



Officer South Fauna Investigation

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Executive Summary

The Officer South Employment Precinct Structure Plan (PSP) area, around 45 km south-east of Melbourne, has been earmarked by the State Government for over 15 years for a large-scale employment and development precinct. The PSP area is located within the Melbourne Strategic Assessment (MSA) area and has a Conservation Area along both main waterways for growling grass frog habitat and assets, with additional support for Australian grayling along Cardinia Creek. The Cardinia Creek area is a site of Biodiversity significance and there are records of protected flora and fauna in the area, including native fish species (Dwarf galaxias and Australian grayling), growling grass frog, southern brown bandicoot and matted flax lily. Melbourne Water, as the responsible authority for floodplain and water management and protection of waterway biodiversity values, is considering these protected species in their strategic planning and development processes.

The development of the PSP is occurring in conjunction with significant urban and commercial developments in neighbouring PSP areas, which are expected to have a cumulative impact on the waterways of the area including Officer South Drain, Gum Scrub Creek, and Cardinia Creek. In order to more accurately plan for potential future development, manage impacts, and determine the conservation and habitat protection requirements for the area, Melbourne Water commissioned Jacobs to conduct a more targeted and up-to-date field survey. This study involved a review of previous data, survey planning and development, and completion of a field survey. The field work included fishing surveys targeting Dwarf galaxias and Australian grayling, as well as water sampling and analysis to detect eDNA of target fish species and Growling grass frog.

The study found that Australian grayling were present in Cardinia Creek in two locations, both within and downstream of the PSP area. These sites had similar characteristics; instream sites with flowing water, riparian and fringing vegetation cover and good water quality. This helped support Australian grayling and other native fish species (Common galaxias, short-finned eel, and tupong) at both sites. Dwarf galaxias were not recorded in any of the fishing surveys, but eDNA results returned 'equivocal' data at two sites along Cardinia Creek. These sites are consistent with the location of previous species records, meaning it is likely that the species is present or has recently moved through the area. Both sites also recorded other native galaxias species (common galaxias and spotted galaxias). Although field surveys did not include the Growling grass frog, water samples collected were analysed for their DNA however there were no samples which returned positive results.

The survey confirmed the presence of Australian grayling within and downstream of the PSP area, and previous records combined with equivocal eDNA results also suggest Dwarf galaxias are present and or move through the Cardinia Creek sites surveyed. The approach taken to date with development planning, which assumes presence of Australian grayling and Dwarf galaxias and appropriate design and management to mitigate impacts, should be continued.

Development in the PSP area will result in altered hydrology and stormwater runoff, which has potential to impact flows and aquatic biodiversity values in Cardinia Creek, Officer South Drain, and Gum Scrub Creek. Current development plans suggest that most of the increased flows from the PSP will be directed to Gum Scrub Creek and Officer South Drain, in order to protect the known values within Cardinia Creek. Changes to flows within Gum Scrub Creek and Officer South Drain should be carefully managed to avoid impacts to existing habitat and remnant vegetation, but could potentially provide additional habitat opportunities.

The reach of Cardinia Creek downstream of the PSP could be impacted if significant additional flows are directed into Officer South Drain, or if there are other significant changes to the hydrology of Cardinia Creek as a result of the PSP development. This survey confirmed the presence of Australian grayling in Cardinia Creek within the PSP as well as downstream of the PSP, outside the MSA area. As the Australian grayling is an EPBC-listed species, this will need to be considered as part of future approval and referral requirements.

Important note about your report

The sole purpose of this report is to provide the results of a field survey and associated reporting in accordance with the scope set out in our proposal of services "Officer South Employment PSP Targeted Fauna Survey" dated 25th November 2020, and later amended to remove the requirement for Matted Flax-Lily surveys.. That scope of services was developed with Melbourne Water, in response to the Melbourne Water request for proposal document "Officer South Employment PSP – Targeted Fauna Surveys for Gum Scrub Creek, Officer South Drain and Tributaries".

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1. Introduction

Jacobs has been engaged by Melbourne Water to conduct field surveys for specific threatened flora and fauna species in the Officer South Employment Precinct Structure Plan (PSP) area, around 45 km south-east of Melbourne. The PSP area has been earmarked by the State Government for over 15 years for a large-scale employment and development precinct.

Considerable planning and development works are already underway in the area, but in many cases this is occurring based on insufficient or out of date field survey data. In order to more accurately plan for potential future development, manage impacts, and determine the conservation and habitat protection requirements for the area, a more targeted and up-to-date field survey is necessary.

This project required a data and literature review to obtain up-to-date information about the relevant species records in the area, development of a survey plan to determine the most appropriate field survey locations and methods, and completion of the field work. This report describes the methods and results for the data review and field survey, and a write up of study findings including implications for further survey, planning and approval processes in the area.

1.1 Background

The Officer South Precinct Structure Plan (PSP) area is located approximately 45 kilometres south-east of Melbourne, on the edge of the Koo Wee Rup swamp and within the municipality of Cardinia. The PSP area is bounded by the Princes Freeway in the north, and by Cardinia Creek in the west and south. The remainder of the southern boundary of the PSP, and part of the eastern boundary, is formed by the boundary of the urban growth area. The eastern boundary is formed by Gum Scrub Creek (Figure 1-1 **Error! Reference source not found.**). The Officer South Drain runs from north to south through the middle of the PSP area, along Officer South Road, and discharges to Cardinia Creek at the southern boundary of the PSP. Western Port Bay (including the Western Port Bay RAMSAR site) is located around 13 kilometres to the south and is the receiving environment for flows from Cardinia Creek and Gum Scrub Creek. The PSP covers an area of approximately 1069 hectares - 317 hectares above Lecky Rd and 752 hectares in the south.



Figure 1-1 Officer South PSP area with marked waterways (Image courtesy Melbourne Water)

The Victorian Planning Authority has targeted Officer South Employment PSP as a streamlined project, looking for efficiency with timelines through co-design approach (PSP 2.0). The PSP area is located within the Melbourne Strategic Assessment (MSA) area and has a Conservation Area along both main waterways for growling grass frog habitat and assets, with additional support for Australian grayling along Cardinia Creek. The Cardinia Creek area is a site of Biodiversity significance and there are records of protected flora and fauna in the area, including native fish species (Dwarf galaxias and Australian grayling), growling grass frog, southern brown bandicoot and matted flax lily. Melbourne Water, as the responsible authority for floodplain and water management and protection of waterway biodiversity values, is considering these protected species in their strategic planning and development processes.

The development of the PSP is occurring in conjunction with significant urban and commercial developments in neighbouring PSP areas, which are expected to have a cumulative impact on the waterways of the area including Officer South Drain, Gum Scrub Creek, and Cardinia Creek. In addition, there are works proposed for the downstream reaches of Cardinia Creek (modified drain sections) to increase the capacity of the creek during high flow events and minimise the impact of flooding in adjacent agricultural properties. These works are not directly related to the upstream PSP developments, but are also likely to impact on conservation values of the creek.

Melbourne Water is also attempting to manage conservation values in this area and reduce impacts to species including growling grass frog and Australian grayling. This area is not covered by the MSA, and potential referral requirements in this area (including requirements under the *Environment Protection and Biodiversity Conservation Act* (EPBC Act) may need to be considered.

1.2 Study scope and objectives

In light of the planning and development works occurring in the area, this study is now required in order to provide additional, updated data to inform Melbourne Water's future planning and biodiversity. Building on the previous body of work, this study has three main components: a review of previous data and reports, a targeted field survey to ground-truth existing records and address data gaps for threatened fish (Dwarf galaxias, and Australian grayling) and a summary report of findings, including provision of advice to Melbourne Water relating to biodiversity values, protection and referral requirements.

The findings of the survey will be considered along with a review and summary of previous studies, and be used to provide advice on actions related to future drainage scheme works including fish habitat protection or creation in Gum Scrub Creek, Officer South Drain, and Cardinia Creek. This may include waterway corridor design, including planning for any reconstruction of waterways, MSA Conservation Areas and provisioning of threatened species habitats. The advice will consider management and mitigation recommendations and staging of works, as well as management actions relating to referral requirements.

1.3 Report Structure

This report sets out the study background, objectives, method and results. A discussion of findings, including implications for future planning and referral processes is also provided. The report is structured as follows:

- Section One – Introduction: Background, Study scope and Objectives
- Section Two – Method: Review, Survey plan and Survey methods
- Section Three – Results: Field survey data and results
- Section Four – Discussion: Project findings and future planning implications
- Section Five – Conclusion
- Section Six – References

2. Method

2.1 Background and literature review

The first component of the study was a background data and literature review. The review covered two key aspects:

- Key threatened fish species – habitat and lifecycle preferences, previous survey efforts and studies, and previous or current records in the area
- PSP area development and plans, as relevant to altered stormwater, hydrology and waterway impacts to Gum Scrub Creek, Cardinia Creek and tributaries

2.1.1 Species records

Due to the nature and scale of development occurring in the region of the PSP area, a number of fauna assessments have previously been undertaken in the area. As part of planning for more up-to-date field surveys in this study, we completed an initial review of previous relevant studies from the area to ascertain the current known species records, and any areas where survey effort had not resulted in species detection. Studies and reports were generally sourced from Melbourne Water, excluding those which were completed by Jacobs and already held in-house. Key species of interest for this study were Dwarf galaxias (*Galaxiella pusilla*), Australian grayling (*Prototroctes maraena*) and Growling grass frog (*Litoria raniformis*).

Full details of each study are included in the References.

In addition to previous studies, we also reviewed the Victorian Biodiversity Atlas (VBA) database for all relevant species records, and any additional records from the Melbourne Water fish database. The review of the fish species records also involved a review of their lifecycle and habitat preferences in order to identify potentially suitable survey locations. Existing species records informed the development of the survey plan, as outlined in Section 2.2. A map of existing survey records (prior to this survey) is included in Appendix F.

2.1.2 PSP Area studies and plans

To determine the most appropriate survey locations, the review included aspects of PSP development and planning, specifically relating to stormwater runoff, hydrology, and the potential for altered streamflows in waterways. An objective of the study was to conduct field surveys to ground truth and update existing records, and to survey additional areas that recent studies have shown may be impacted by hydrological changes but have not been surveyed as they are outside the PSP boundaries.

The studies listed in Section 2.1.1 were also a key source of information for the characterisation of existing hydrological conditions, streamflows, and proposed future works and impacts. Additional data and information was sourced from recent and ongoing Jacobs studies, and through information provided by Melbourne Water.

