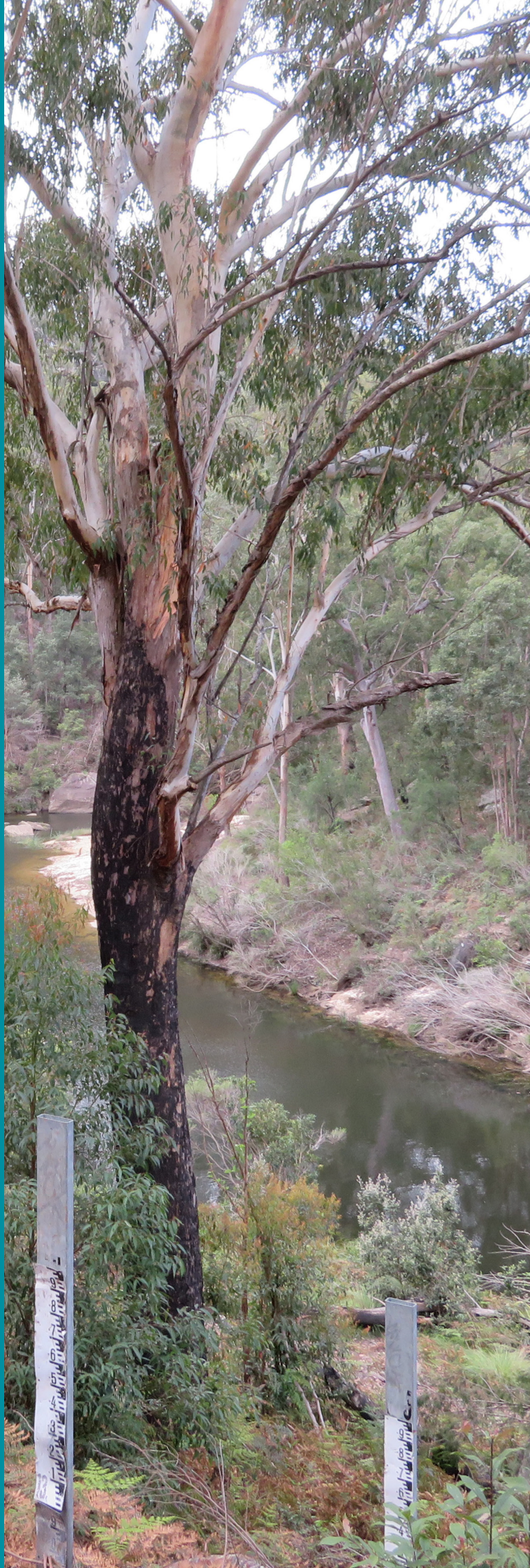
CAMPBELLTOWN

PLATYPUS PALS

UNDERSTANDING AQUATIC FAUNA POPULATIONS IN THE GEORGES RIVER THROUGH CITIZEN SCIENCE AND eDNA

Acknowledgement to Country

We acknowledge the traditional custodians of the land, the Dharawal people and their unique and spiritual connections to the land. We also respectfully acknowledge Elders past and present for the role they continue to play in guiding future generations.



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Project Acknowledgements

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Executive Summary

The Georges River is a place of cultural significance and pristine beauty, extending from the headwaters of Dharawal National Park to Botany Bay, where the river meets the sea. The Georges River Catchment is home to an array of unique plants and animals, some of which are highly cryptic and difficult to locate using traditional survey methods.

Through the use of a new cutting edge scientific technique called environmental DNA (*eDNA*), we have confirmed that the Upper Georges River is home to a small, isolated population of platypus (Ornithorhynchus anatinus) and a population of endangered Macquarie perch *(Macquaria australasica).*

The project involved various project partners including Platypus and Macquarie perch experts, academics, local and state government staff, not-for profit originations and community members, all with a key focus rediscovering the presence and distribution of these important animals to ensure their protection into the future.

This report forms part of the Platypus Pals Project. The primary aim of the project was to involve the community in water samples to detect Platypus DNA across the extent of the Upper Georges River with an additional focus on community education to educate residents in avoiding behaviors that are detrimental to the survival of the Platypus in Campbelltown.

The data gathered in this project will help identify important habitat for these species in Campbelltown. It will also allow us to better target education for residents who could unknowingly affect platypus and Macquarie perch through their recreational activities. This project is a keystone to help us protect these important native species into the future.

Project Background

In 2019 we received funding to undertake the Platypus Pals Project (*the Project)* which focused on surveying for platypus *(Ornithorhynchus anatinus)* using eDNA methods to determine their presence and extent in the Upper Georges River. The Project also included community education following the survey to minimise known impacts to the population such as the use of Opera House Yabby Traps, irresponsible recreational fish practices and impacts to water quality.

During the survey program design, it was apparent that there were other species of interest in the Georges River that could be incorporated into the program whilst collecting water samples for eDNA testing to deliver increased information in a cost efficient manner. These included the native 'Endangered' Macquarie perch *(Macquaria australasica)* and exotic species including European carp *(Cyprinus carpio)* and red-eared slider turtle *(Trachemys scripta elegans).*

Detecting the Hidden Using eDNA

A key challenge for biodiversity conservation is the ability to detect species. Determining the presence or absence of a species is integral to making informed management decisions.

Unfortunately, detecting species, particularly in an aquatic environment, can be difficult, time consuming, expensive, and often highly invasive. Analysis of environmental DNA (*eDNA)* is a relatively new, cheap, quick and non-invasive method for detecting species *(Rees et al.2014; McColl-Gausden et al. 2019; Thomsen and Willerslev 2015).* As the name suggests, eDNA refers to the genetic material that an organism leaves behind in its environment.

