, Wellington.

O'Hanlon, R., Choiseul, J., Corrigan, M., Catarame, T. and Destefanis, M., 2016. Diversity and detections of Phytophthora species from trade and non-trade environments in Ireland. EPPO Bulletin, 46(3), pp.594-602.

Pau'Uvale, A., Dewan, C., Mora, H., Waipara, N., and Bellgard, S.E. (2011). Kauri killer on the loose? – a study of PTA vectors and hygiene treatments. Liggins Institute, Auckland University.

Podger, F.D., and Newhook, F.J. 1971. Phytophthora cinnamomi in indigenous plant communities in New Zealand. New Zealand Journal of Botany 9, 625-638.

Randall, S. 2011: Fishing for Phytophthora: A year-long investigation into the diversity of Phytophthora species in the Waitakere Ranges, Auckland, NZ. Unpublished MSc Thesis, University of Auckland.

Scott, P.M. and Williams, N.M. 2014. Phytophthora diseases in New Zealand forests. NZ Journal of Forestry 59(2), 14-21.

Studholme D.J., McDougal, R.L., Sambles, C., Hansen, E., Hardy, G., Grant, M, Ganley, R.J., Williams, N.M. 2016. Genome sequences of six Phytophthora species associated with forests in New Zealand. http://dx.doi.org/10.1016/j.gdata.2015.11.015.

Waipara, N.W., Hill S., Hill, L.M.W., Hough, E.G., Horner, I.J., 2013. Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. New Zealand Plant Protection 66: 235-241.

Weir, B.S., Paderes, E.P., Anand, N., Uchina, J., Pennycook, S.R., Bellgard, S.E., Beever, R.E., 2015. A taxonomic revision of Phytophthora Clade 5 including two new species, *Phytophthora agathidicida* and P. cocois. Phytotaxa ISSN 1179-3155. Phytotaxa 205 (1): 021–038.

Winkworth, R., Nelson, B.C.W., Bellgard, S.E., Probst, C.M., McLenachan, P.A., Lockhart, P.J. 2020. A LAMP at the end of the tunnel: a rapid, field deployable assay for the kauri dieback pathogen, *Phytophthora agathadicida.* PLOS One. 15(1): e0224007.https://doi.org/10.1371/journal. pone.0224007

9. Appendix

Appendix 1 – Selected bibliography of this team's kauri dieback related outputs

Technical reports

Horner, I.J., Arnet, M., **Bellgard, S.E.**, Probst, C.M., Paynter, Q. 2019. Heat deactivation of oospores of *Phytophthora agathidicida* in soil, plant, and gravel. Technical Report for Kauri Dieback Program (KDP), MPI.

Bellgard, S.E., Probst, C.M. 2018. 18883: Oospore deactivation of Phytophthora agathidicida: desktop review: alkaline-based solution. Biosecurity New Zealand Technical Paper 2018/02.

Bellgard, S.E., Probst, C.M. 2018. Oospore deactivation of oospores of *Phytophthora agathidicida:* desk top review: alkaline based solutions. Technical Report for KDP, MPI.

Hill, L.M.W., Stanley, R., Hammon, C., Waipara, N.W. 2017 An investigation into the distribution of kauri dieback, and implications for its future management, within the Waitakere Ranges Regional Park. Auckland Council Technical Surveillance Report 40pp.

Hill, L.M.W., Stanley, R.: Waipara, N.W. 2017 A study to evaluate the effectiveness of various commercially available agri-products as potential biological control agents of kauri dieback (Phytophthora agathidicida). Auckland Council Technical Research Report 40pp.

Bellgard, S.E., Weir, B.S., Pennycook, S.R., Paderes E.P., Than, D.J., Anand, N. and Beever, R.E.† 2014. Specialist research into the biology, pathology, ecology, and detection of PTA. Landcare Report.

Hill, L.M.W., Davis, A., Waipara, N.W. 2012. An investigation into the distribution and spread of kauri dieback in the Waitakere Ranges. Auckland Council Technical Surveillance Report 80pp.

Bellgard, S.E., Dick, M.A., Horner, I.J. 2011. Analysis of kauri dieback soil samples, Phase 1 Final Report. MAF Biosecurity New Zealand on behalf of the Kauri Dieback Joint Agency Response (KDJAR).

Beever, R.E., **Bellgard, S.E.**, Dick, M.A., Horner, I.J., Ramsfield, T.D. 2010. Detection of Phytophthora taxon Agathis (PTA): Final Report. Prepared for MAF, Biosecurity New Zealand on behalf of the KDJAR.

Bellgard, S.E., Paderes, E.P., Beever, R.E. 2010. Kauri Dieback: Kauri Hygiene - small project for KDJAR on behalf of MAF, BNZ

Peer-reviewed papers

Winkworth, R., Nelson, B.C.W., **Bellgard, S.E.**, Probst, C.M., McLenachan, P.A., Lockhart, P.J. 2020. A LAMP at the end of the tunnel: a rapid, field deployable assay for the kauri dieback pathogen, *Phytophthora agathidicida.* PLOS One. 15(1): e0224007.https://doi.org/10.1371/journal. pone.0224007

Bradshaw, R.E., **Bellgard, S.E.**, et al. 2019. *Phytophthora agathidicida*: Research progress, cultural perspectives and knowledge gaps in the control and management of kauri dieback in New Zealand. Plant Pathology – Special Edition. Doi: 10.1111/ppa.13104

Bellgard, S.E., Padamsee, M., Probst, C.M., Lebel, T., Williams, S.E. 2016. Visualising the early infection of *Agathis australis* by *Phytophthora agathidicida* using microscopy and fluorescent in situ hybridisation. Forest Pathology doi: 10.1111/efp.12280.

Padamsee, M., Johansen, R.B., Stuckey, S.A., Williams, S.E., Hooker, J.E., Burns, B.R., **Bellgard, S.E.** 2016. The arbuscular mycorrhizal fungi colonising roots of New Zealand Kauri Agathis australis. Fungal Biology 34, 1-11.

Bellgard, S.E., Pennycook, S.R., Weir, B.S., Ho, W., Waipara, N.W. 2015. Phytophthora agathidicida. Forest Phytophthoras 6(1). doi: 10.5399/osu/fp.5.1.3748

Jamieson, A.: Bassett, I.E.: **Hill, L.M.W.:** Hill, S.: Davis, A.: Waipara, N.W.: Hough, E.G.: Horner, I.J.: (2014) Aerial surveillance to detect kauri dieback in New Zealand. New Zealand Plant Protection 67: 60–65.

Than, D.J., Hughes, K.J.D., Boonham, N., Tomlinson, J.A., Woodall, J.W., **Bellgard, S.E.** 2013. A TaqMan real time PCR assay for Phytophthora 'taxon Agathis', in soil, pathogen of kauri in New Zealand. Forest Pathology doi: 10.1111/efp.12034

Waipara, N.W.; Hill, S.; **Hill, L.M.W.**; Hough, E.G. Horner, I.J. (2013). Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. New Zealand Plant Protection 66: 235–241.

Bellgard, M.I., **Bellgard, S.E.** 2011. A bioinformatic framework for plant pathologist to deliver global food security outcomes. Australasian Plant Pathol. DOI 10.1007/s13313-011-0099-x

Bellgard, S.E., Williams, S.E. 2011. Response of mycorrhizal diversity to current climatic changes. Diversity 3, 8-90.

Webber, J., Vettraino, A-M, Chang, T.T., **Bellgard, S.E.**, Brasier, C.M., Vannini, A. 2011. Isolation of Phytophthora lateralis from Chamaecyparis foliage in Taiwan. Forest Pathology doi: 10.1111/j.1439-0329.2011.00729.x

Book chapters

Bellgard, S.E., Weir, B.S. and Pennycook, S.R. 2018. Phytophthora root and collar rot of Agathis australis NZ kauri. Compendium of Conifer Diseases Second Edition. Ed.by EM Hansen et al. APS Disease Compendium Series. APS, St Paul Minnesota.