2.2 Survey plan

The background review was a key component in the development of the survey plan, which was developed to ensure a targeted approach to the field survey and provide relevant data for future planning and approval processes. The survey plan set out the most appropriate sites, target species and techniques for field surveys which involved water collection for analysis of eDNA, and fish survey using netting or electrofishing techniques as appropriate to each site. The review identified the potential nature and location of impacts to flow and waterways, and helped to identify survey locations.

Potential survey locations were selected on the basis of:

- Ground truthing existing records, particularly where these were based on older VBA records that had not been verified recently
- Confirmation of species presence in previously recorded locations, particularly where there have been significant changes to habitat or hydrology since the date of the record
- Locations which may provide suitable habitat that had not been previously surveyed, or other gaps in survey location and effort
- Locations outside the PSP area which had not previously been surveyed, but which may be impacted by hydrological changes as a result of PSP development
- A suitable diversity of waterbody habitat types, including instream and off stream, and general habitat type and conditions
- Species requirements and preferred or potential habitat.

2.3 Survey methods

The field survey included two key aspects – collection of water samples for laboratory eDNA analysis, and fishing surveys using appropriate netting or electrofishing techniques at each site.

Water samples collected in the field were sent to EnviroDNA laboratory and analysed for the presence of DNA from the two target fish species (Dwarf galaxias and Australian grayling), as well as Growling grass frog. The use of eDNA sampling provides additional scope for assessing a large number of sites, targeting multiple species, and providing an additional weight of evidence for the fish surveys. eDNA sampling also allows assessment of waterways which may not be modelled as highly suitable habitat, but where previous data indicates potential movement of fish through these waterways, and potential exists for future habitat creation or enhancement in these waterways.

2.3.1 eDNA sampling methodology

Water samples were collected from each survey site in accordance with methods provided by the eDNA laboratory, EnviroDNA. At each site, a fresh sample pack was opened including clean latex gloves, disposable sampling syringe, filter unit, and labels and ziplock bags for sample storage. Water samples were generally collected from the water's edge using a sampling bottle on a pole to avoid entering the water, in order to minimise safety risks as well as potential contamination of samples. The sampling pole was rinsed with bleach solution between sites to avoid contamination, and rinsed several times with stream water at each site prior to drawing the sample to filter. Each sample was collected in accordance with a standard procedure involving drawing sampled water from sampling bottle into the syringe and expelling the water through the filter unit. The filter unit retains the eDNA from the sampled water and was removed from the syringe after water had been pushed through. Filter units were removed with gloved hands and placed into labelled zip lock bags and stored on ice before transporting to laboratory for analysis. It should be noted that the laboratory protocol is to push up to 500 ml (10 syringes worth) of water through the filter unit. However, this was not possible due to the turbidity of the samples water in this survey, meaning that water volumes were significantly less than 500 ml at most sites and the laboratory processing and analysis was affected, likely compromising the accuracy of results.

2.3.2 Fishing methodology

At each site, fish surveys were carried out using the appropriate fishing methods based on site conditions, habitat and target fish species. The prescribed survey methods for Australian grayling and Dwarf galaxias are detailed in the Survey Guidelines for Australia's Threatened Fish (Australian Government 2011) ([Department of Sustainability Environment Water Population and Communities 2011](#)) and a range of these techniques were used in the current survey. Details of methods used at each site including further technical information (electrofishing effort time etc) are provided in Table 2-1.

Backpack electrofishing

A range of fish species can be sampled using backpack electro fishers in wadable water. A 500 watt E-Fish backpack electrofisher was used to survey suitable habitat at two sites during the current project.

Box nets/bait traps

Bait traps were set at each site overnight. The bait traps consist of a 2 mm mesh and are approximately 250 mm x 250 mm x 450 mm (Figure 2-1). This size rating complies with restrictions set by DELWP/Fisheries Victoria (i.e. that a single bait trap must not be larger than 500 mm x 350 mm x 250 mm with an entrance not larger than 65 mm and mesh between 10 mm and 40 mm). Bait traps have a small aperture and are essentially a passive trap. The traps are generally set for an overnight period and retrieved the next morning.

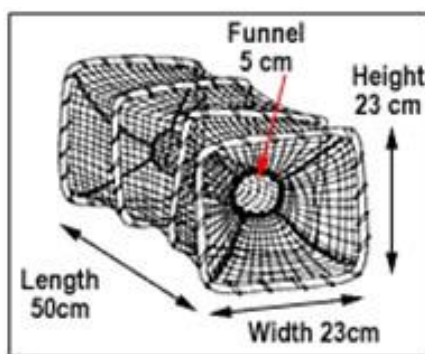


Figure 2-1 Typical bait trap configuration.

Fyke nets

Fyke nets (Figure 2-2) are single or double wing (600 mm drop passive trap devices) with a 2 - 20 mm mesh size that are set in and checked at intervals of no greater than 12 hours. Fyke nets are generally set overnight and entrapped fish released the next morning when checked. Risks to non-target species through the use of fyke nets are mitigated by placing a float in the cod end of the fyke net. This prevents the complete submersion of the net and ensures that any semi-aquatic fauna trapped can swim through to the floated end until removal from the net. The cod end of the net is tied out of the water against the bank.

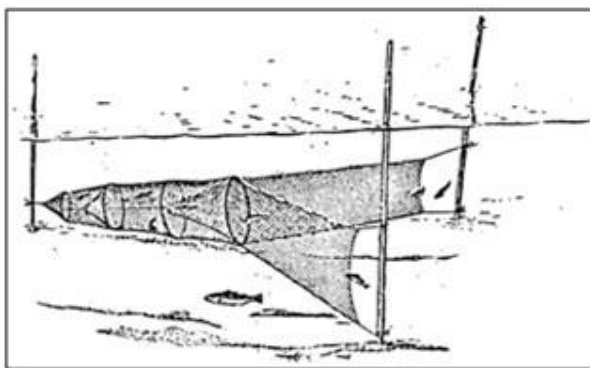


Figure 2-2 Diagram showing double wing fyke net design

Dip Netting

Active dip netting through suitable habitat such as submerged vegetation is a preferred method for capturing small bodied species such as Dwarf galaxias. This survey technique is undertaken in wadable habitat.

Seine Netting

Seine nets (Figure 2-3) are towed by two people in areas with no obstructions (e.g. snags and extensive macrophyte growth). The seine net used was 20m long with a 1.2m drop and made of 2-4mm stretch mesh. The two ends of the seine net are drawn toward the bank/shore and the fish are gathered in the cod end or middle of the net. The fish are then collected from the net, placed in a bucket for identification

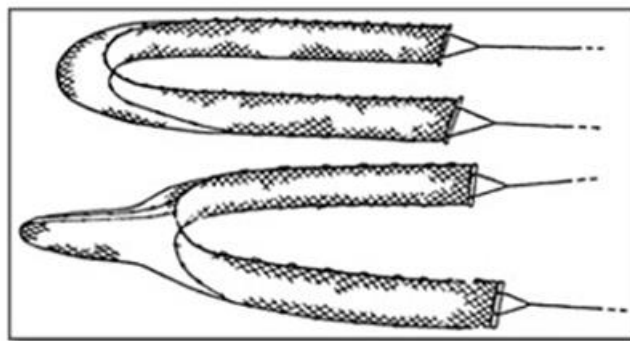


Figure 2-3 Diagram of two seine net types commonly used in fish surveys

Each of these fishing methods was used during this field survey, in accordance with the habitat and waterway conditions at each site, and the target species. A summary of methods at each site is shown in Table 2-1.

Table 2-1: Fishing methods summary

Site	eDNA	Dip Netting (10 min)	Bait traps (Number)	Fyke nets (number)	Electrofishing (time/mins)	Seine (2mm x 20m)
1	Yes			1		
2	Yes					yes
3	Yes		8	2		
4	Yes	yes	8			
5	Yes					yes
6	Yes	yes	8	2	6	
7	Yes	yes	8	2		
8	Yes					yes
9	Yes	yes	8			
10	Yes					yes
11	Yes	yes	8			
12	Yes					
13	Yes	yes	8	2		
14	Yes	yes	8	2		
15	Yes		8	2	6	
16	Yes					
17	Yes					

Site	eDNA	Dip Netting (10 min)	Bait traps (Number)	Fyke nets (number)	Electrofishing (time/mins)	Seine (2mm x 20m)
18	Yes					
19	Yes	yes	8	1		
20	Yes	yes	8			
21	No	yes	8	1		

All fish captured were removed from nets, identified and counted. Native species were returned to the waterway, and noxious species including Eastern gambusia were euthanised in line with animal ethics approved guidelines (using AQUIS solution).

2.3.3 Water quality data

In situ water quality parameters were recorded at each fish netting site using a calibrated YSI Proplus multimeter to measure pH, dissolved oxygen (%), dissolved oxygen (mg/l), conductivity ($\mu\text{S}/\text{cm}$), specific conductivity ($\mu\text{S}/\text{cm}$), temperature ($^{\circ}\text{C}$) and a HACH 2100Q turbidity meter was used to measure turbidity (NTU).

3. Results

3.1 PSP area habitat and waterways

As described in Section 1.1, the PSP area is bounded by the two main waterways in the area - Cardinia Creek to the west and south and Gum Scrub Creek to the east. Officer South Drain runs from north to south through the middle of the PSP area.

The PSP sits on the edge of the historic footprint of Koo Wee Rup Swamp, with flatness and cut drains coexisting alongside high value threatened species habitats and corridors. The catchment upstream has experienced extensive urban development, while land to south supports agriculture. The PSP area is characterised by a highly modified landscape, largely cleared for agricultural purposes and generally dominated by exotic vegetation. Remnant native vegetation is generally restricted to road reserves and riparian corridors. Nonetheless, the area retains significant environmental values and there are several species of national conservation significance have previously been recorded in the area, particularly along Cardinia Creek. Key aquatic species of note are the Dwarf galaxias and the Australian grayling, both EPBC-listed species. Growling grass frogs have also been recorded in the area.

Cardinia Creek retains significant ecological values, including native riparian vegetation and the presence of threatened fish including Australian grayling. A fishway has been constructed in Cardinia Creek to ameliorate the impacts of flow disruption and movement barriers caused by the Cardinia Drop structure.

Officer South Drain is a cut drain which runs alongside Officer South Road, and outfalls to Cardinia Creek in the south of the PSP. There is some established Swampy Woodland EVC along the roadside, but the area is highly modified and largely cleared of native vegetation.