Quantitative comparisons with traditional sampling methods indicate that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species *(Biggs et al. 2015; Lugg et al. 2018; Smart et al. 2015; Thomsen et al. 2012; Valentini et al. 2016*), enabling effective detection of species at low densities. While the primary objective of this project was to investigate platypus occurrence throughout the Georges River and tributaries, samples were also screened for a native fish (*Macquarie perch),* an invasive fish (*European carp),* and an invasive turtle *(red-eared slider).*

The Sampling Method

In September 2020, water samples were collected from 19 sites by Campbelltown City Council staff, experts, non-for profit groups and community members following sampling protocols developed by EnviroDNA with further sampling undertaken at 11 sites in February 2021.

At each site, two samples were collected by passing 200-400 ml water *(average 329 ml)* through a 0.22 µm filter *(Sterivex)* that has high DNA retention *(Spens et al. 2017)*. Filtration of samples was undertaken on-site to reduce DNA degradation during transport of whole water samples (*Yamanaka et al. 2016)*.

Clean sampling protocols were employed to minimise contamination between sites including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. Filters were stored out of sunlight and refrigerated or on ice for a maximum of 48 hrs before being transported to the laboratory for processing.

DNA was extracted from the filters using a commercially available DNA extraction kit *(Qiagen DNeasy Blood and Tissue Kit)*. Real-time quantitative Polymerase Chain Reaction *(qPCR)* assays were used to amplify the target DNA, using a species-specific probe targeting a small region of the mitochondrial DNA of each target species.

Available gene sequences were compared between related taxa *(including humans)* and a probe sequence selected to detect the target species. Where possible, further in-vitro *(tissue samples)* testing was undertaken on the target species and closely related co-occurring species to check for cross-amplification of non-target DNA. Assays were performed in triplicate on each sample. Positive and negative controls were included for all assays as well as an Internal Positive Control *(IPC)* to detect inhibition *(Goldberg et al. 2016)*. At least two positive PCR's *(out of six assays undertaken for each site)* were required to classify the site as positive for the presence of the target species. To minimise false positives, sites were considered equivocal if only 1 assay returned a positive result, indicating very low levels of target DNA.

While trace amounts of DNA may indicate the target species is actually present in low abundance, it may also arise from sample contamination through the sampling or laboratory screening process *(minimised through strict protocols and negative controls)*, facilitated movement of DNA between waterbodies *(i.e. water birds, recreational anglers, water transfers, predator scats)*, or dispersal from further upstream.

The Results

The results from the survey efforts and eDNA analysis are summarised below (*Tables 1-4)* with locations of sampling shown in Figures 1-3.

Platypus

Platypus *(Ornithorhynchus anatinus)* are one of only four existing monotreme *(egg-laying mammal)* species and are found in fresh-water habitats from The Wet Tropics of Queensland through a band of Eastern Australia to Tasmania. In the last 30 years the range of the platypus has contracted by approximately 22% *(UNSW)* with an associated decline in population numbers. This is primarily due to habitat loss, pollution, accidental trapping using opera house traps and the ongoing effects of climate change. This has resulted in the species being listed as 'Near-Threatened' by the International Union of Conservation Nature *(IUCN)*, 'vulnerable' in South Australia, 'threatened' in Victoria and under recommendation to be listed in NSW. In the Georges River specifically, previous Platypus studies were completed in in 2008 and 2012, coordinated by the National Parks Association Macarthur Branch with tentative sightings recorded. A previous unpublished eDNA detection was also recorded at Site 16 (*EnviroDNA).*

Platypus eDNA sampling was undertaken at 19 sites within the Georges River Catchment during September 2020, with further targeted sampling undertaken in February 2020 at 11 targeted sites where positive or equivocal results were detected in the first round of sampling. In the first round of survey, trace amounts of Platypus eDNA were detected at four sites *(13, 14, 15, 17)* clustered in the lower Georges River (*Figure 1).* The second round of surveys in February 2021 failed to detect platypuses elsewhere in the catchment *(11 sites*). This indicates a small, low density, and presumably isolated, population in this area. No platypus eDNA was detected in the upper reaches or tributaries despite historical records *(>10 years; Atlas of Living Australia, BioNet)* in the region.