Beever, R.E., McKenzie, E.H.C., Pennycook, S.R., **Bellgard, S.E.**, Dick, M.A., and Buchanan, P.K. 2012. Phylum Oomycota: Water Moulds, Downy Mildews. Chapter 11: Volume III, New Zealand Inventory of Biodiversity Ed by D.P. Gordon, Canterbury University Press.

Conference presentations

Bellgard, S.E., Paderes, E.P, Probst, C.M. 2019. Root infections will necessitate the long-term management of site infected with *Phytophthora agathidicida.* 22nd Biennial Conference of the APPS, 25-28th November 2019. Melbourne, Australia.

Bellgard, S.E., Padamsee, M., Williams, S.E. 2018. Connecting microscopic interactions with macroscopic ecological impacts. 14th Great Yellowstone Ecosystem Biennial, Big Sky, Montana. September 2018

Bellgard, S.E., Probst, C.M., Parry, D., Jacobs, R., Waipara, N.W., Kelly, L., Ratcliffe, S., Weir, B.S, Pattison, N. 2017. "Unlocking a Nation of Curious Minds": a science participatory platform for the next generation of *Phytophthora* scientists. SciPlant Conference, Brisbane, September 2017.

Bellgard, S.E., Probst, C.M., Williams, R., Winks, C.J., Williams, N.M. 2016. Testing kauri for tolerance to P. agathidicida. Meeting of Working Party 7.02.09, Phytophthoras in Forest and Natural Ecosystems IUFRO, Sapa, Vietnam.

Bellgard, S.E., Pattison, N., Probst, C.M., Weir, B.S., Jacobs, R., Ratcliffe, S., Kelly, L., Waipara, N.W. 2016. A participatory science platform to engage the next generation of Phytophthora scientists. Meeting of Working Party 7.02.09, Phytophthoras in Forest and Natural Ecosystems IUFRO, Sapa, Vietnam.

Weir, B.A., Johnston, P.R., Park, D., Then, D.J., **Bellgard, S.E.** 2012. Species concept for PTA. Meeting of Working Party 7.02.09 Phytophthoras in Forest and Natural Ecosystems IUFRO, Cordoba, Spain.

Beever, R.E., Waipara, N.W., **Bellgard, S.E.**, Ramsfield, T., Horner, I.J., Dick, M.A., Tsai, S., Park, D., Johnston, P.R., Than, D.J. 2011. Kauri (Agathis australis) under threat from Phytophthora. Phytophthoras in Forest and Natural Ecosystems IUFRO 27.02.09, Rotorua, New Zealand.

Bellgard, S.E., Beever, R.E., Paderes, E.P. 2010. Comparative efficacy of disinfectants against Phytophthora Taxon Agathis (PTA). Phytophthoras in Forest and Natural Ecosystems IUFRO 27.02.09, Rotorua, New Zealand.

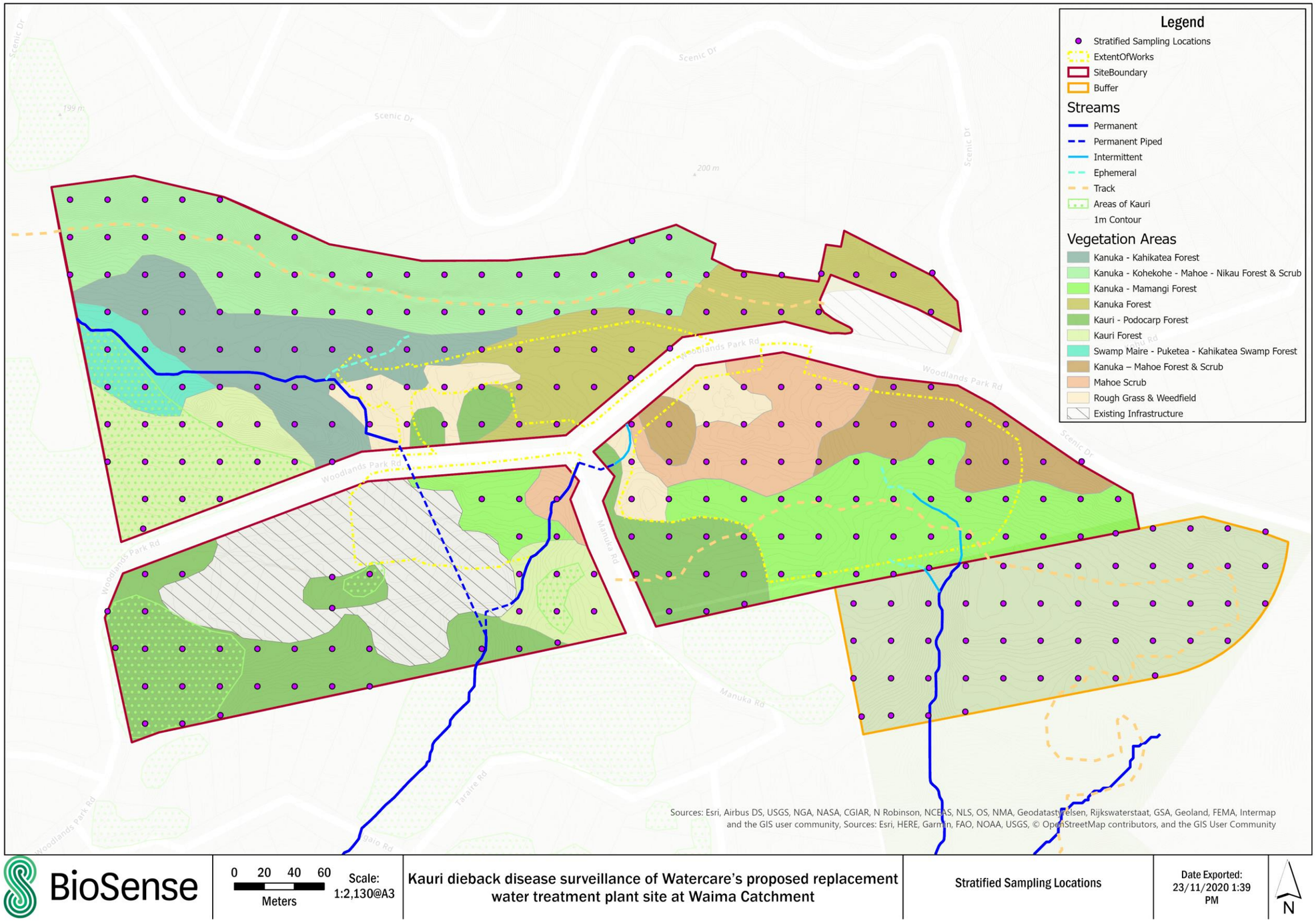
Appendix 2 – Selected bibliography of this team's kauri dieback related outputs

This team has carried out or have been involved in all the kauri dieback surveillance projects across since 2010 including, but not limited to, the following:

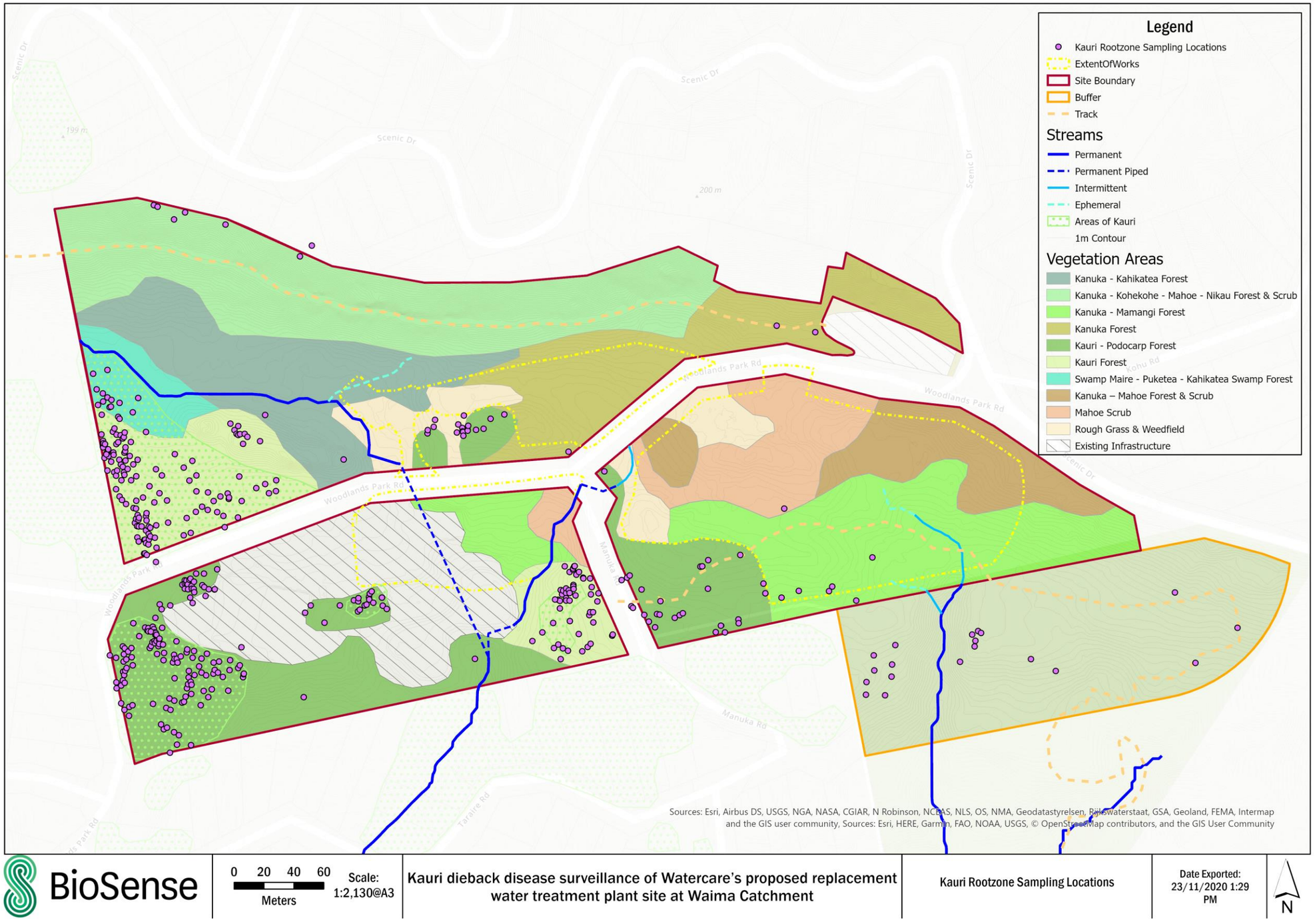
2010 – Investigation into kauri dieback symptomology and design of survey parameters.
2010 to 2018 – Design and implementation of Auckland Council's active kauri dieback surveillance plan.
2010 to 2018 – Design and implementation of Auckland Council's passive kauri dieback surveillance plan.
2010 – Aerial surveillance of Waitakere Ranges.
2010 – Groundtruthing and sampling of Waitakere Ranges.
2011 to 2012 – On-track survey of kauri health along the track network across Auckland Council-managed parks.
2014 – Aerial surveillance of Waiheke and Ponui Islands.
2015 – Design of RFP for PDH student to investigate the use of multispectral surveillance a tool for kauri dieback detection.
2015 – Technical support for investigation of the use of multispectral surveillance.
2015 – Field support for investigation of the use of multispectral surveillance.
2015 to 2018 – Progress review of investigation of the use of multispectral surveillance.
2016 – Aerial surveillance of Waitakere Ranges.
2016 to 2017 – Groundtruthing and sampling of Waitakere Ranges.
2016 to present – Designed, sort funding for and implemented Kauri Rescue.
2017 – Aerial survey of Hunua Ranges and Awhitu Peninsula.
2017 – Groundtruthing and sampling of Hunua Ranges and Awhitu Peninsula.
2018 – Aerial survey of Northern Auckland.
2018 – Groundtruthing and sampling of Northern Auckland.
2018 – Identification of kauri dieback with 60m of Tane Mahuta, Waipoua Forest.
2019 – Northland Kauri Cone Collection project as part of the Healthy Trees, Healthy Future programme.
2019 – Phosphite treatment and survey of kauri dieback area in Coromandel.
2019 – Phosphite treatment and survey of kauri dieback area in Auckland.
2019 – Groundtruthing and sampling of 170 potential kauri dieback sites across Northland.
2019 – Groundtruthing and sampling of 20 potential kauri dieback sites across Coromandel.
2020 – Phosphite treatment and survey of kauri dieback area in Coromandel.
2020 – Groundtruthing and sampling of potential kauri dieback sites across DOC land in Northland

This does not include the numerous workshops, events and seminars on kauri dieback and kauri dieback surveillance.

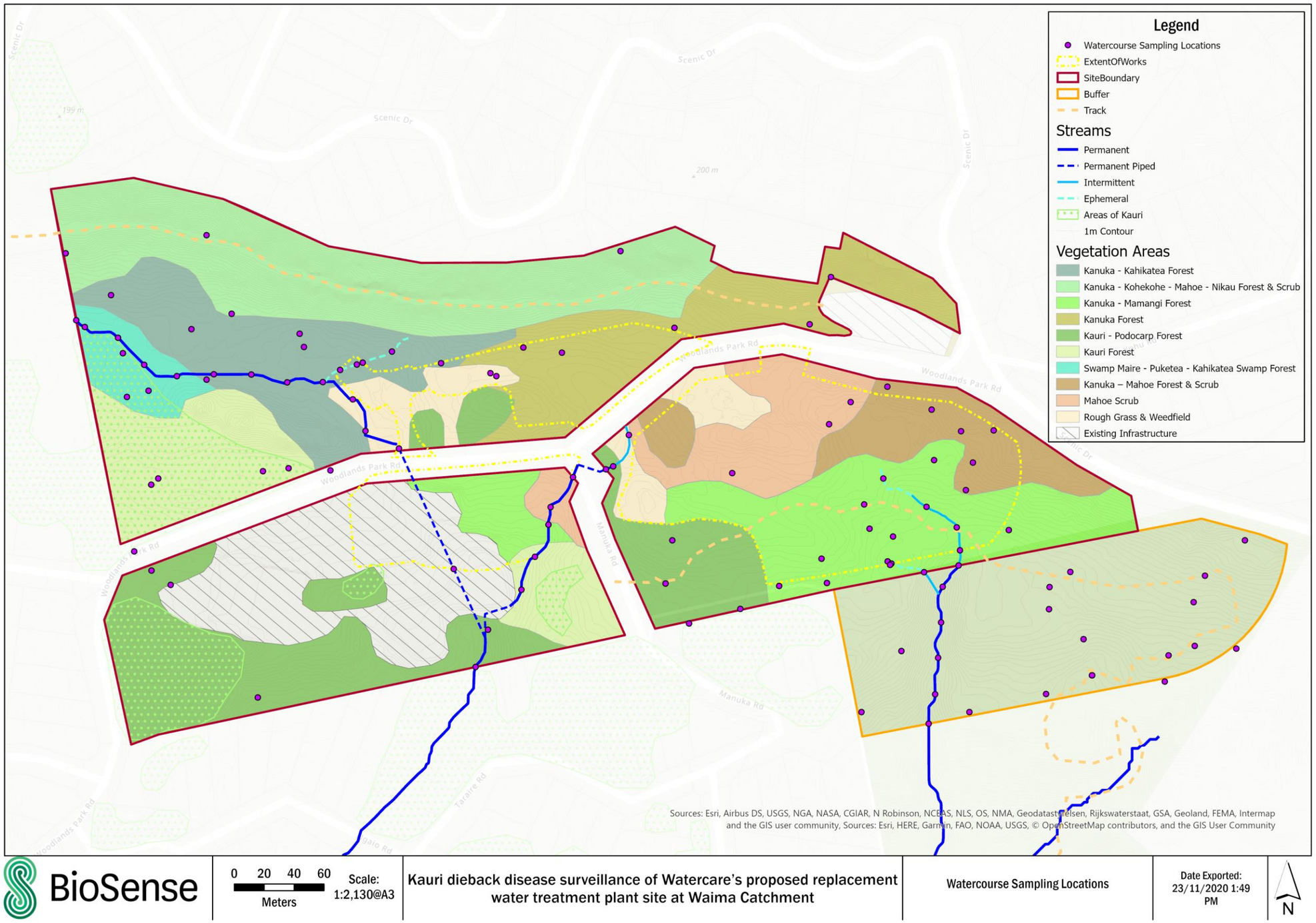
Appendix 3 – A map of stratified sampling locations