Gum Scrub Creek is also a cut farm drain through most of the PSP area, though a more natural reconstructed form is retained north of the Princes Freeway. The creek retains patches of remnant or established vegetation.

3.2 Key species records and habitat

3.2.1 Habitat requirements

The habitat requirements of each 'target' fish species were considered in the survey planning to ensure that suitable potential habitat areas were included in the survey. Both species are known to occur within the PSP and surrounding area; the location of the fauna records reflects the different habitat requirements and lifecycle of the two species.

Dwarf galaxias is a small native galaxid species, reaching only 35–40 mm. Its preferred habitat is shallow, slow-flowing or swamp like waterways which contain either permanent or intermittent water, with abundant submerged and emergent vegetation. Fish are typically found in vegetated areas but have also been seen in open water areas. Dwarf galaxias can also tolerate dry periods well, and can travel overland between different habitat areas in very shallow water (~2cm). They complete their entire lifecycle in freshwater and are thought to die after spawning. The species is vulnerable to direct impacts from loss of habitat, including drainage of wetland areas and clearance of vegetation.

Australian grayling is a medium fish, generally growing to around 200 mm. The species is migratory. Most of its lifecycle is spent in freshwater, but following spawning (in freshwater), larvae hatch from eggs and are swept downstream to estuarine/marine areas. At around six months of age, juveniles migrate back up freshwater rivers where they remain for the remainder of their life. Spawning typically occurs in late summer – early winter, initiated by an increase in flow volume and rate, and possibly lower temperatures. The requirement for increased

flows to trigger spawning, and suitable flows to enable the return upstream migration means that the species is vulnerable to impacts associated with changes in stream flow and velocity.

3.3 Hydrology and development review

Potential impacts to aquatic species arise from direct disturbance within the PSP (including loss of habitat from development, drainage, vegetation clearance, or construction of stormwater treatment wetlands) as well as indirect impacts as a result of changes to streamflow, hydrology and hydraulics.

Jacobs have recently completed an investigation of potential impacts of the Officer South and other adjacent PSPs on the aquatic values of Cardinia Creek, including an assessment of changes to stormwater inflows and associated changes to hydrology and hydraulics in the creek. The objective of this study was to examine the potential impacts of the development on the streamflow and habitat requirements of Dwarf galaxias and Australian grayling in Cardinia Creek.

The investigation indicated that the section of Cardinia Creek abutting the PSP will be relatively protected from stormwater inputs, with the majority of the DSS catchments directed to Officer South Drain and Gum Scrub Creek. Current planning assumes that Officer South Drain will need to be reconstructed along the eastern side of the road, with a suggested 50-100m corridor for hydraulic requirements. Where possible, to reduce stormwater volumes through this waterway, stormwater will be directed towards Gum Scrub Creek.

Initial drainage planning has indicated that Gum Scrub Creek will receive significantly increased flows as a result of the PSP development and will require some re-modelling. Reengagement of floodplain areas and a proposed a minimum 100m buffer width on each bank has been proposed. The study also indicated that there is the potential for some impacts to the Cardinia Creek channel downstream of the Officer South Drain outfall if suitable low flow retardation is not provided within the Officer South PSP.

In addition, there are works proposed for the downstream reaches of Cardinia Creek (modified drain sections) to increase the capacity of the creek during high flow events and minimise the impact of flooding in adjacent agricultural properties. These works are not directly related to the upstream PSP developments, but are also likely to impact on conservation values of Cardinia Creek, particularly the use of the lower reaches of the creek by Australian grayling as part of their migrations. This area is not covered by the MSA and EPBC referral requirements in this area may need to be considered. Conservation values and flow impacts will need to be considered more broadly in the context of future development surrounding Cardinia Creek.

3.4 Survey plan and sample site selection

3.4.1 Review and potential survey locations

Following our review of previous studies and database records, and in consultation with Melbourne Water, we developed a preliminary sampling and survey plan based on the target species, likely habitat, and areas of potential impacts to waterways from the PSP development.

The potential survey locations considered included the major waterways and tributaries of the PSP region, including off-stream water bodies and areas both upstream and downstream of the PSP, namely:

- Officer South Drain (from the Princes Freeway to Cardinia Creek).
- Lower Gum Scrub Creek (from the Princes Freeway to the southern boundary of the PSP, plus other targeted sites downstream to Bullarto Road and including a wetland area east of Cardinia Road)
- Other roadside drains and off-stream habitats (e.g. farm dams)

- Additional locations on Gum Scrub Creek and Officer South Drain to the north of the PSP

Potential survey sites were selected based on the following factors:

- The location of historical records of target species (records which require updating/ground truthing)
- Potentially suitable instream or wetland habitat that may support target species, based on initial site inspections and aerial imagery. This includes areas upstream of, or adjacent to the PSP if they support habitat that is not well represented within the PSP. For example, off channel wetlands north of the PSP along Officer South Drain and Gum Scrub Creek, and wetlands adjacent to Cardinia Creek.
- Areas that may be either directly or indirectly impacted as a result of PSP development. Areas within the PSP may be directly impacted through loss of habitat as a result of future development, including construction of stormwater treatment wetlands and drainage works. Areas downstream of the PSP including Cardinia Creek may be impacted by changes in hydrology and drainage within the PSP areas, leading to changes to stream hydrology and hydraulics and impacts to Australian grayling populations.
- Areas which are outside the PSP but that support target species and may act as a source population. Source populations from could recolonise waterways in the PSP area when prevailing conditions permit, or following the construction of wetlands associated with the PSP.
- A diversity of habitat types (larger creek, smaller tributary or drain, wetlands, farm dams) that also contain suitable water levels and flow conditions for access and sampling.
- Off channel natural and constructed wetlands and farm dams that may support Dwarf galaxias and Growling grass frogs based on site inspection and aerial imagery. This includes farm dams associated with current or historical drainage lines.
- Landholder permission and access to sites. Potential sites were identified based on habitat suitability and data gaps, and also the ability to access the site. Additional potential sites were selected to ensure that if access to some sites was not possible, then 20 sites could still be sampled. Potential sites were located on a mixture of public, Melbourne Water or developer owned land, as well as private land. Permission to access sites on private land was sought through Melbourne Water.

A draft sample plan was provided to Melbourne Water for review, and for discussion at a meeting held on 22nd of April 2021.

The final survey sites were refined on the basis of suitability, variety, access, and water levels. Some potential survey sites could not be sampled as permission to access the site could not be obtained from the landholder. In addition, potential survey sites along Officer South Drain could not be sampled due to a lack of water. A preliminary site inspection was carried out in December 2020 to identify potential survey locations; there was no flow in the drain at that time, nor during the subsequent site survey dates.

3.4.2 Sample sites

The final field survey sites are shown in

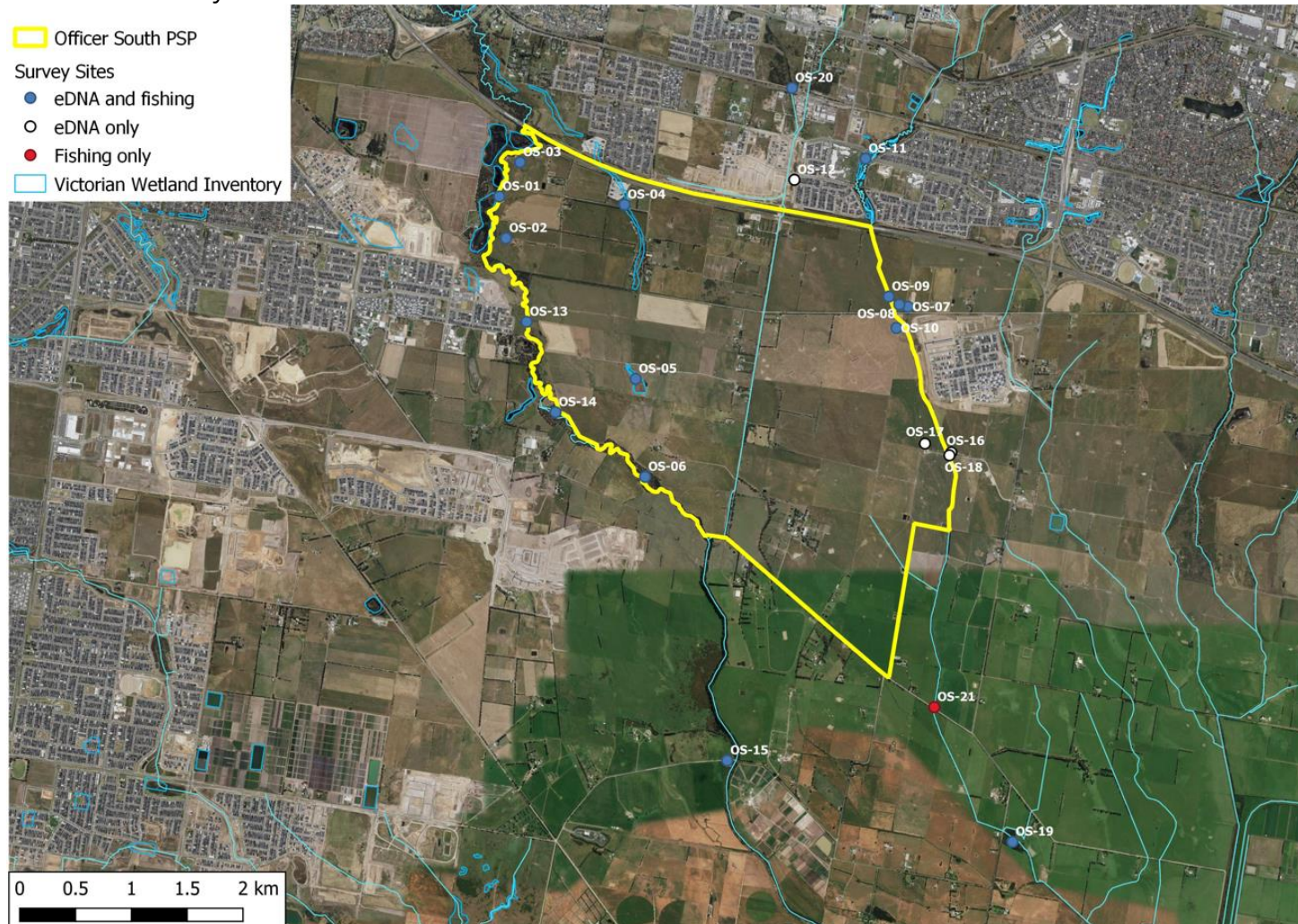


Figure 3-1 and summarised in Table 3-1. Figure 3-1 shows sites where both eDNA sampling and fishing surveys were completed.