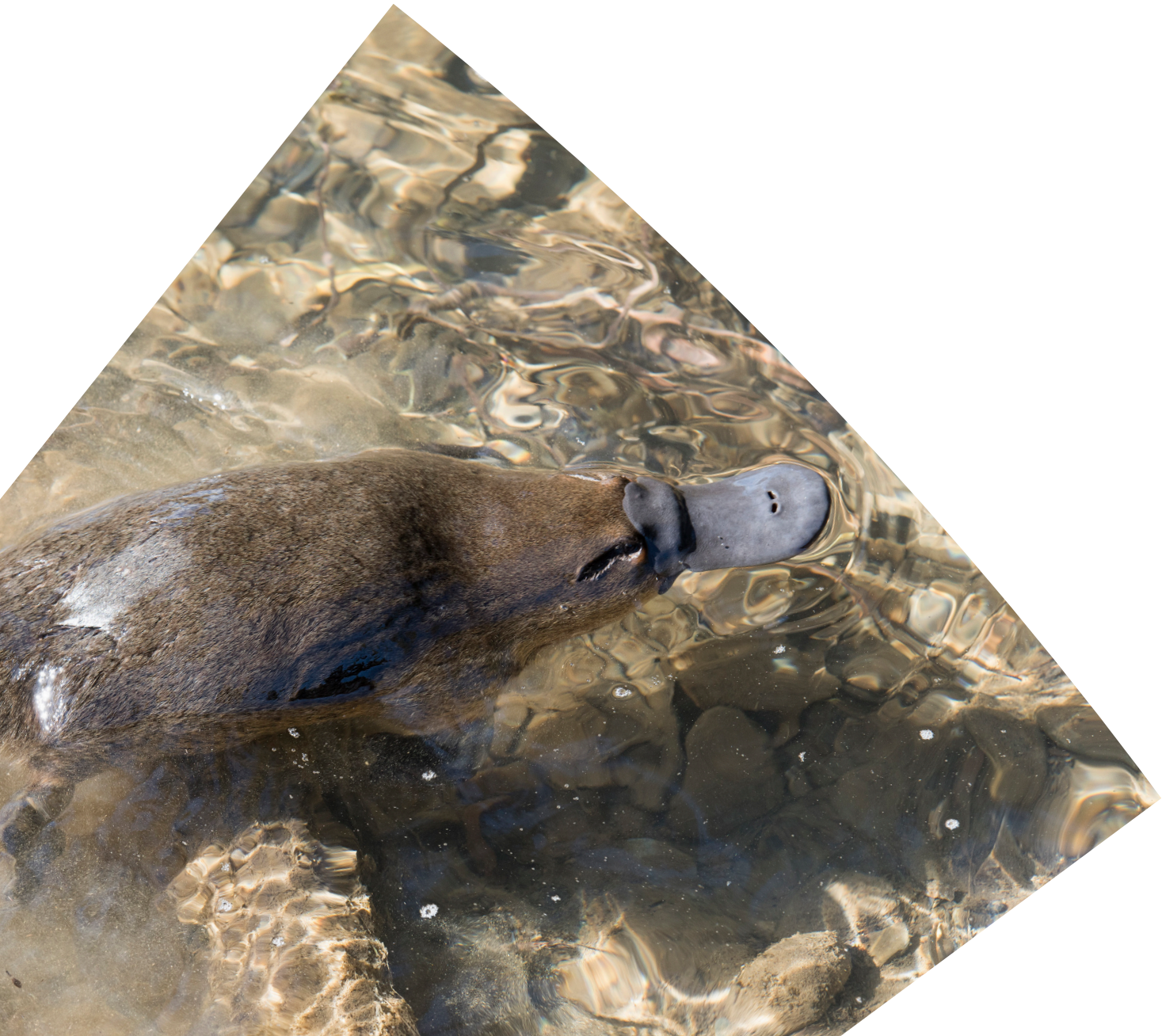

Table 1. Results for eDNA analysis of water samples for platypus *(Ornithorhynchus anatinus).*

Site Code	Waterway	Latitude	Longitude	Date Sampled	Positive assays	Test Result
2	O'Hare's Creek	-34.199194	150.870978	28/9/20	0/6	Negative
3	O'Hare's Creek	-34.184598	150.854152	28/9/20	0/6	Negative
4	O'Hare's Creek	-34.175408	150.84041	28/9/20	0/6	Negative
5	Stokes Creek	-34.182154	150.830083	28/9/20	0/6	Negative
6	Stokes Creek	-34.21174	150.844952	28/9/20	0/6	Negative
7	O'Hare's Creek	-34.163737	150.83778	27/9/20	0/6	Negative
8	Stokes Creek	-34.163808	150.826156	27/9/20	0/9	Negative
9	Georges River	-34.137235	150.800473	27/9/20	0/6	Negative
10	Georges River	-34.240157	150.823367	27/9/20	0/6	Negative
11	Georges River	-34.202293	150.79535	27/9/20	0/6	Negative
12	Georges River	-34.162642	150.794861	27/9/20	0/6	Negative
13	Georges River	-34.10873	150.818842	27/9/20	1/6	Equivocal
14	Georges River	-34.093249	150.83375	27/9/20	1/6	Equivocal
15	Georges River	-34.080296	150.865319	27/9/20	1/6	Equivocal
16	Georges River	-34.043402	150.892303	27/9/20	0/6	Negative
17	Georges River	-34.007935	150.888067	27/9/20	1/6	Equivocal
18	Georges River	-34.000156	150.9123	27/9/20	0/6	Negative
19	Georges River	-33.970063	150.912241	27/9/20	0/6	Negative
20	Georges River	-33.950934	150.91297	27/9/20	0/6	Negative
1	Georges River	-33.993729	150.908349	22/2/21	0/6	Negative
2	Georges River	-34.017397	150.906725	22/2/21	0/6	Negative
3	Myrtle Creek	-34.020004	150.885458	22/2/21	0/6	Negative
4	Peter Meadows Creek	-34.041888	150.890882	22/2/21	0/6	Negative
5	Punchbowl Creek	-34.049291	150.896938	22/2/21	0/6	Negative
6	Georges River	-34.062222	150.878333	22/2/21	0/6	Negative
7	Georges River	-34.116475	150.810291	22/2/21	0/6	Negative
8	Georges River	-34.130273	150.798509	22/2/21	0/6	Negative
9	Georges River	-34.139508	150.799257	22/2/21	0/6	Negative
10	Georges River	-34.147195	150.800965	22/2/21	0/6	Negative
11	Georges River	-34.156253	150.800702	22/2/21	0/6	Negative

Table 1. Results for eDNA analysis of water samples for platypus *(Ornithorhynchus anatinus).*