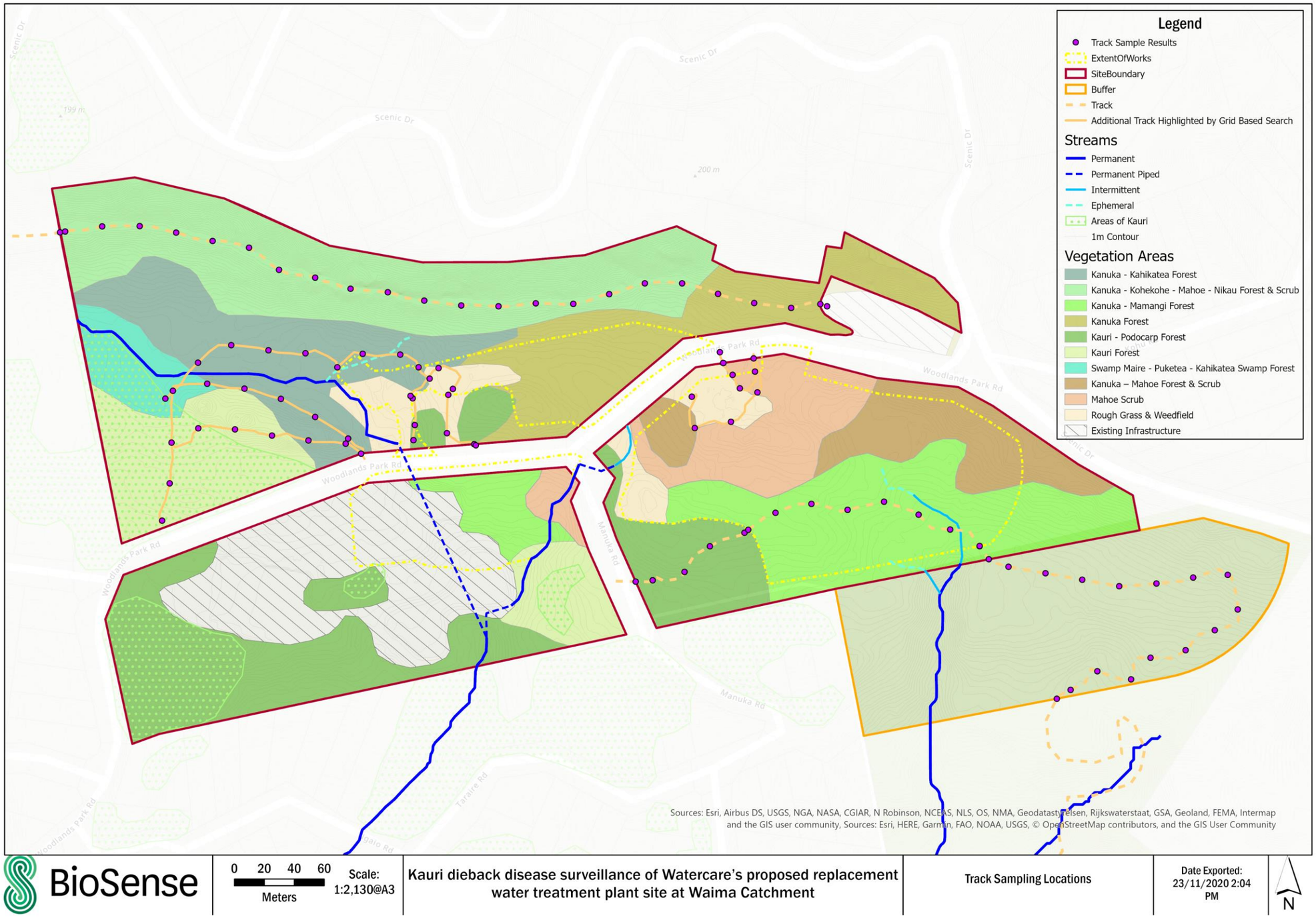
Appendix 4 – A map of the kauri rootzone sampling locations



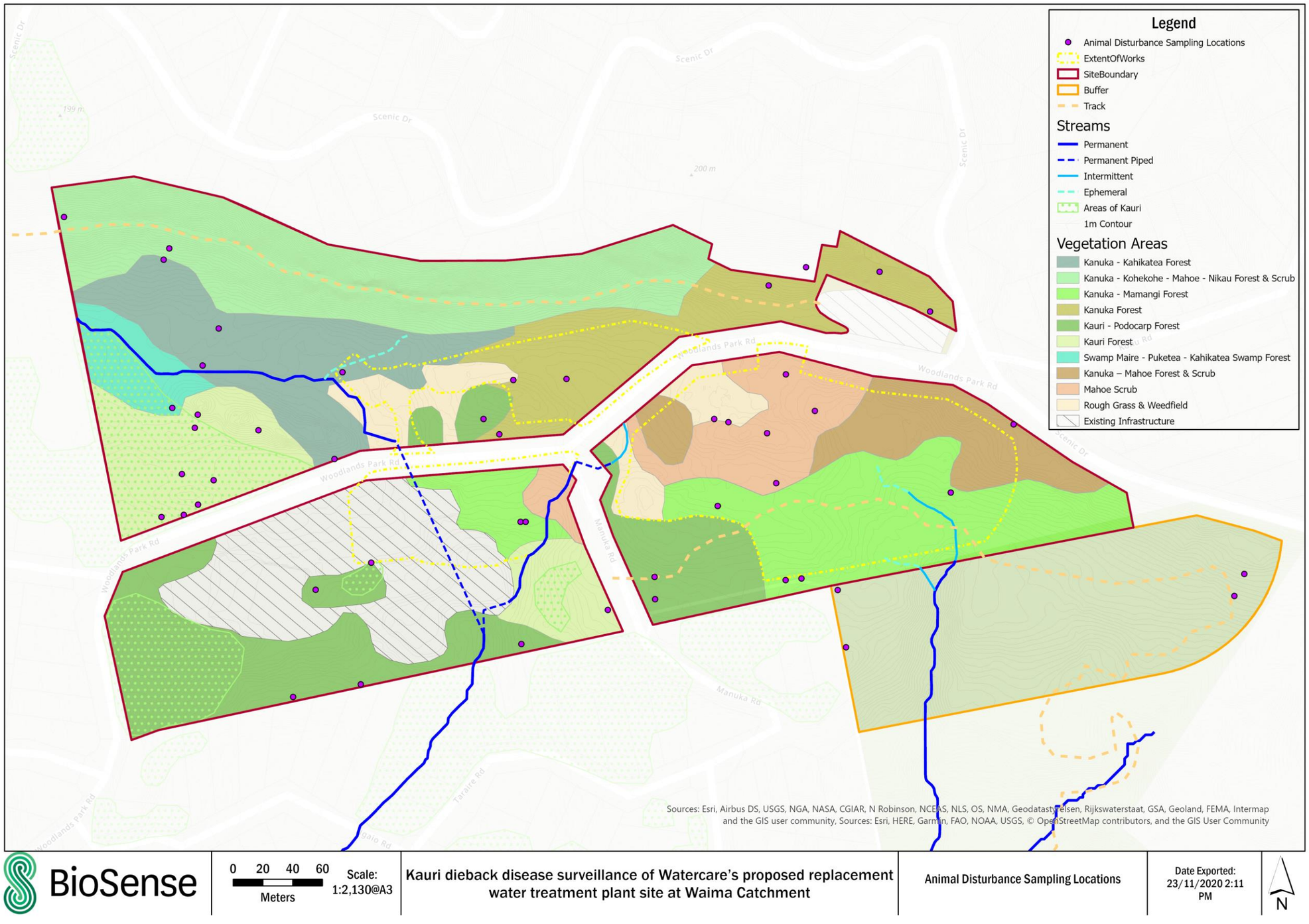
Appendix 5 – A map of watercourse sampling locations