Figure 3-1 Field survey sites

Table 3-1 Field site details

Site	Type	Catchment	Latitude	Longitude
OS-01	Creek	Cardinia Creek	-38.073895	145.377597
OS-02	Farm dam	Cardinia Creek	-38.077245	145.378284
OS-03	Farm dam	Cardinia Creek	-38.071145	145.379722
OS-04	Dam	Cardinia Creek	-38.074548	145.390384
OS-05	Large farm dam	Cardinia Creek	-38.088544	145.391526
OS-06	Creek	Cardinia Creek	-38.096405	145.392495
OS-07	Dam	Gum Scrub Creek	-38.082747	145.419343
OS-08	Dam	Gum Scrub Creek	-38.082567	145.418464
OS-09	Creek	Gum Scrub Creek	-38.081934	145.417386
OS-10	Dam	Gum Scrub Creek	-38.084449	145.418166
OS-11	Stormwater wetland	Gum Scrub Creek	-38.070852	145.415017

OS-12	Instream stormwater wetland	Officer South Drain	-38.072552	145.407744
OS-13	Creek	Cardinia Creek	-38.083958	145.380387
OS-14	Creek	Cardinia Creek	-38.091219	145.383339
OS-15	Creek	Cardinia Creek	-38.119182	145.400879
OS-16	Farm dam	Gum Scrub Creek	-38.094445	145.423775
OS-17	Farm dam	Gum Scrub Creek	-38.093736	145.421082
OS-18	Creek	Gum Scrub Creek	-38.094682	145.423583
OS-19	Shallow instream wetland	Gum Scrub Creek	-38.125707	145.43003
OS-20	Stormwater wetland	Officer South Drain	-38.065175	145.407507
OS-21	Gum Scrub Creek	Gum Scrub Creek	-38.114896	145.422036

4. Field survey results

The summary results for each site including site description, photographs and field survey results are presented in this Section. General habitat notes were collected at each site, including potential suitability of habitat for the target fish species (Dwarf galaxias and Australian grayling), growling grass frog, and southern bandicoot.

This section also presents fish survey catch data, including the number and species of fish caught at each site. Species detected included the target threatened species (Australian grayling and Dwarf galaxias), other native species (tupong, common galaxias, spotted galaxias, southern pygmy perch, flatheaded gudgeon, short-finned eel) and exotic species (Brown trout, eastern gambusia, European carp, and goldfish). Where very large numbers of eastern gambusia were recorded, approximate counts are provided.

Results of eDNA analysis are also presented in this section. Each water sample was analysed for eDNA of three species – Australian grayling, Dwarf galaxias, and Growling grass frog. Where results are listed as 'negative', this means negative for all three species, unless otherwise stated. eDNA and fish survey catch data are included in the summary results for each site in this section.

In terms of the target species, Australian grayling were recorded in the fish survey at two sites (Sites 6 and 15), but Dwarf galaxias were not captured in the fish survey at any site. There were two sites which recorded equivocal eDNA results for Dwarf galaxias (Sites 3 and 14), and no other positive eDNA samples for Australian grayling or Growling grass frog. These summary results for target species are shown in the colour-coded survey sites map in Figure 4-1.

Finally, water quality results are summarised in this section for each site where in-situ water quality readings were taken. Full water quality data is included in Appendix D, which also shows the water quality objectives for the Rivers and Streams of the area, as per the Environment Reference Standard. The objectives are for Rivers and Streams and not intended to apply to constructed stormwater and agricultural drains, wetlands, or off-stream farm dams. Many of the sample sites fall into those categories; water quality objectives are provided for high level general reference and comparison only.

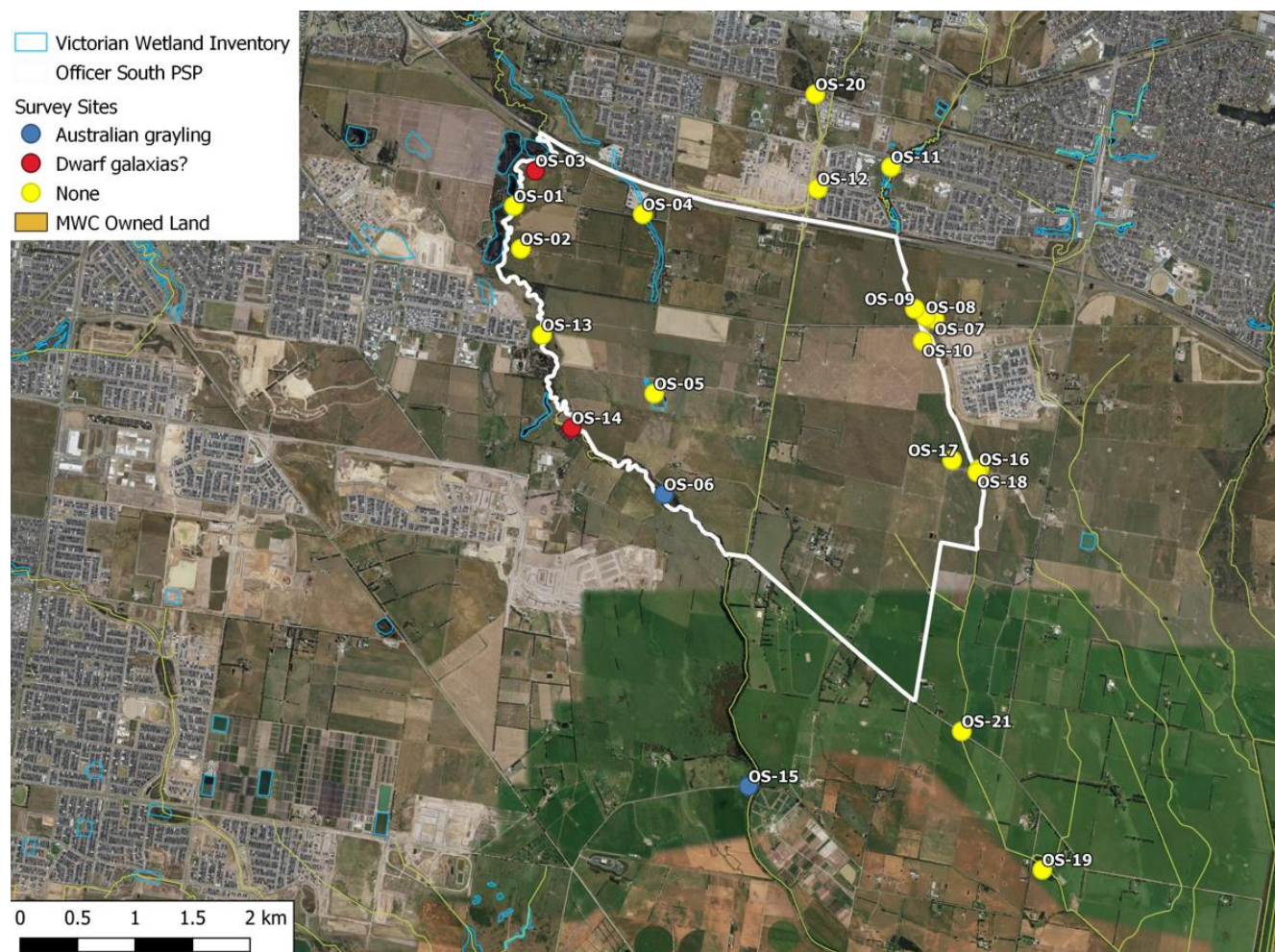


Figure 4-1 Map of survey sites with summary results. Red dots indicate sites which recorded equivocal eDNA results for Dwarf galaxias, blue dots indicate sites where Australian grayling were captured in fish surveys. Yellow dots are sites where there were no positive results for Australian grayling, Dwarf galaxias or Growling grass frog.

4.1 Site One

Site OS-1 was located on Cardinia Creek, in a narrow muddy section accessed via private property on Handford Lane. The understory vegetation was dominated by blackberry thicket. There was a thick mid-storey of small shrubs and trees (a mix of native and exotic), with some larger trees. The channel was narrow and flowing rapidly, and contained a large amount of instream wood and small snags. Banks were predominantly muddy with some small undercut areas (Table 4-1).

Habitat at this site has the potential to support the following target species: Southern brown bandicoot, Australian Grayling, Dwarf galaxias (movement only), Growling grass frog (movement only).

A single native fish (flathead gudgeon) was caught during the fishing survey, and the water samples tested negative for eDNA from the three target species.

Table 4-1 Site One: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-1	Creek	Cardinia Creek	Fyke net	1 flathead gudgeon	Negative



Water quality was characterised by cool temperature (12 degrees), consistent with the ambient temperature and time of day, neutral pH and relatively high dissolved oxygen (93% saturation, 9.89 mg/L) (Table 4-2). Turbidity, conductivity, dissolved oxygen (DO) saturation and pH values all met the water quality objectives (WQOs) as outlined in Section 4 and Appendix D.

Table 4-2 Site One: Summary water quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-01	12	93.6	9.89	205.5	7.47	12.1	11.00

4.2 Site Two



Site OS-2 was a farm dam located on the same private property as Site OS-1, accessed from Handford Lane. The dam had negligible fringing vegetation, and some floating vegetation on fringe. Edges were muddy, with significant pugging (Table 4-3). Water was noticeably highly turbid. A large fish, suspected to be a carp, was observed breaking the surface.

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey resulted in the catch of a large number of exotic species – more than 60 Eastern gambusia, 3 large European carp, and 31 goldfish. Water samples tested negative for eDNA from the three target species.

Table 4-3 Site Two: Summary results and photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-2	Farm Dam	Cardinia Creek catchment	Seine	>60 eastern gambusia 3 European carp 31 goldfish	Negative

Compared to Site OS-01 which was on the same property but located on Cardinia Creek rather than in an off-stream dam, the water at Site OS-02 was warmer (16 degrees), with much lower DO (64% saturation, 6.27 mg/L) (Table 4-4). Turbidity (209 NTU) was an order of magnitude higher than both the Cardinia Creek site and the water quality objective for streams. Dissolved oxygen (DO) saturation and turbidity did not meet the water quality objectives (WQOs).