Figure 1. Results for eDNA screening for platypus

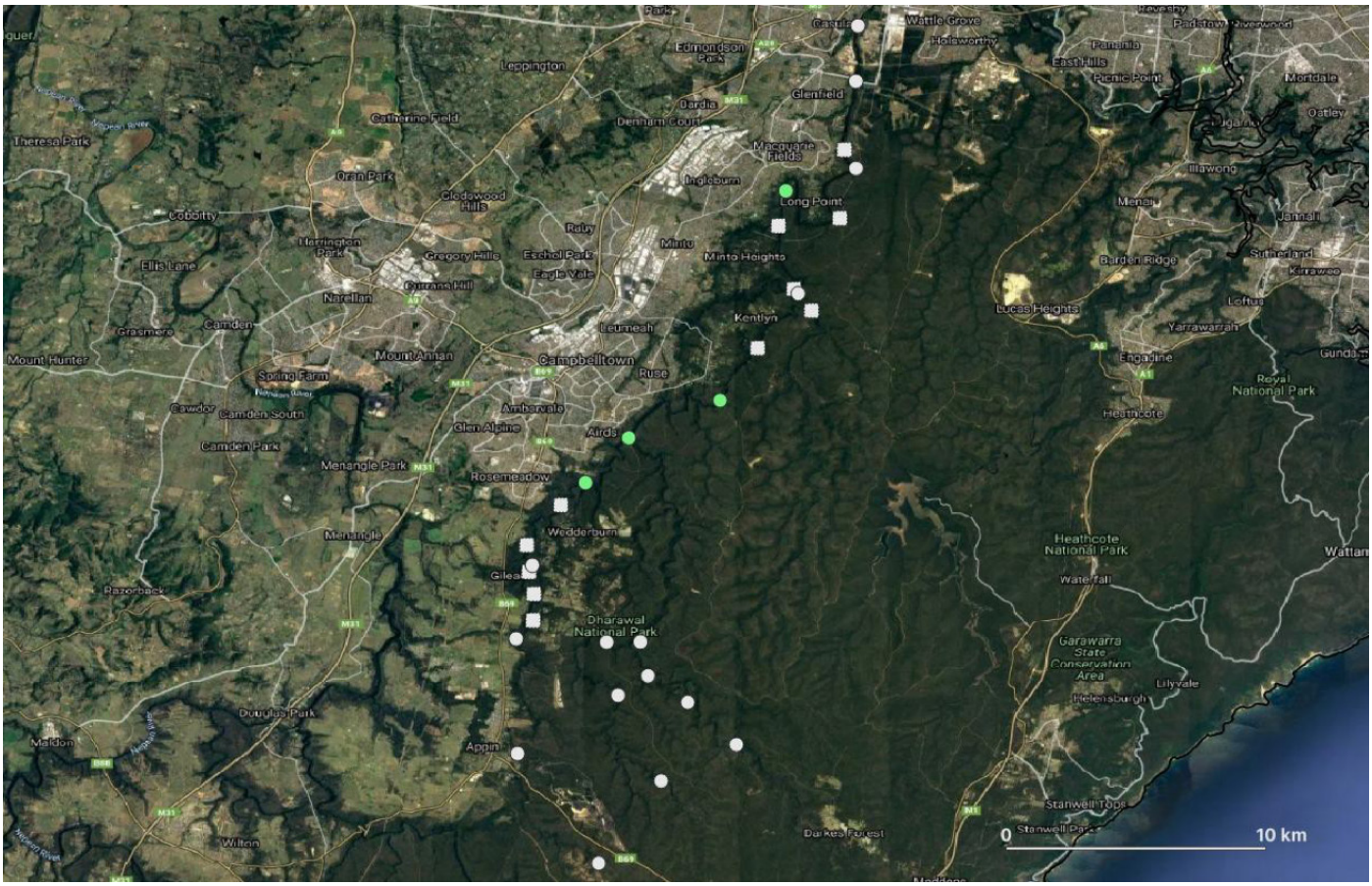

Figure 1. Results for eDNA screening for platypus (pale green = equivocal, grey = negative) in September 2020 (circles) and February 2021 *(squares).* Small red markers indicate recent *(<10yrs)* platypus records from Atlas of Living Australia *(www.ala.org)*

Macquarie perch

Macquarie perch *(Macquaria australasica)* are listed as Endangered under the Fisheries Management Act 1995 and have suffered decline due to overfishing by anglers, habitat degradation through siltation, and regulation of flow and "thermal pollution" by dams. The species had an original range through both the upland river sections of the Murray-Darling Basin and the rivers of the Shoalhaven, Georges and Hawkesbury-Nepean River systems. In the Georges River specifically, Macquarie perch records had been previously observed however, confirmation of these and their overall extent in the Georges River had not been verified for many years.

Macquarie perch eDNA sampling was undertaken at 19 sites within the Georges River Catchment during September 2020, with further targeted sampling undertaken in February 2020 at 11 targeted sites where positive or equivocal results were detected in the first round of sampling. In the first round of survey, Macquarie perch were detected at four sites clustered in the lower Georges River *(14, 15, 16, 17)* with a further two sites returning equivocal results *(7, 13)*. Interestingly, one equivocal site (*Site 7)* was in a tributary of the upper reaches, quite distant from the other results. The second round of survey returned another two positive and two equivocal sites in the same area *(Figure 2)*.