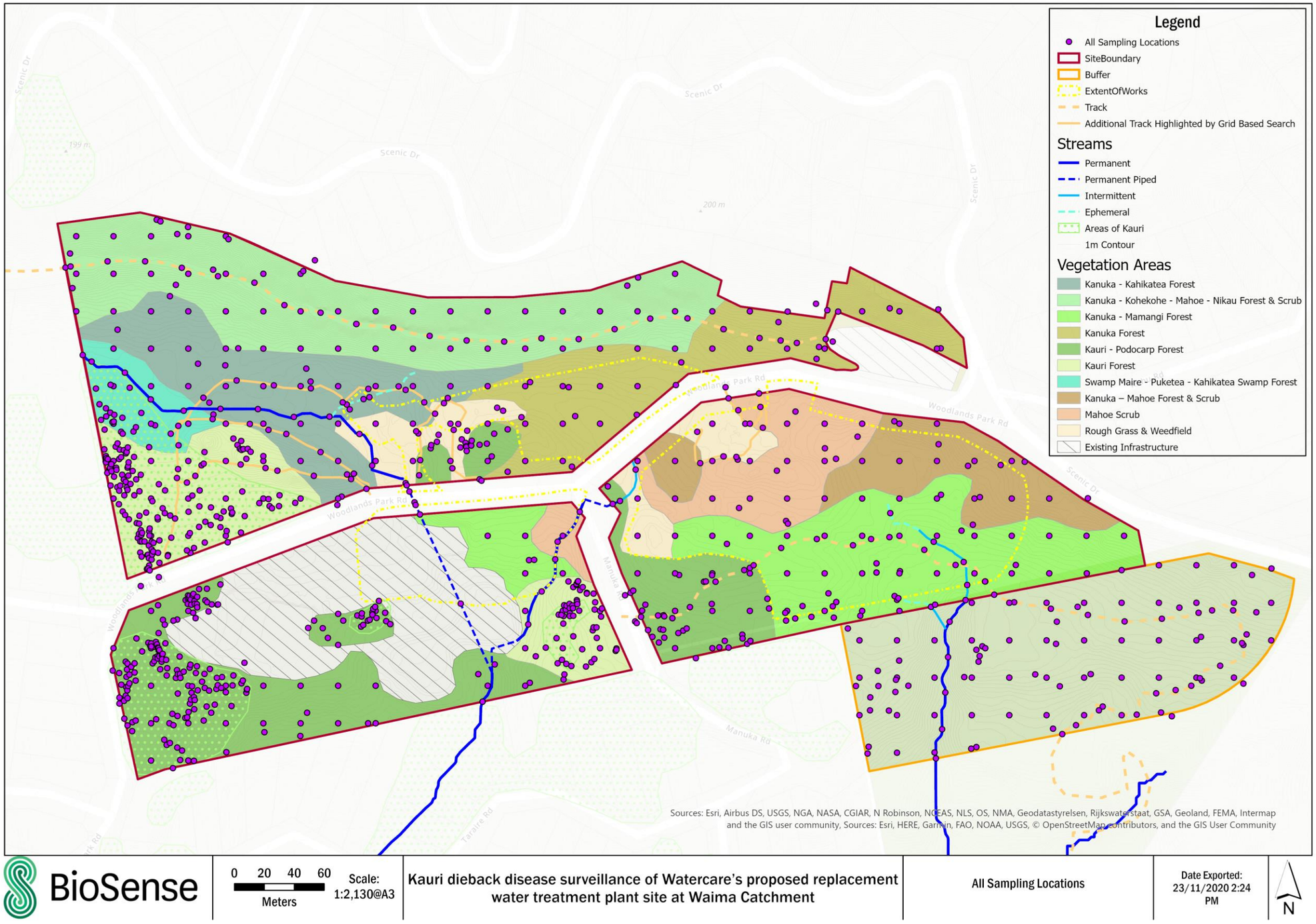
Appendix 6 – A map of the track sampling locations



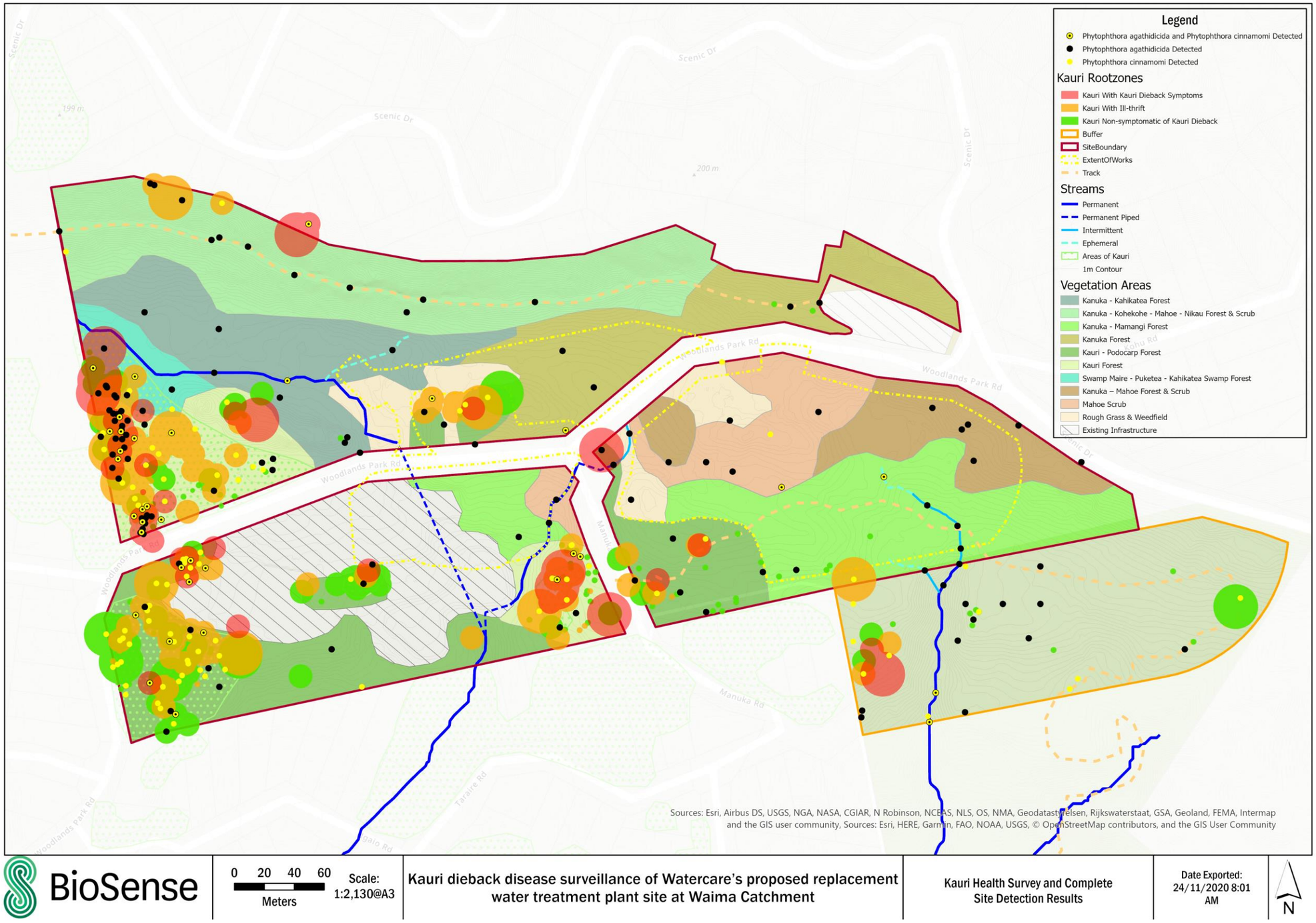
Appendix 7 – A map of the animal disturbance sampling locations