Table 4-4 Site Two: Summary Water Quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-02	16.1	64	6.27	227.2	7.3	209	11.30

4.3 Site Three

Site OS-3 was another dam site on the same private farming property as OS-1 and OS-2.



The site is a shallow farm dam/ wetland with some fringing vegetation, generally low (<10 cm high), but with some tussock grasses. The water surface was entirely covered by a thick layer of *Azolla*. There was a sparse cover of swamp paperbarks emerging from the dam and also fringing the dam. Some pugging was evident around the edges of the water (Table 4-5). The wetland was located close to the creek.

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site resulted in the catch of one common galaxias and one spotted galaxias. Water samples tested negative for eDNA from Australian grayling and Growling grass frog, and returned an **equivocal** result for Dwarf galaxias.

Table 4-5 Site Three: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-3	Farm dam	Cardinia Creek catchment	Bait traps Fyke nets	1 common galaxias 1 spotted galaxias	Equivocal for Dwarf galaxias Negative for Australian grayling and Growling grass frog

Water quality at this site was characterised by very low dissolved oxygen levels, likely as a result of the thick *Azolla* covering the water surface (Table 4-6).

Table 4-6 Site Three: Summary Water Quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-03	11.5	13	1.31	345.2	7.5	819	10.30

4.4 Site Four

Site four was located on a private property on Handford Lane, just south of the Princes Freeway and adjacent to the large freeway service station site. The site was a small dam on an old drainage line, which now forms a chain of ponds and small wetlands, including the stormwater treatment wetlands associated with the service station site immediately upstream. The dam was well vegetated, with lots of floating vegetation and some floating algae. There were also dense patches of emergent vegetation (predominantly Typha), as well as dense fringing vegetation that extends into the water and was dominated by cooch grass and also included some tussocks. There was a small area of open water in the centre of the dam (Table 4-7).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site was conducted using bait traps and dip netting; no fish were caught. Water samples tested negative for eDNA from the three target species.

Table 4-7 Site Four: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-4	Dam	Cardinia Creek catchment	Dip netting Bait traps	None	Negative




Water quality at this site was characterised by moderately low dissolved oxygen levels, though DO levels would be expected to fluctuate during the diurnal cycle (Table 4-8). Turbidity at the site (36.9 NTU) was just above the WQO for rivers and streams; and pH and conductivity both met WQOs.

Table 4-8 Site Four: Summary Water Quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-04	14.2	45	4.56	347.6	7.1	36.9	1200

4.5 Site Five

Site five was a large farm dam accessed through a private property on the western side of Officer South Rd, between Leckie Rd and Patterson Rd.

The dam was large, with gently sloping muddy edges and considerable pugging. There was no floating, emergent or fringing vegetation. The dam appeared to be located at the downstream end of a small, local ephemeral drainage line (Table 4-9).

Habitat at this site is generally of low quality currently but has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site was conducted using a seine net, as appropriate for a large, shallow open water site with no vegetation; 28 goldfish were caught. Water samples tested negative for eDNA from the three target species.

Table 4-9 Site Five: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-5	Large farm dam	Cardinia Creek catchment	Seine net	28 Goldfish	Negative



Dissolved oxygen at this site was relatively high and within the range set for the WQOs (Table 4-10). As noted previously, DO levels are highly variable over the diurnal cycle due to respiration and photosynthesis by aquatic algae and plants, and are likely to be higher if measured in sunny conditions, and or during the afternoon (as at this site). Turbidity levels exceed the WQOs and pH was slightly elevated and only just within the range set out in the WQOs.

Table 4-10 Site Five: Summary Water Quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-05	17.9	96.1	8.98	224.1	7.86	158	1440

4.6 Site Six

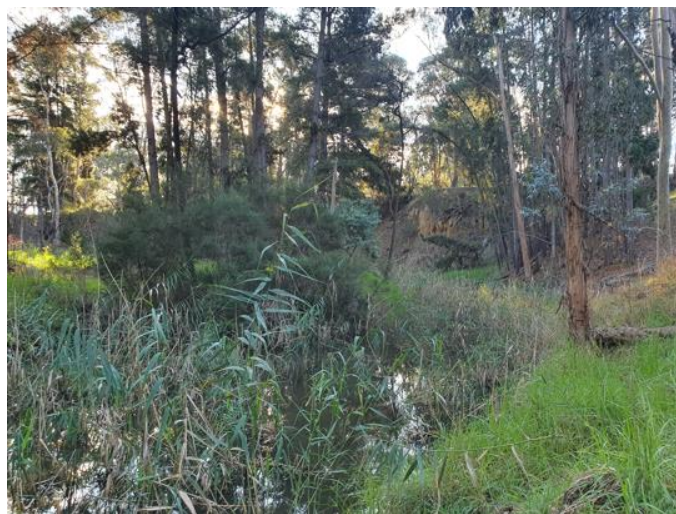
Site Six was located on Cardinia Creek, and accessed through a property on the western side of Officer South Road, near Patterson Rd. The site was located at a constructed fishway on a broad section of Cardinia Creek, downstream of the drop structure. The creek channel is relatively broad at this location, but quite deeply incised. The creek was shallow and flowing over rock and pebble substrate, with some backwater and pool habitat also present. Rock beaching was present at the fishway, and the site was well vegetated. Fringing grass vegetation was present upstream and downstream of the site, extending into the channel in some places. There were dense thickets of emergent vegetation (phragmites) upstream and downstream of the fishway. There was some limited mid-storey vegetation and a moderate canopy of generally small native trees.

Habitat at this site has the potential to support the following target species: Southern brown bandicoot, Australian Grayling, Dwarf galaxias (movement only) and Growling grass frog (movement only).

The fishing survey at this site was conducted using a variety of techniques including electrofishing and netting, to survey the variety of habitats present. Five different native fish species were captured, including Australian grayling (Table 4-11). Water samples tested negative for eDNA from the three target species.

Table 4-11 Site Six: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-6	Creek	Cardinia Creek	Dip netting Bait traps Fyke nets Electrofishing	1 Australian grayling 7 Common galaxias 1 Flathead gudgeon 3 Short-finned eel 3 Tupong	Negative



Water quality at the site was generally good, reflecting the relatively unimpacted and well vegetated on-stream site. Dissolved oxygen was relatively high (Table 4-12). DO saturation, conductivity and turbidity all met WQOs. However, pH was unusually high (13) and well outside the WQO range (6.4 – 7.9).

Table 4-12 Site Six: Summary Water Quality results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-06	14.5	106.4	10.76	321.7	13	7.7	1600

4.7 Site Seven

Site Seven was a dam located on Development Victoria land, on Lecky Road. The dam was close to Gum Scrub Creek and fenced from cattle. The dam had relatively steep banks which were bare and muddy in some parts and vegetated (tussocks, sedges and grasses) in other areas. The pond supports a high cover of floating and emergent vegetation. During the eDNA sampling, and numerous small fish (thought to be eastern gambusia) were observed.

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey used dip netting, bait traps and fyke nets and a large number of native and exotic fish were caught. Over 700 Eastern gambusia were caught, as well as one goldfish. Native species caught were short-finned eel, and common galaxias (Table 4-13). Water samples tested negative for eDNA from the three target species.

Table 4-13 Site Seven: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-7	Dam	Gum Scrub Creek	Dip netting Bait traps Fyke nets	2 Common galaxias >700 Eastern gambusia 1 Goldfish 2 Short-finned eel	Negative
					

Water quality at Site Seven met WQOs for all parameters (Table 4-14).

Table 4-14 Site Seven: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-07	16.5	87.2	8.33	417.1	7.34	31.8	1500


4.8 Site Eight

Site Eight was another dam located close to Site Seven, on the Development Victoria land, close to Gum Scrub Creek. Unlike Site Seven, Site Eight was unfenced, and cattle access and pugging was evident. The perimeter of the dam was predominantly bare mud, with pugging in most areas. There was some fringing vegetation, which was dominated by blackberries. The water was shallow and visibly turbid (Table 4-15).

Habitat at this site is generally of low quality but has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site recorded over 300 Eastern gambusia, and 3 goldfish. Native common galaxias were also caught. Water samples tested negative for eDNA from the three target species.

Table 4-15 Site Eight: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-8	Dam	Gum Scrub Creek	Seine net	2 Common galaxias >300 Eastern gambusia 3 Goldfish	Negative
					

The water quality at this site was characterised by much higher turbidity than Site Seven, reflecting the lack of fringing vegetation, areas of bare muddy banks, and evident disturbance from cattle access. Turbidity and conductivity levels exceeded the WQO limits. The DO saturation was also close to exceeding the upper limit of the WQO range (Table 4-16).

Table 4-16 Site Eight: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-08	19.7	125.2	10.96	575	7.09	92.5	1600

4.9 Site Nine

Site Nine was an on-stream site located on Gum Scrub Creek, close to Site Seven and Eight. The sampling location on Gum Scrub Creek was a series of well-vegetated runs and small, more open pools just upstream of Lecky Road (Table 4-17).

Run sections supported some submerged and emergent vegetation (primarily *Persicaria*), and fringing vegetation on runs and pools was a mix of tussocks, sedges and grasses. The water was turbid, and cattle pugging was evident, particularly in the pool sections. The creek was flowing well in the run sections.

Habitat at this site is generally of low quality but has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site recorded 39 Eastern gambusia, one goldfish, and one native common galaxias. Water samples tested negative for eDNA from the three target species.

Table 4-17 Site Nine: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-9	Creek	Gum Scrub Creek	Dip netting Bait traps	1 Common galaxias 39 Eastern gambusia 1 Goldfish	Negative




Water quality at this site showed that conductivity was higher than at nearby sites and did not meet WQOs. Other parameters (DO, turbidity, and pH) all met WQOs (Table 4-18).

Table 4-18 Site Nine: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-09	15.8	104.1	10.12	767	7.17	31.1	1330

4.10 Site Ten

Site Ten was a farm dam next to Gum Scrub Creek, accessed from a private property on the south side of Lecky Road. The dam was relatively large with significant open water areas. The dam fringe was bare in parts, with approximately 50% cover of fringing vegetation including grasses, sedges and reeds. There was some floating vegetation in edge areas, and patches of emergent vegetation, predominantly *Typha*. During eDNA sampling, water appeared relatively clear compared to other sites (Table 4-19).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

The fishing survey at this site was conducted using a seine net. It recorded over 500 Eastern gambusia, and one short-finned eel. Water samples tested negative for eDNA from the three target species.