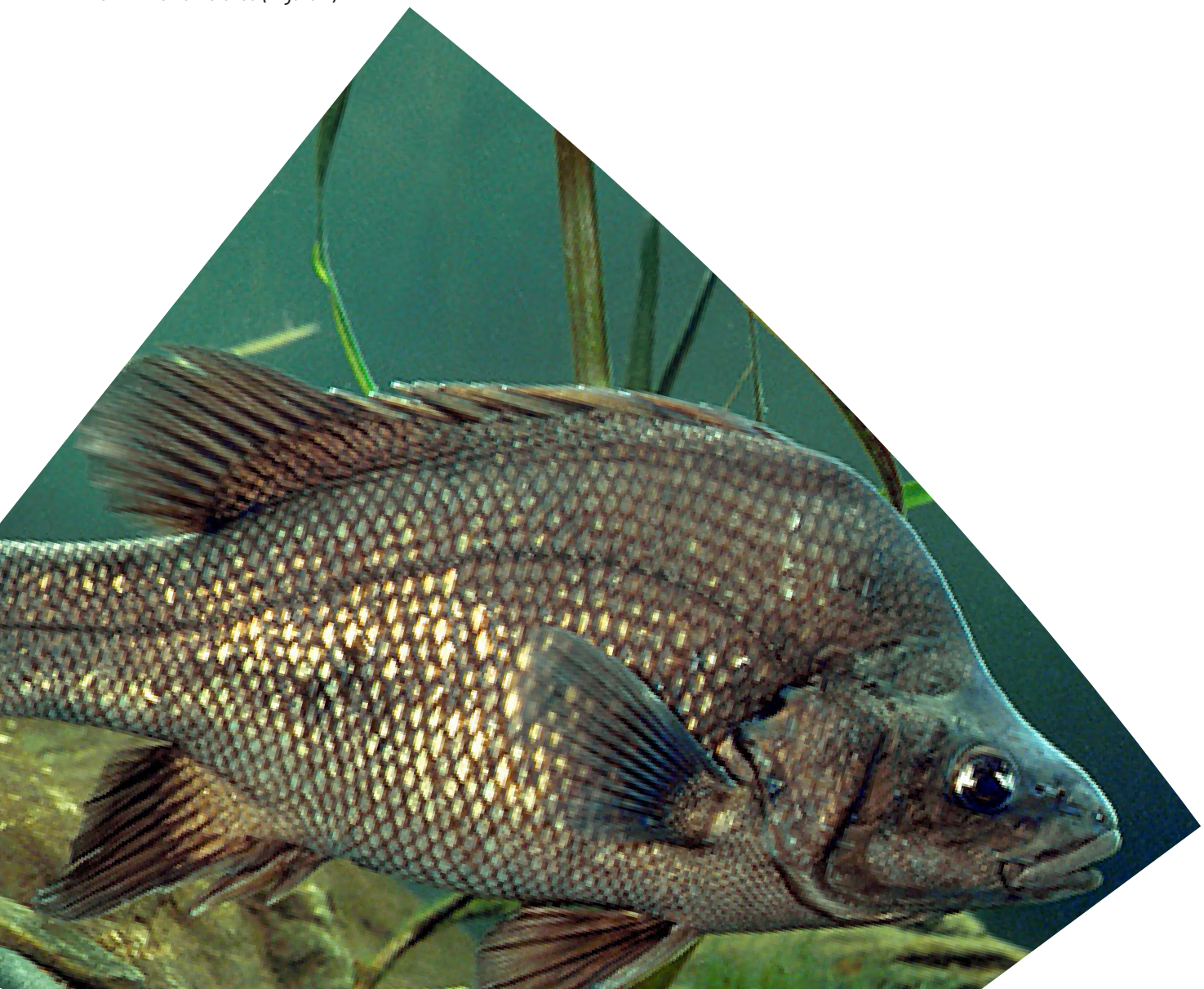

Table 2. Results for eDNA analysis of water samples for Macquarie perch *(Macquaria australasica).*

Site Code	Waterway	Latitude	Longitude	Date Sampled	Positive assays	Test Result
2	O'Hare's Creek	-34.199194	150.870978	28/9/20	0/6	Negative
3	O'Hare's Creek	-34.184598	150.854152	28/9/20	0/6	Negative
4	O'Hare's Creek	-34.175408	150.84041	28/9/20	0/6	Negative
5	Stokes Creek	-34.182154	150.830083	28/9/20	0/6	Negative
6	Stokes Creek	-34.21174	150.844952	28/9/20	0/6	Negative
7	O'Hare's Creek	-34.163737	150.83778	27/9/20	1/6	Equivocal
8	Stokes Creek	-34.163808	150.826156	27/9/20	0/9	Negative
9	Georges River	-34.137235	150.800473	27/9/20	0/6	Negative
10	Georges River	-34.240157	150.823367	27/9/20	0/6	Negative
11	Georges River	-34.202293	150.79535	27/9/20	0/6	Negative
12	Georges River	-34.162642	150.794861	27/9/20	0/6	Negative
13	Georges River	-34.10873	150.818842	27/9/20	1/6	Equivocal
14	Georges River	-34.093249	150.83375	27/9/20	6/6	Positive
15	Georges River	-34.080296	150.865319	27/9/20	4/6	Positive
16	Georges River	-34.043402	150.892303	27/9/20	3/6	Positive
17	Georges River	-34.007935	150.888067	27/9/20	6/6	Positive
18	Georges River	-34.000156	150.9123	27/9/20	0/6	Negative
19	Georges River	-33.970063	150.912241	27/9/20	0/6	Negative
20	Georges River	-33.950934	150.91297	27/9/20	0/6	Negative
1	Georges River	-33.993729	150.908349	22/2/21	0/6	Negative
2	Georges River	-34.017397	150.906725	22/2/21	1/6	Equivocal
3	Myrtle Creek	-34.020004	150.885458	22/2/21	2/6	Positive
4	Peter Meadows Creek	-34.041888	150.890882	22/2/21	0/6	Negative
5	Punchbowl Creek	-34.049291	150.896938	22/2/21	2/6	Positive
6	Georges River	-34.062222	150.878333	22/2/21	0/6	Negative
7	Georges River	-34.116475	150.810291	22/2/21	0/6	Negative
8	Georges River	-34.130273	150.798509	22/2/21	1/6	Equivocal
9	Georges River	-34.139508	150.799257	22/2/21	0/6	Negative
10	Georges River	-34.147195	150.800965	22/2/21	0/6	Negative
11	Georges River	-34.156253	150.800702	22/2/21	0/6	Negative