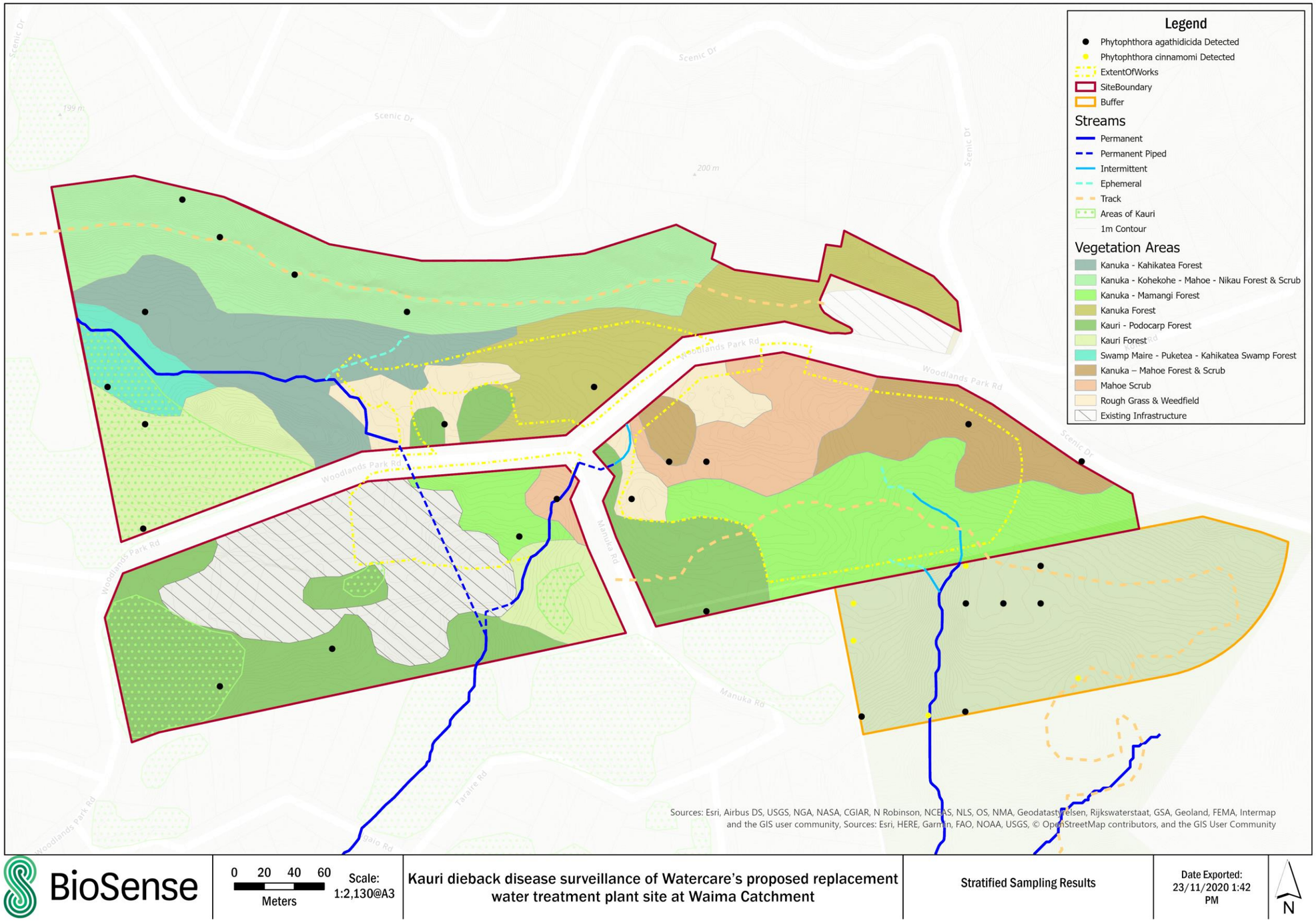
Appendix 8 – A map of all sampling locations



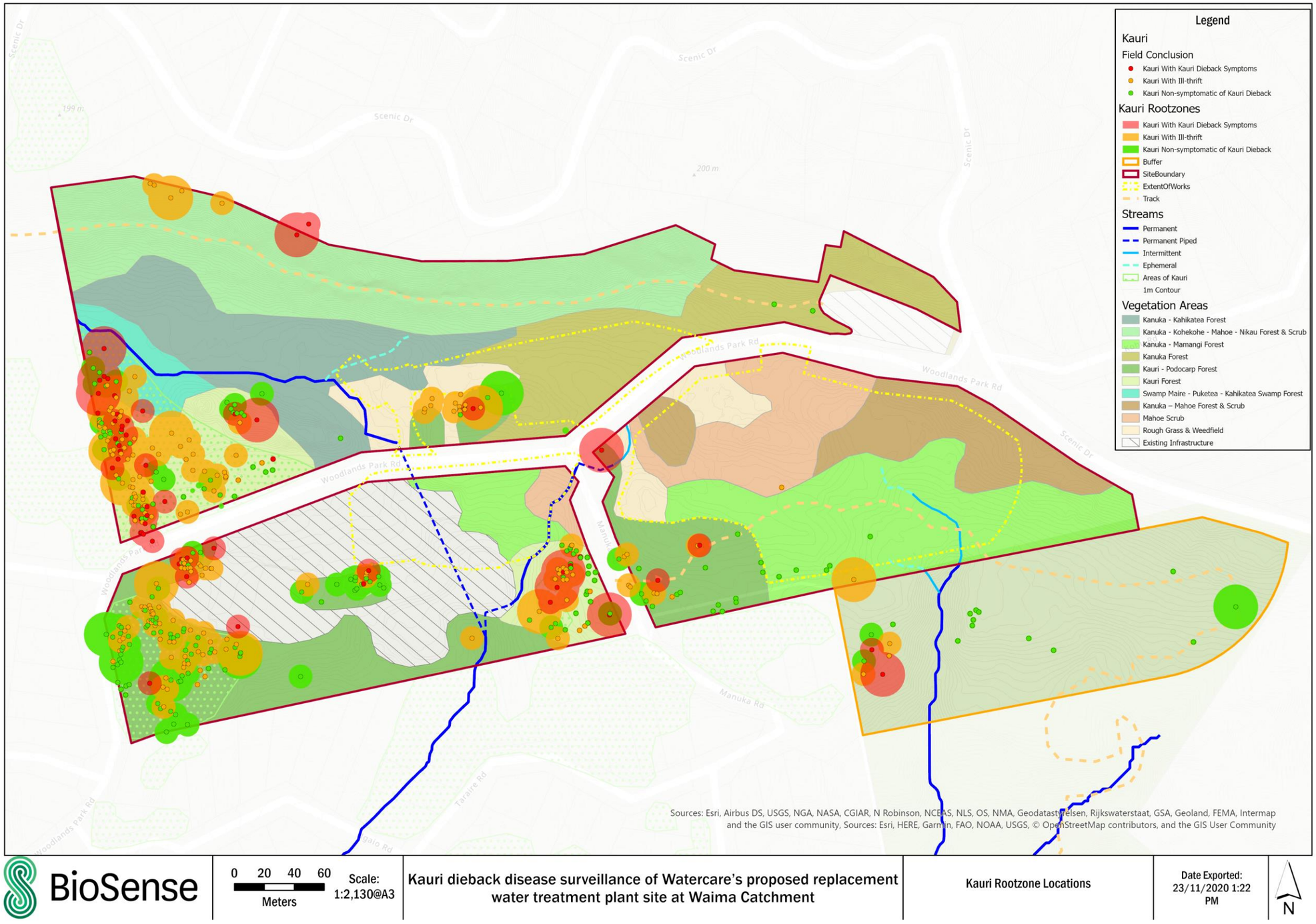
Appendix 9 - A map of the kauri health survey and complete site detection results