Table 4-19 Site Ten: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-10	Dam	Gum Scrub Creek	Seine	> 500 Eastern gambusia 1 Short-finned eel	Negative
					

Dissolved oxygen levels were relatively high, and within WQO range as were all other parameters (Table 4-20).

Table 4-20 Site Ten: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-10	19.4	127.2	11.57	333.9	7.73	22.2	1400

4.11 Site Eleven



Site Eleven was a large, constructed stormwater wetland next to Gum Scrub Creek. The site was located to the north of the Princes Freeway, adjacent to Officer Secondary College and immediately to the south of Bridge Road. The site was reached via pedestrian access from Bridge Road. The site supported a very high cover of floating and submergent vegetation (primarily water ribbon) and dense, tall fringing vegetation. Some emergent vegetation was also noted. The wetland fringes and surrounding area had a relatively dense cover of trees and tall shrubs. The water appeared to be deep and relatively clear at the time of the eDNA sampling (Table 4-21).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog, and Southern brown bandicoot (though the site is quite isolated from other habitat).

The fishing survey at this site resulted in the capture of more than 150 Eastern gambusia, as well as two short-finned eel. Water samples tested negative for eDNA from the three target species.

Table 4-21 Site Eleven: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-11	Stormwater wetland	Gum Scrub Creek	Dip netting Bait traps	> 150 Eastern gambusia 2 Short-finned eel	Negative

Water quality at the site had a recorded conductivity of 2058 $\mu\text{S}/\text{cm}$, which was significantly higher than surrounding sites, and around four times higher than the WQO for streams. With the exception of the conductivity, water quality was otherwise good at this site and all other parameters met WQOs. The DO concentration was relatively high, and turbidity was relatively low (in accordance with visual observations) (Table 4-22).

Table 4-22 Site Eleven: Summary Water Quality Results

Site	Temperature ($^{\circ}\text{C}$)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Turbidity (NTU)	Time
OS-11	16.3	101.1	9.76	2058	7.41	14.2	1300

4.12 Site Twelve



Site Twelve was a newly constructed wetland on Officer South Drain, immediately to the north of the Princes Freeway. The wetland is on private (developer-owned land) and access was via the site office and an induction process. The site has only recently been constructed, and is part of stormwater treatment associated with adjacent and upstream urban development. The wetland is edged with jute matting with some smaller rocky areas, and is in the process of being vegetated (planting currently underway). The site is located at the junction of the main Officer South Drain and other smaller stormwater drains, and outflow from the site flows into Officer South Drain and under the freeway.

The site is an artificial wetland which is recently constructed and offers minimal habitat currently. Habitat at this site has the potential to support the following target species once revegetated: Dwarf galaxias, Growling grass frog. The site was not fished due to low fish habitat potential and access constraints. As there was no fish survey

completed, water quality data was not collected. Water samples tested negative for eDNA from the three target species (Table 4-23).

Table 4-23 Site Twelve: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-12	Instream stormwater wetland	Officer South Drain, north of Princes Freeway	Not fished	None	Negative

4.13 Site Thirteen

Site Thirteen was an on-stream site located on Cardinia Creek. The site is on Melbourne Water owned land south of Princes Freeway and Grices Road, and between Soldiers Road to the west and Officer South Road to the east.

The creek at the sample site was narrow and deeply incised, and had moderate flow at the time of sampling. There was substantial remnant natural riparian and fringing vegetation, including some larger eucalypt trees that overhang the creek. The channel itself was contained numerous woody snags and instream vegetation (Table 4-24).

Habitat at this site has the potential to support the following target species: Southern brown bandicoot, Australian grayling, Dwarf galaxias (movement only) and Growling grass frog (movement only).

The fishing survey at this site resulted in the capture of four different native fish species, though these did not include the target species (Australian grayling, Dwarf galaxias). Water samples tested negative for eDNA from the three target species.

Table 4-24 Site Thirteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-13	Creek	Cardinia Creek	Dip netting Bait traps Fyke nets	21 Common galaxias 1 Flatheaded gudgeon 2 Short-finned eel 1 Southern pygmy perch	Negative



Water quality at the site was met WQOs for streams for every parameter (Table 4-25).

Table 4-25 Site Thirteen: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-13	11.5	89.8	9.56	288.1	7.06	13.3	1400

4.14 Site Fourteen

Site Fourteen was also located on Cardinia Creek, just downstream of drop structure, on Melbourne Water land. Access was on foot through the gate at the corner of Smiths Lane and Thompsons Road. The creek at this location is quite broad (5 – 10 m wide), with low steep banks in a shallow valley. The site has a good cover of fringing and emergent vegetation, including an expansive dense patch of phragmites. The creek was flowing, and water appeared turbid. There is a high cover of riparian vegetation, primarily small trees and large shrubs, with a more patchy mid-storey.

Habitat at this site has the potential to support the following target species: Southern brown bandicoot (though groundcover is patchy), Australian grayling, Dwarf galaxias (movement only) and Growling grass frog (movement only).

The fishing survey at this site recorded both native and exotic species, including the Eastern gambusia as well as native gudgeon and galaxias species (Table 4-26).

The water samples from this site tested negative for eDNA from Australian grayling and Growling grass frog, but equivocal for Dwarf galaxias.

Table 4-26 Site Fourteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-14	Creek	Cardinia Creek	Dip netting Bait traps Fyke nets	1 Common galaxias 76 Eastern gambusia 16 Flathead gudgeon	Equivocal for Dwarf galaxias Negative for Australian grayling and Growling grass frog.



At Site Fourteen, dissolved oxygen levels were significantly lower than at Sites 13 and 15, located upstream and downstream on Cardinia Creek. The DO levels of 42.7% saturation and 4.49 mg/L at this site (Table 4-27) were around half of the DO levels at upstream and downstream sites. In addition, turbidity was also much higher (around double) than at the upstream and downstream sites. Water quality at this location may be affected by the operation of the drop structure or other catchment factors that differ from upstream and downstream sites. Water quality may also have influenced the fish survey results at this site, which showed a lower diversity and abundance of native fish than other nearby sites.

Table 4-27 Site Fourteen: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-14	13.0	42.7	4.49	183.9	6.96	28.7	1200

4.15 Site Fifteen

Site Fifteen was located on Cardinia Creek, downstream of Site Fourteen, at the Chasemore Road crossing. Access to the site was from the road crossing. At this location, the creek was quite broad (5 – 10 m wide) in a shallow valley setting with good cover of fringing vegetation (mostly grasses). At the time of eDNA sampling the creek was flowing well. There is a riffle section / fishway just downstream of the road crossing at this site, and a larger

pool section upstream. There was a high cover of riparian vegetation, primarily small trees and large shrubs, with a mid-storey that was denser than Site 14.

Habitat at this site has the potential to support the following target species: Southern brown bandicoot, Australian grayling, Dwarf galaxias (movement only) and Growling grass frog (movement only).

The fishing survey resulted in detection of five species, four of which were native. The native species catch included 7 Australian grayling (Table 4-28). Water samples tested negative for eDNA from the three target species.

Table 4-28 Site Fifteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-15	Creek	Cardinia Creek at Chasemore Road crossing	Bait traps Fyke nets Electrofishing	7 Australian Grayling 6 Brown trout 3 Common galaxias 5 Short-finned eel 4 Tupong	Negative




Water quality at this site was similar to Site Thirteen, further upstream, and met WQOs for streams for every parameter (Table 4-29).

Table 4-29 Site Fifteen: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-15	11.6	84	8.88	208.6	7.18	15.8	1000

4.16 Site Sixteen

Site Sixteen was a farm dam immediately adjacent to Gum Scrub Creek, and accessed through a private property on Cardinia Road. The dam is fenced off from cattle and has good cover of fringing vegetation that extends into

the water (Table 4-30). There were some small patches of emergent *Typha* and *Phragmites*. Fish (thought to be Eastern gambusia and carp) could be seen breaking the water.

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

There was no fishing survey conducted at this site due to access constraints at the time of survey, and as a result there was no water quality data recorded.

Table 4-30 Site Sixteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-16	Farm dam	Gum Scrub Creek	Not fished	None	Negative



4.17 Site Seventeen

Site Seventeen was another farm dam on the same property as Site Sixteen, also located immediately adjacent to Gum Scrub Creek. This dam was on the western side of the creek, while Site Sixteen was on the eastern side. Site Seventeen was also immediately adjacent to Gum Scrub Creek and fenced off from cattle. The dam was large and comprised mostly open water, with good cover of fringing vegetation extending into the water in places (Table 4-31).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog.

As with Site Sixteen, access constraints at the time of the fish survey meant that no fishing survey was conducted at this site and no water quality data was recorded.

Table 4-31 Site Seventeen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-17	Farm dam	Gum Scrub Creek	Not fished	None	Negative



4.18 Site Eighteen


Site Eighteen was located on the same property as Site Sixteen and Seventeen, but was an on-stream site (on Gum Scrub Creek) rather than a dam. The site location was Gum Scrub Creek at the farm road/driveway crossing. Sampling was conducted both upstream and downstream of the road crossing.

The creek at this location sloped steeply down a small 1.5 metre high barrage and culvert under the road. The creek was fringed with a thick cover of blackberry and swamp paperbark, with some emergent *Persicaria*. At the road culvert the creek was a wider slower flowing pool, which flowed over the sloped barrage into a narrow (<1 m wide), fast flowing section downstream (Table 4-32).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog, Southern brown bandicoot.

As with Site Sixteen and Seventeen, access constraints at the time of the fish survey meant that no fishing survey was conducted at this site and no water quality data was recorded.

Table 4-32 Site Eighteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-18	Creek	Gum Scrub Creek	Not fished	None	Negative
					


4.19 Site Nineteen

Site Nineteen was a shallow instream wetland located on Gum Scrub Creek and accessed from a private landholder driveway on Cardinia Road. The site was a small branch of Gum Scrub Creek that flowed through a marshy area next to a patch of swamp paperbark scrub. The marsh supported a high cover of grasses, sedges and rushes, as well as some patches of blackberry. While the area is fenced, there is evidence of cattle damage and pugging in parts. Flow was quite low, with only small areas flowing water and some shallow standing water. It is likely that most of the site would cease to flow and dry out in most parts, when conditions are drier.

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog, Southern brown bandicoot.

The fishing survey at this site recorded one common galaxias, one Eastern gambusia and one short-finned eel. Water samples tested negative for eDNA from the three target species (Table 4-33).