Table 2. Results for eDNA analysis of water samples for Macquarie perch *(Macquaria australasica).*

Figure 2. Results for eDNA screening for Macquarie perch

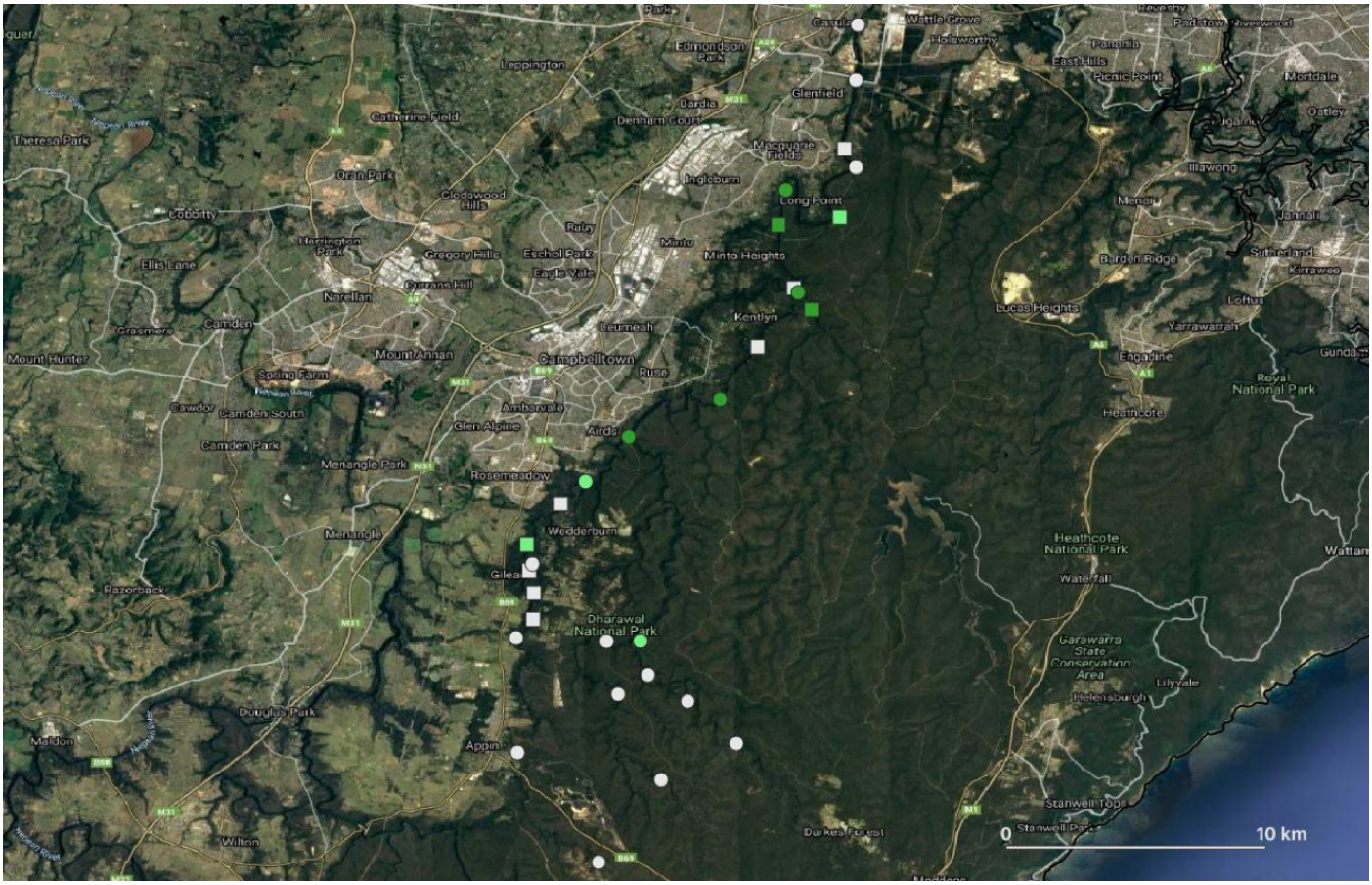

Figure 2. Results for eDNA screening for Macquarie Perch *(dark green = positive, pale green = equivocal, grey = negative)* in September 2020 (*circles)* and February 2021 *(squares).*

European Carp

European carp *(Cyprinus carpio)* is an exotic species of fish that has been in Australia for over 100 years with infestations established in all states and territories, except the Northern Territory. They completely dominate freshwater fish communities and compete with native species for valuable food resources. European carp also cause poor water quality through increased turbidity and erosion of stream banks through their feeding habits, which can also effect recreation and irrigation in rural areas. In the Georges River specifically, the extent of European Carp upstream was unknown, this study shows that distribution is widespread in the open sections of the upper river.