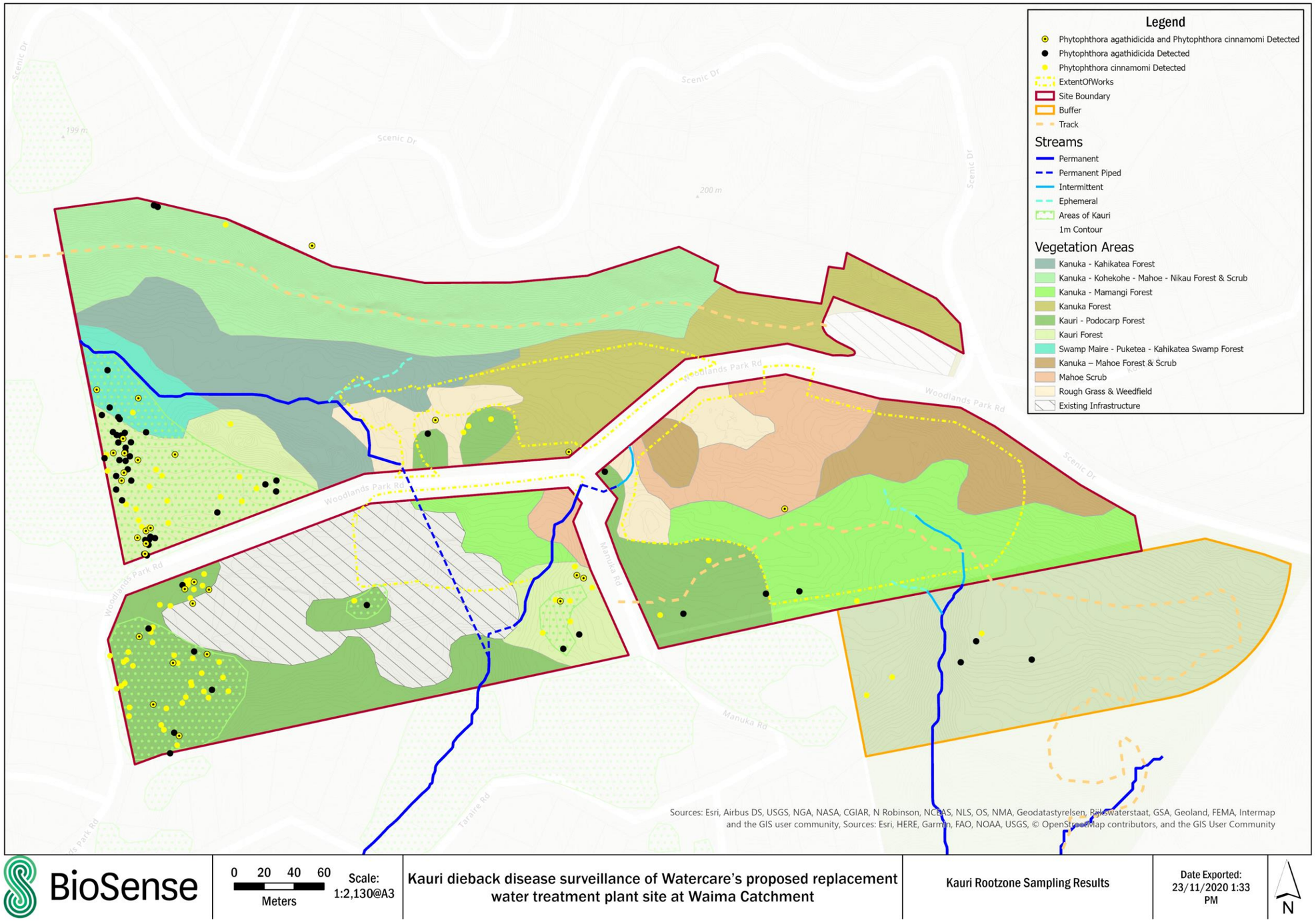
Appendix 10 - A map of the stratified sampling detection results



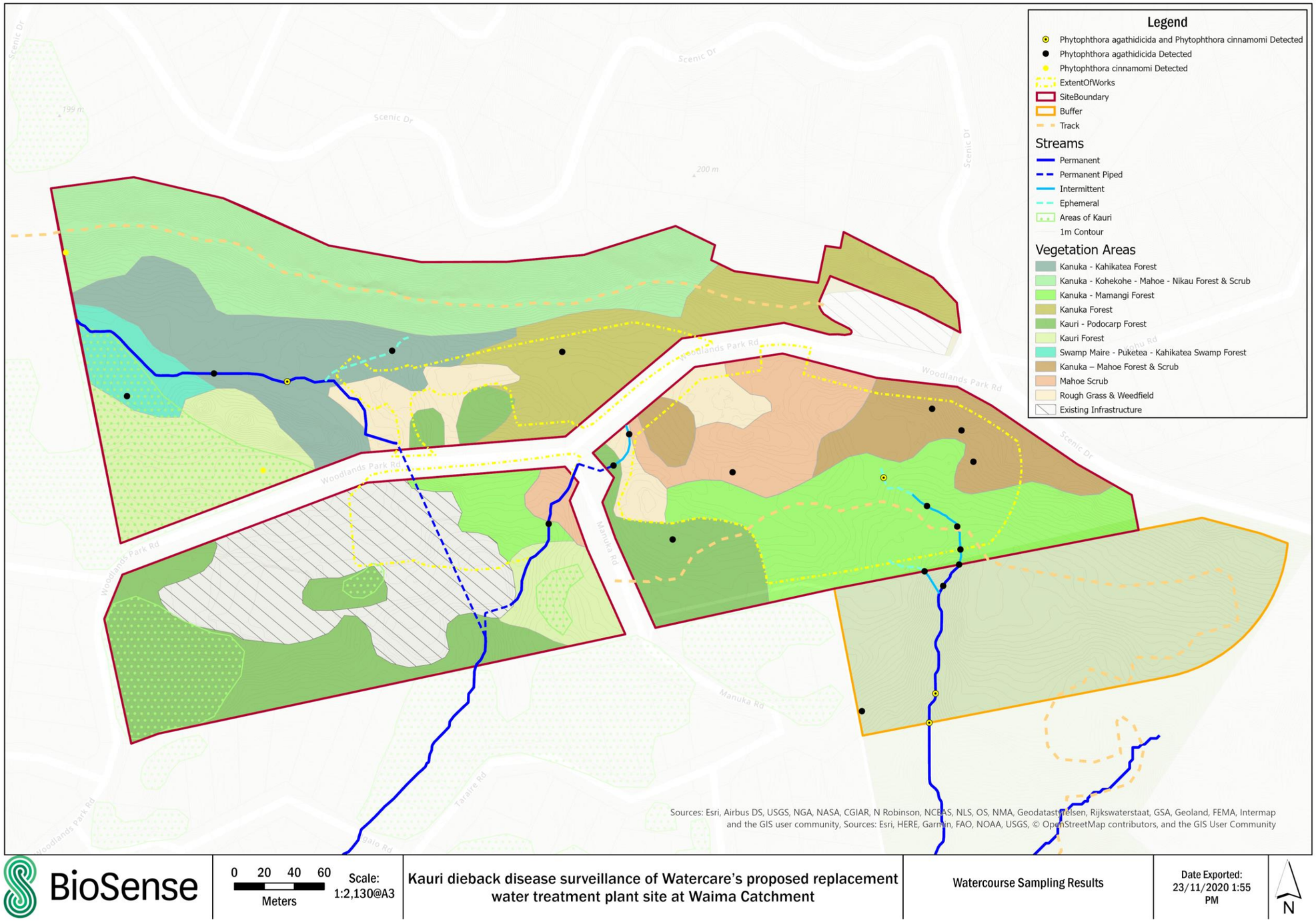
Appendix 11 – A map of kauri and kauri dieback status