Table 4-33 Site Nineteen: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-19	Shallow instream wetland	Gum Scrub Creek	Dip netting Bait traps Fyke nets	1 Common galaxias 1 Eastern gambusia 1 Short-finned eel	Negative
					

Water quality at the site did not meet WQOs for streams for most parameters (DO saturation, conductivity and turbidity). Turbidity and DO levels were close to WQOs but conductivity levels (829 $\mu\text{S}/\text{cm}$) were well above the guideline value of <500 (Table 4-34).

Table 4-34 Site Nineteen: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Turbidity (NTU)	Time
OS-19	12.7	68.8	7.34	829	7.62	35.5	1000

4.20 Site Twenty

Site Twenty was a narrow, constructed stormwater wetland on Officer South Drain, on public land adjacent to the railway line. The wetland supported a very high cover of emergent floating and submergent vegetation and dense tall fringing vegetation. There was also a relatively dense cover of surrounding trees and tall shrubs. The water appeared deep and relatively clear (in low light conditions at dusk) (Table 4-35).

Habitat at this site has the potential to support the following target species: Dwarf galaxias, Growling grass frog, Southern brown bandicoot.

Fishing survey at this site recorded only Eastern gambusia (>700 individuals), consistent with the impacted, urbanised constructed wetland habitat. Water samples tested negative for eDNA from the three target species.

Table 4-35 Site Twenty: Summary Results and Photos

Site number	Site type	Location	Methods	Catch data	eDNA
OS-20	Stormwater wetland	Officer South Drain	Dip netting Bait traps	> 700 Eastern gambusia	Negative



Water quality at the site met WQO for DO and conductivity, but not pH and turbidity which were slightly higher than the guidelines for streams (Table 4-36).

Table 4-36 Site Twenty: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	pH	Turbidity (NTU)	Time
OS-20	17.8	110.6	10.38	470.3	8.06	36.4	1400

4.21 Site Twenty One

Site Twenty One was located on Gum Scrub Creek at the Patterson Rd crossing, and was accessed from Patterson Rd. The creek is highly modified at this location and has been channelised. The creek was ~1.5m deep, and 2 m wide at the top of the bank. Water depth was approximately 0.6 m at the time of the fishing survey and the channel wetted width was approximately 1m. The riparian cover comprised blackwoods and eucalypts with a dense ground cover of box thorn, blackberries and other exotic weed species. This site was an additional site chosen for fishing survey due to access constraints at some other sites previously sampled for eDNA; no eDNA samples were taken at this site. The fishing survey at this site recorded 2 native species – common galaxias, and short-finned eel (Table 4-37).

Table 4-37 Site Twenty One: Summary Results

Site number	Site type	Location	Methods	Catch data	eDNA
OS-21	Creek	Gum Scrub Creek	Dip netting Bait traps Fyke nets	2 Common galaxias 2 Short-finned eel	No sample

Water quality at the site met all WQOs for streams, with the exception of conductivity which was significantly higher than the guideline limit of 500 $\mu\text{S}/\text{cm}$ (Table 4-38).

Table 4-38 Site Twenty One: Summary Water Quality Results

Site	Temperature (°C)	Dissolved oxygen (% saturation)	Dissolved oxygen (mg/l)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Turbidity (NTU)	Time
OS-21	12.8	73.5	7.56	899	7.64	23.7	900

5. Discussion

The field survey results showed a variety of native and exotic fish present in the waterways around the PSP, and were largely consistent with previous surveys and expectations.

5.1 Waterways and habitat

Cardinia Creek is known to support significant ecological values. In this survey, sites along Cardinia Creek (including instream sites and off stream sites such as dams and wetlands) represented the most suitable, quality, fish habitat and comprised the bulk of the fish records. There were 8 sites surveyed on Cardinia Creek, 3 off stream and 5 instream, and fish were captured in the fishing surveys at every site. Instream sites supported a larger and more diverse native fish population than the off stream sites. The five instream Cardinia Creek sites recorded a number of native fish species including several galaxias species, flathead gudgeon, tumpung, short-finned eel, and southern pygmy perch. The three sites which recorded only native species (no exotic species) were all instream sites on Cardinia Creek. Of the Cardinia Creek sites that recorded **only** exotic fish species, both were farm dams. The third farm dam site, however, recorded only native species – the common and spotted galaxias.

The only two sites where Australian grayling were recorded in this survey were also both on Cardinia Creek (Site 6 and Site 15, see below for further discussion). Cardinia Creek sites (3 and 14) were also the only sites that recorded equivocal DNA results for Dwarf galaxias. There were no other equivocal or positive DNA results for any species.

Gum Scrub Creek is a highly impacted waterway, in the form of channelised farm drains for much of its length in the area of the PSP. The impacted nature of Gum Scrub Creek was consistent with the fishing survey results, which recorded fewer native fish than Cardinia Creek sites, no target species in either fish surveys or eDNA results, and a high abundance and diversity of exotic species. All Gum Scrub Creek sites (dam, wetland and creek) that were sampled in the PSP (sites 7 – 11) recorded high numbers of Eastern gambusia, a noxious pest species, and most also recorded goldfish.

In comparison to Cardinia Creek, the Gum Scrub Creek sites provided less suitable habitat and a far lower number and diversity of native fish caught. Fish were recorded at each of the seven sites surveyed on Gum Scrub Creek, however only two native species were recorded in total (common galaxias and short-finned eel) and exotic species were recorded at every site except one. Compared to Cardinia Creek sites, the Gum Scrub Creek sites recorded a much **greater abundance and diversity of exotic species**, and a **lower abundance and diversity of native species**. Only two native species were recorded, both of which are common, and tolerant of impacted waterway conditions. The survey results for Gum Scrub Creek sites also reflected the mix of site types – compared to Cardinia Creek, there was a higher proportion of dams and constructed wetlands (5 sites) compared to instream sites (2 sites). As with Cardinia Creek, the instream sites on Gum Scrub Creek recorded less exotic fish and more natives – the only site on Gum Scrub Creek which did not record any exotic species was an instream site.

Officer South Drain through the PSP could not be surveyed due to a lack of flows, though two sites to the north of the PSP on Officer South Drain were sampled. One site (Site 12) was a stormwater wetland that was in the process of construction and vegetation. The site offers low habitat value in its current condition, and no fish survey was conducted; eDNA samples from the site were negative for all species. The other site (Site 20) was also a stormwater wetland, though well established and vegetated, which recorded over 700 Eastern gambusia and no other fish species.

A summary of fish survey results, highlighted by waterway, is included in Appendix B.

5.2 Target species

Australian grayling

In terms of target species, Australian grayling were recorded at only two sites, (Site 6 and Site 15, see Table 5-1), both located on Cardinia Creek. Site Six was located on Cardinia Creek downstream of the drop structure, with good cover of mostly native riparian and instream vegetation. Several other native fish species were also recorded at this site, suggesting the site represents generally good habitat and water quality conditions and minimal impacts. The creek was flowing well at the time of sampling and a range of instream habitat types was present, including riffle, edge and pool areas. Australian grayling had previously been recorded near this location (See Appendix F)

Site Fifteen was also located on Cardinia Creek, and was similar in most characteristics to Site Six. Site Fifteen was also located in a reach of the creek that was flowing well at the time of survey, and contained a variety of riffle/fishway and edge/backwater habitats, with good riparian and fringing vegetation cover and good water quality. In addition to seven Australian grayling, Site Fifteen recorded several other native fish species (Common galaxias, short-finned eel, and tupong). This is consistent with the species also recorded at Site Six. Site Fifteen is located on Cardinia Creek outside of the PSP and was included in the survey as this reach of Cardinia Creek may be impacted by works within the PSP (See Section 3.3). There are no previous species records at this site, as most survey effort has been within the PSP area.

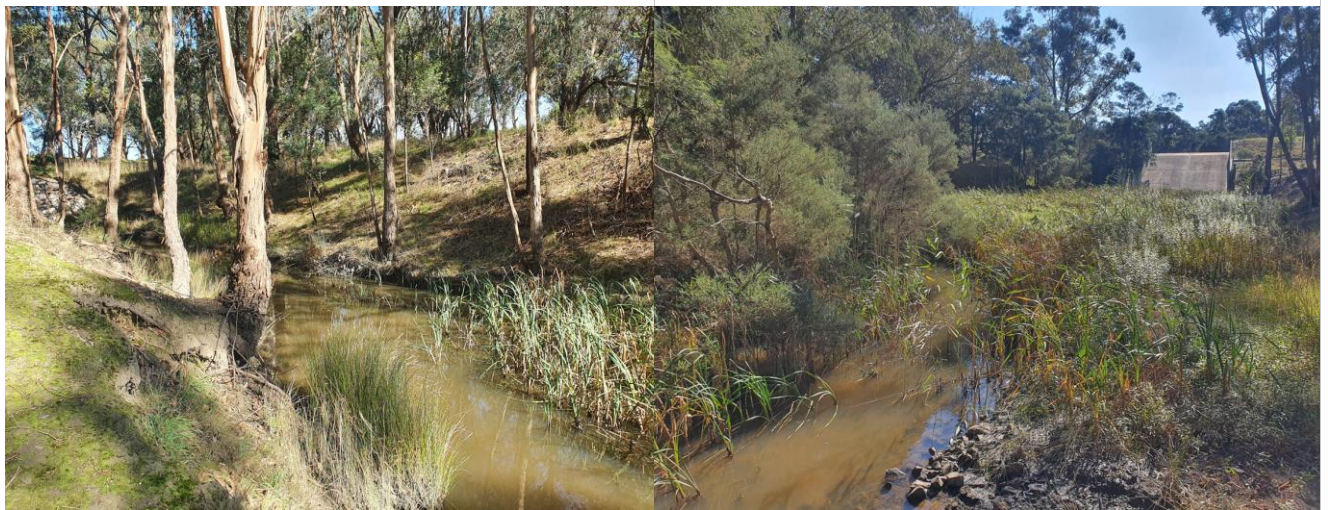
No Australian grayling were recorded at sites further upstream, though there have been previous species records near Site 14. Site 14 appeared from the field survey to provide suitable habitat for Australian grayling, though water quality was poor compared to nearby sites. None of the water samples tested positive for Australian Grayling eDNA, even at sites where the fish were caught in the field survey.