European carp eDNA sampling was undertaken at 19 sites within the Georges River Catchment during September 2020, with further targeted sampling undertaken in February 2020 at 11 targeted sites where positive or equivocal results were detected in the first round of sampling. In the first round of sampling, European carp were detected at two sites in the lower reaches *(13, 20)* with a further three sites returning equivocal results *(7, 14, 19)*. Two of these sites were also in the lower reached but one was in a tributary of the upper reaches *(Figure 3)*. The second round of survey failed to detect carp elsewhere in the catchment *(11 sites)*.

European Carp

Table 3. Results for eDNA analysis of water samples for European carp *(Cyprinus carpio).*

Site Code	Waterway	Latitude	Longitude	Date Sampled	Positive assays	Test Result
2	O'Hare's Creek	-34.199194	150.870978	28/9/20	0/6	Negative
3	O'Hare's Creek	-34.184598	150.854152	28/9/20	0/6	Negative
4	O'Hare's Creek	-34.175408	150.84041	28/9/20	0/6	Negative
5	Stokes Creek	-34.182154	150.830083	28/9/20	0/6	Negative
6	Stokes Creek	-34.21174	150.844952	28/9/20	0/6	Negative
7	O'Hare's Creek	-34.163737	150.83778	27/9/20	1/6	Equivocal
8	Stokes Creek	-34.163808	150.826156	27/9/20	0/9	Negative
9	Georges River	-34.137235	150.800473	27/9/20	0/6	Negative
10	Georges River	-34.240157	150.823367	27/9/20	0/6	Negative
11	Georges River	-34.202293	150.79535	27/9/20	0/6	Negative
12	Georges River	-34.162642	150.794861	27/9/20	0/6	Negative
13	Georges River	-34.10873	150.818842	27/9/20	4/6	Positive
14	Georges River	-34.093249	150.83375	27/9/20	1/6	Equivocal
15	Georges River	-34.080296	150.865319	27/9/20	0/6	Negative
16	Georges River	-34.043402	150.892303	27/9/20	0/6	Negative
17	Georges River	-34.007935	150.888067	27/9/20	0/6	Negative
18	Georges River	-34.000156	150.9123	27/9/20	0/6	Negative
19	Georges River	-33.970063	150.912241	27/9/20	1/6	Equivocal
20	Georges River	-33.950934	150.91297	27/9/20	6/6	Positive
1	Georges River	-33.993729	150.908349	22/2/21	0/6	Negative
2	Georges River	-34.017397	150.906725	22/2/21	0/6	Negative
3	Myrtle Creek	-34.020004	150.885458	22/2/21	0/6	Negative
4	Peter Meadows Creek	-34.041888	150.890882	22/2/21	0/6	Negative
5	Punchbowl Creek	-34.049291	150.896938	22/2/21	0/6	Negative
6	Georges River	-34.062222	150.878333	22/2/21	0/6	Negative
7	Georges River	-34.116475	150.810291	22/2/21	0/6	Negative
8	Georges River	-34.130273	150.798509	22/2/21	0/6	Negative
9	Georges River	-34.139508	150.799257	22/2/21	0/6	Negative
10	Georges River	-34.147195	150.800965	22/2/21	0/6	Negative
11	Georges River	-34.156253	150.800702	22/2/21	0/6	Negative

Table 3. Results for eDNA analysis of water samples for European carp *(Cyprinus carpio).*