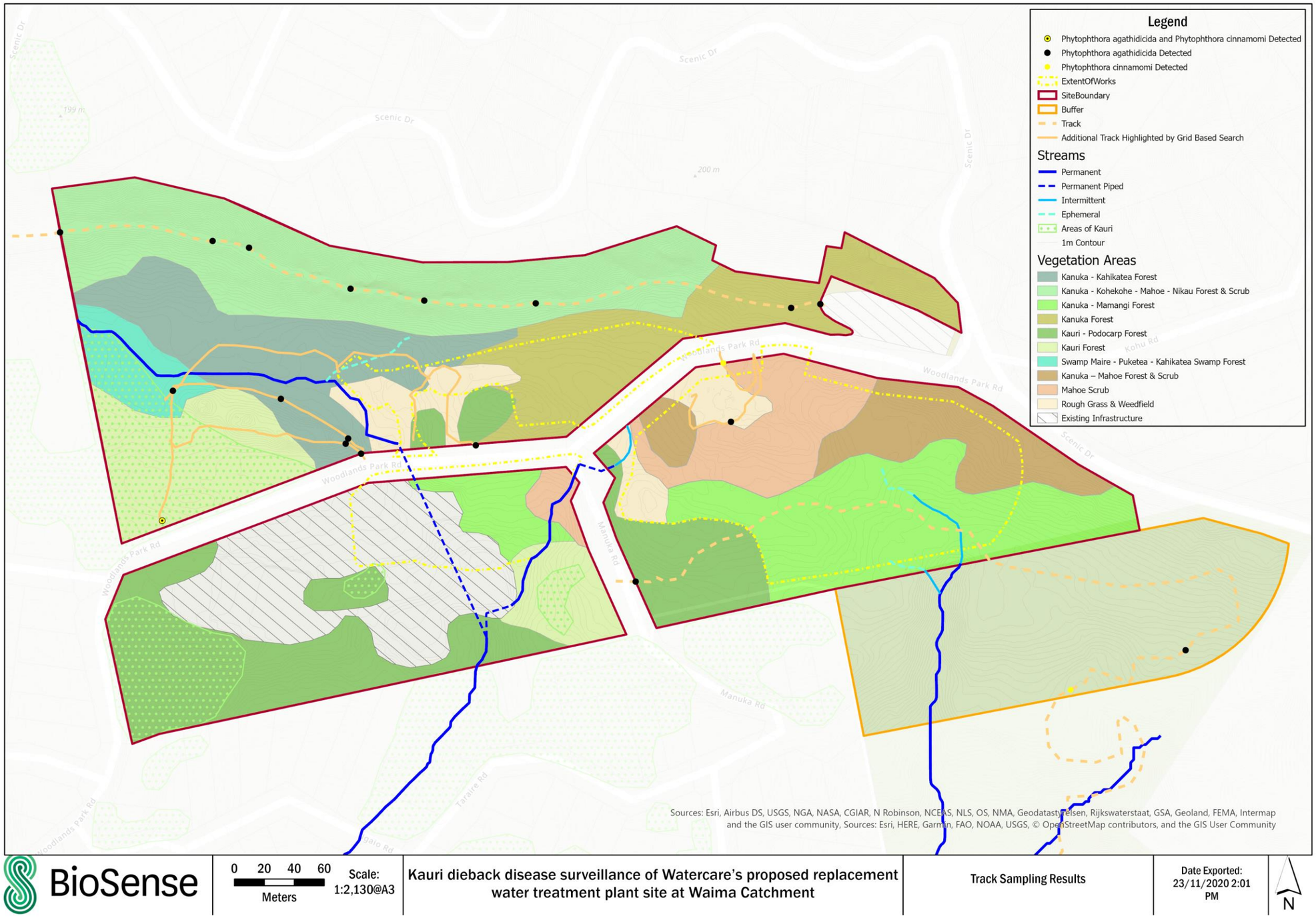
Appendix 12 - A map of the kauri rootzone sampling detection results



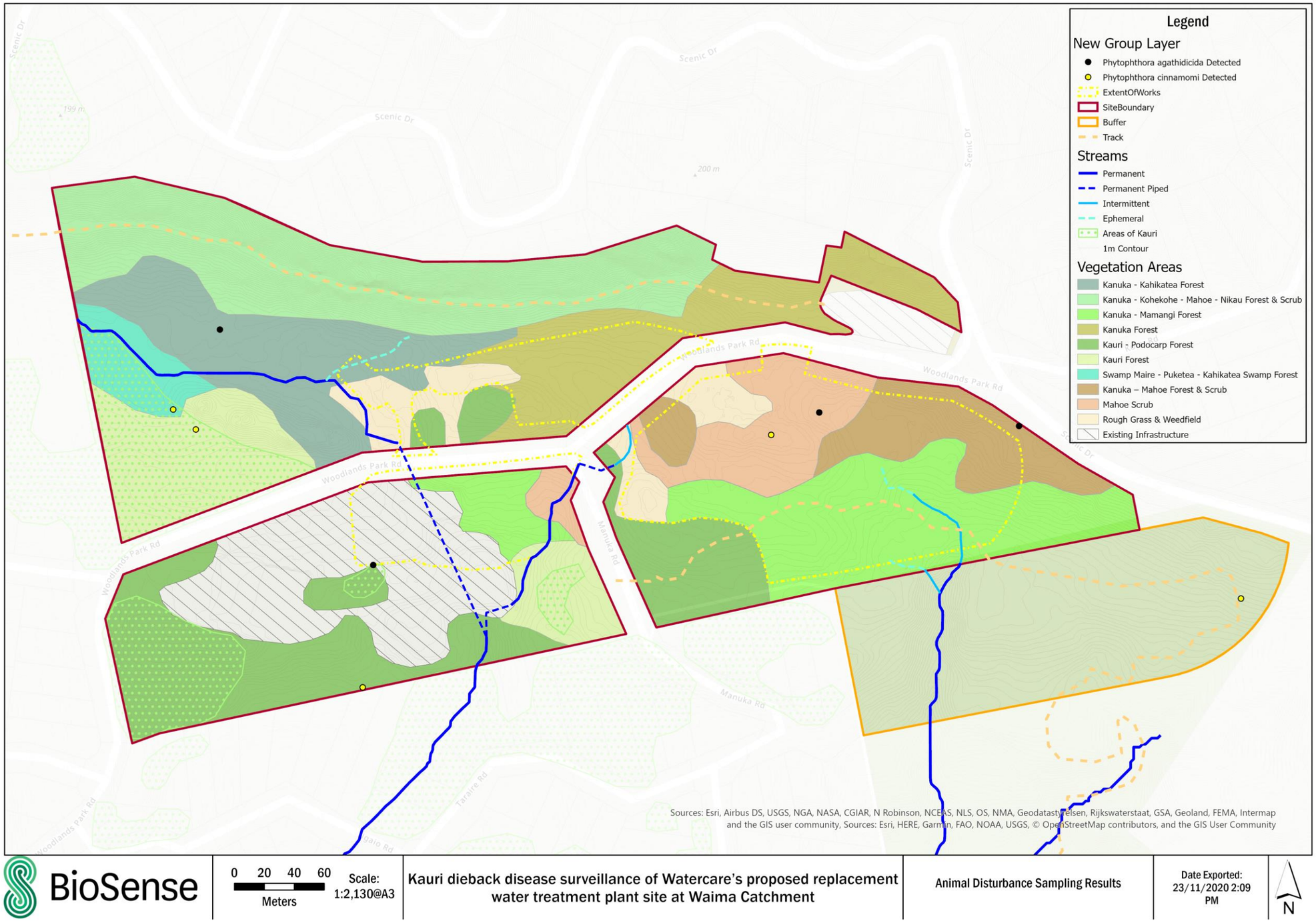
Appendix 13 – A map of the watercourse sampling detection results



Appendix 14 – A map of the track sampling detection results



Appendix 15 - A map of the animal disturbance sampling detection results



Appendix 16 - A map of *Phytophthora agathidicida* and *Phytophthora cinnamomi* detections with a 30 m buffer