Figure 3. Results for eDNA screening for European carp

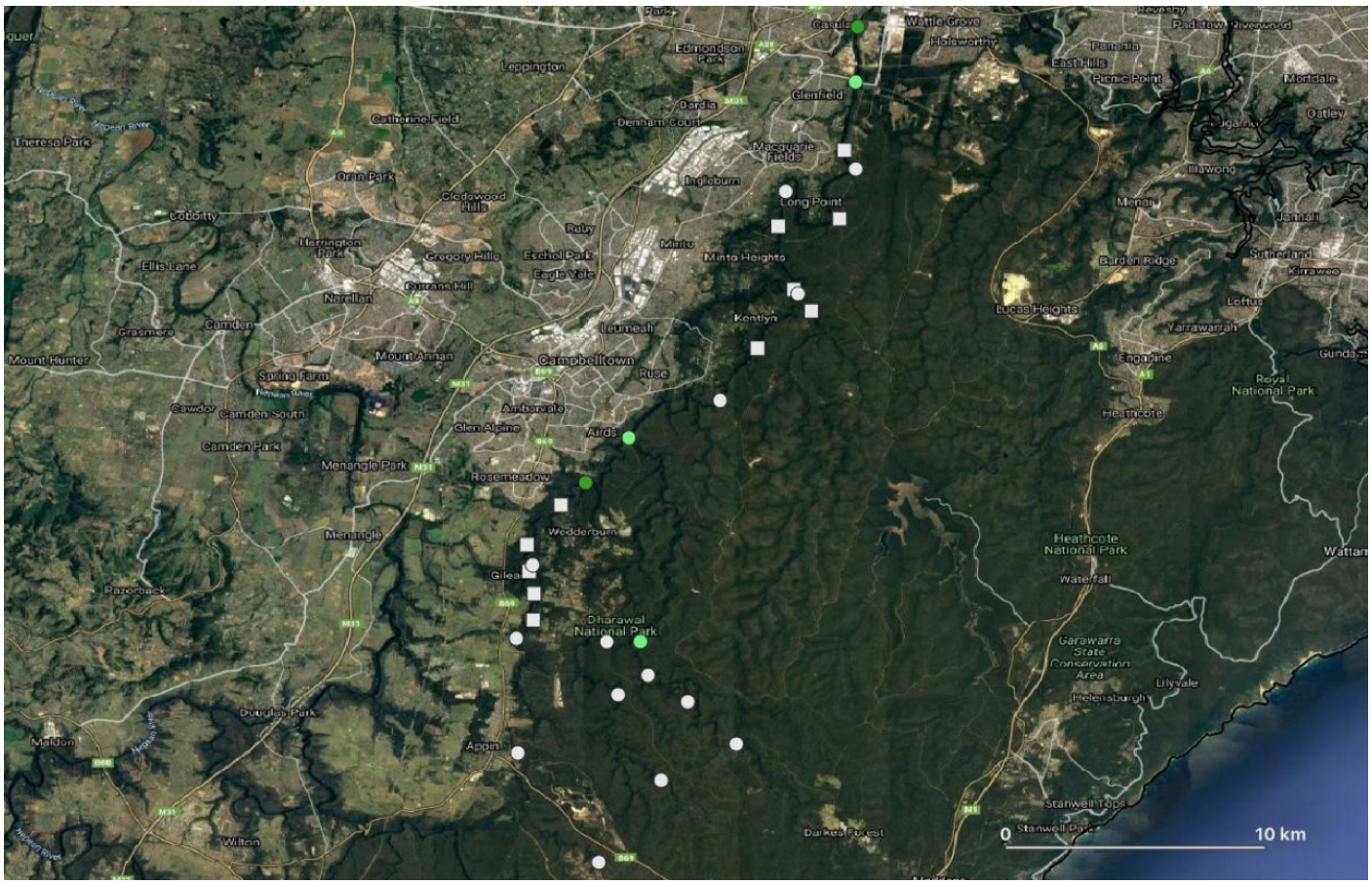

Figure 3. Results for eDNA screening for European carp *(dark green = positive, pale green = equivocal, grey = negative*) in September 2020 (*circles*) and February 2021 (*squares*).

Red-eared Slider Turtle

The red-eared slider turtle *(Trachemys scripta elegans)* is listed by the International Union for Conservation of Nature (*IUCN*) as 'one of the world's worst invasive alien species' and is a priority pest species in NSW. Red-eared slider turtles are considered an environmental pest due to the competition with native turtles for valuable food resources, nesting areas and basking sites. Sightings of red-eared slider turtles have been recorded in the Campbelltown LGA previously however; no sightings had been recorded in the Georges River Catchment.

Red-eared slider turtle eDNA sampling was undertaken at 8 sites within the Georges River Catchment during September 2020 with a primary focus on recreation locations adjacent to urban areas where it's likely that releases may occur. Red-eared slider turtle was not detected at any of the sites investigated.

Table 4. Results for eDNA analysis of water samples for red-eared slider turtle *(Trachemys scripta elegans).*

Site Code	Waterway	Latitude	Longitude	Date Sampled	Positive assays	Test Result
2	O'Hare's Creek	-34.199194	150.870978	28/9/20	–	–
3	O'Hare's Creek	-34.184598	150.854152	28/9/20	–	–
4	O'Hare's Creek	-34.175408	150.84041	28/9/20	–	–
5	Stokes Creek	-34.182154	150.830083	28/9/20	–	–
6	Stokes Creek	-34.21174	150.844952	28/9/20	–	–
7	O'Hare's Creek	-34.163737	150.83778	27/9/20	–	–
8	Stokes Creek	-34.163808	150.826156	27/9/20	–	–
9	Georges River	-34.137235	150.800473	27/9/20	–	–
10	Georges River	-34.240157	150.823367	27/9/20	–	–
11	Georges River	-34.202293	150.79535	27/9/20	–	–
12	Georges River	-34.162642	150.794861	27/9/20	–	–
13	Georges River	-34.10873	150.818842	27/9/20	0/6	Negative
14	Georges River	-34.093249	150.83375	27/9/20	0/6	Negative
15	Georges River	-34.080296	150.865319	27/9/20	0/6	Negative
16	Georges River	-34.043402	150.892303	27/9/20	0/6	Negative
17	Georges River	-34.007935	150.888067	27/9/20	0/6	Negative
18	Georges River	-34.000156	150.9123	27/9/20	0/6	Negative
19	Georges River	-33.970063	150.912241	27/9/20	0/6	Negative
20	Georges River	-33.950934	150.91297	27/9/20	0/6	Negative

Table 4. Results for eDNA analysis of water samples for red-eared slider turtle *(Trachemys scripta elegans).*

Future Recommendations

If greater confidence is required in detection of species targeted under the sampling program, further sampling at equivocal sites to confirm the presence or absence of the target species is recommended. In addition, further sampling across the catchment is recommended to help determine the presence of the species at a site *(i.e. resident or transient)* and to note potential changes in distribution following the severe drought conditions prior to sampling 2020.

Further to this increased community awareness and education around the importance of conservation of these species is recommended, particularly to curb human behaviors that are detrimental to their ongoing survival including irresponsible use of Opera House Yabby Traps and disposal of fishing line.

Other recommendations include onsite signage to create awareness for river users, programs with schools and local retailers and increased reporting and liaison with key user groups to continue to assist in monitoring species distribution.

Longer-term considerations for water quality and quantity need to be considered by relevant stakeholders as these species and their food sources such as macroinvertebrates rely on this for their health and survival.

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