Table 5-1 Photos of Sites Six and Fifteen, the two sites which recorded Australian grayling

Site Six**Site Fifteen****Dwarf Galaxias**

No dwarf galaxias were recorded in any of the fishing surveys, but eDNA results returned 'equivocal' data for 2 sites, meaning it is likely that the species is present or has recently moved through the area. The sites where equivocal results were recorded were Site 3 (a farm dam on Cardinia Creek) and Site 14 (on Cardinia Creek). Both of these sites also recorded other native galaxias species (common galaxias and spotted galaxias). The farm dam site was located close to the creek, and the habitat (densely vegetated, still and shallow water) represents potential suitable habitat for Dwarf galaxias.

Table 5-2 Photos of Sites Three and Fourteen, the two sites which recorded Dwarf galaxias

Site Three**Site Fourteen**

Although no Dwarf galaxias were captured in the fishing survey, the equivocal eDNA results for these two sites is consistent with previous species records. There are two records for Dwarf galaxias along Cardinia Creek in the PSP area; the records are located in fairly close proximity to Site 3 and 14 (see Appendix F), suggesting the species is likely to be present in (or move through) the area.

Growling grass frogs

The field survey did not include Growling grass frog, but water samples collected were analysed for their DNA. None of the samples returned positive results. As noted previously, the eDNA samples were somewhat compromised by sampling and analysis issues, so the lack of positive results does not necessarily mean there were no Growling grass frog present at, or moving through the sites. There were suitable habitat conditions at a number of sites, but previous survey records were overwhelmingly located to the east of the PSP (See Appendix F). These areas are not closely hydrologically connected to works in the PSP and were not surveyed.

5.3 Impact management and planning

As expected, the survey recorded the presence of several native fish species in Cardinia Creek, both within and to the south of the PSP area. The approach taken to date with development planning, which assumes presence of Australian grayling and Dwarf galaxias and appropriate design and management to mitigate impacts, should be continued. The survey confirmed the presence of Australian grayling within and downstream of the PSP area, and previous records combined with equivocal eDNA results also suggest Dwarf galaxias are present in, or move through the Cardinia Creek sites.

Any changes to flows in Cardinia Creek have the potential to impact the migration of Australian grayling, which relies on specific flow regime triggers, including timing, volume and velocity. There is also the potential for significantly altered flows to affect stream hydraulics, resulting in increased flow velocity, erosion and scour of bed and banks, and associated physical or geomorphic impacts. Fishways that have been installed to assist with Australian grayling migration could also be 'drowned' or dried out if there is a significant alteration to flow volumes, creating further barriers to migration. Flows into Cardinia Creek therefore need to be carefully managed to avoid potential impacts to the Australian grayling population. Current proposals suggest that most of the additional flows from the PSP development will be directed to Officer South Drain and Gum Scrub Creek, in order to minimise hydrologic and hydraulic impacts on Cardinia Creek and the EPBC-listed species it supports.

Directing the majority of flows to Officer South Drain or Gum Scrub Creek will mitigate many of the potential flow-related impacts in Cardinia Creek through the PSP. However, significant changes to flows in Officer South drain also have the potential to impact the flow regime in Cardinia Creek between the lower boundary of the PSP (where Officer South Drain outfalls into Cardinia Creek) and Western Port Bay. Other cumulative impacts from drainage works in the lower reaches of the creek also need to be managed to ensure that this reach remains protected, and with a flow and water quality regime that facilitates Grayling migration. The survey confirmed the presence of Australian grayling in Cardinia Creek below the PSP, which was not unexpected, though there are no recent survey records in this area. This reach of the creek falls outside of the PSP area and is therefore not covered by the MSA agreement. However, the reach has potential to be impacted by development within the PSP, and contains an EPBC-listed species (Australian grayling). This lends additional weight to the consideration of EPBC referral requirements in the reach.

Current planning assumes that Officer South Drain will need to be reconstructed along the eastern side of the road, with a suggested 50-100m corridor for hydraulic requirements, which represents a significant change. Officer South Drain offers minimal habitat value in current condition, but this could be improved if it received additional flows as a result of the PSP development, and appropriate vegetation was established or maintained. The drain outfalls into Cardinia Creek at the lower boundary of the PSP area, so hydrological effects on the drain also need to be considered in terms of potential impacts to Cardinia Creek. There could be potential for fish species in Cardinia Creek to move up into additional habitat on Officer South Drain if the works resulted in additional flow connections, removal of barriers to movement, and suitable flow volumes. Hydrological connection of the drain from the areas north of the PSP with more consistent and reliable flows to Cardinia Creek may also result in the spread of exotic species. For example, the very high numbers of Eastern gambusia present in the stormwater wetlands on Officer South Drain to the north of the PSP, and the potential for spread of exotic species through new hydrological connections would need to be carefully managed.

Initial drainage planning has indicated that Gum Scrub Creek will receive significantly increased flows as a result of the PSP development and will require some re-modelling. Where possible, stormwater will be directed towards Gum Scrub Creek. While Gum Scrub Creek is heavily modified in some reaches, the re-modelling work is likely to be quite substantial and has the potential to impact on remnant riparian vegetation (mapped Swamp Scrub, Swampy Riparian Woodland and Swampy Woodland EVCs). There is also potential to support establishment of additional fish habitat through re-vegetation and managed flows along the re-modelled channel and associated wetland areas, which could provide good habitat for Dwarf galaxias and Growling grass frog. This is particularly

important if the construction of treatment wetlands elsewhere in the PSP results in loss of Dwarf galaxias and Growling grass frog habitat from natural wetland, drainage areas or anabranches.

The most critical further investigations required are related to drainage and hydrology including detailed analysis of proposed flows in each waterway, nature and extent of remodelled channels, flood extents, and wetland construction and location.

6. Conclusion

- The survey confirmed that Australian grayling are present in Cardinia Creek. Grayling were recorded at two sites on Cardinia Creek; one located within the PSP (where Cardinia Creek forms the western boundary of the PSP) and one outside the PSP area, on Cardinia Creek around 2 kilometres south of the PSP boundary, and the confluence of Officer South Drain and Cardinia Creek.
- This finding is consistent with previous surveys and confirms the need to assume presence of Australian grayling in the creek and to plan, design and manage drainage and waterway works in a way that minimises risks and impacts to the species to the greatest extent possible
- Cardinia Creek retains native fish values and was the only waterway where target species (Australian grayling and Dwarf galaxis) were recorded in either fish surveys or (equivocal) eDNA results. If development impacts (in the form of altered stormwater runoff) are to be directed to Gum Scrub Creek and Officer South drain, this will help to minimise impacts to Cardinia Creek. However, as Officer South Drain flows into Cardinia Creek there is potential for sites in Cardinia Creek downstream of the confluence to be impacted by significant changes in Officer South Drain.
- Changes to hydrology and drainage works in Officer South drain have the potential to impact on conditions in Cardinia Creek after the confluence of the drain with Cardinia Creek at the southern edge of the PSP. At the time of site inspections and surveys in December 2020 and in April 2021, there were no flows in Officer South Drain despite relatively wet conditions and good flows in surrounding waterways. This suggests that there are currently minimal flows conveyed through the drain, and if development was to result in a significant increase in flows through the drain this could a) be a significant change to the drain itself, and potentially compromise riparian vegetation or habitat along the drain and b) be of sufficient magnitude to affect flows in Cardinia Creek downstream of the drain, including changes to timing and velocity of flows.
- The field survey did not detect presence of any target species in instream or off stream sites along Gum Scrub Creek, which is a highly modified waterway in parts. PSP development plans currently suggest that most of the stormwater flows from the PSP will be directed to Gum Scrub Creek. This process would need to be carefully managed to avoid any impacts to existing habitat and maximise potential opportunities for the establishment of new habitat.

7. References

Ecology & Heritage Partners (2011). Ecological assessment of the Officer South Drain and Lower Gum Scrub Creek Development Services Schemes. Report prepared for Melbourne Water.

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Biosis Research (2008). Growling Grass Frog Conservation Management Plan for VicUrban Development at Officer, Victoria.



Biosis Research (2005). Pakenham Bypass: Conservation Management Plan for the Growling Grass Frog *Litoria raniformis*, Pakenham, Victoria.



Appendix A. Sample site summary




Site description and photos




Site	Type	Catchment	Site description	Photo
OS-01	Creek	Cardinia Creek	<p>Cardinia Creek at a narrow muddy section of the creek. Undertstorey vegetation dominated by blackberry thicket. Thick mid-storey of shrubs and small trees. Some larger trees. Lots of in stream woody debris and small snags. Banks predominantly muddy. Creek flowing quite fast.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none">• Southern brown bandicoot• Australian Grayling• Dwarf galaxias (movement only)• Growling grass frog (movement only)	
OS-02	Farm dam	Cardinia Creek	<p>Farm dam with negligible fringing vegetation, some floating vegetation on fringe. muddy edges with lots of pugging. Very turbid. A fish (carp?) was observed breaking the surface.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none">• Dwarf galaxias• Growling grass frog	




Site	Type	Catchment	Site description	Photo
OS-03	Farm dam	Cardinia Creek	<p>Farm dam with some fringing vegetation, generally low (<10 cm high), but with some tussocks grasses. Surface entirely covered by azolla. Sparse cover of swamp paperbarks emerging from the dam and also fringing the dam. Some pugging present at the dam, but lots of low vegetation.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> Dwarf galaxias Growling grass frog 	
OS-04	Dam	Cardinia Creek	<p>A small dam on old drainage line which forms a chain of ponds/small wetlands. Dam well vegetated, with lots of floating vegetation (mainly cooch grass) and some floating algae. Dense patch of emergent vegetation (Typha). Dense fringing vegetation that extends into the water dominated by cooch grass but also some tussocks. Small area of open water.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> Dwarf galaxias Growling grass frog 	
OS-05	Large farm dam	Cardinia Creek	<p>Large farm dam with gently sloping muddy edges and considerable pugging. No floating, emergent or fringing vegetation. Located at the downstream end of an ephemeral drainage line.</p> <p>Habitat has the potential to support the following target species, but is generally of low quality.</p> <ul style="list-style-type: none"> Dwarf galaxias Growling grass frog 	



Site	Type	Catchment	Site description	Photo
OS-06	Creek	Cardinia Creek	<p>At a recently constructed fishway on a broad section of Cardinia Creek, but quite deeply incised. Rocky beaching at the fishway with some vegetation starting to grow through the rocks. Upstream and downstream there is fringing vegetation of grass, that extents into the water at points. Quite dense thickets of emergent vegetation (phragmites) upstream and downstream of the fishway. Limited mid-storey, and a moderate canopy of generally small trees.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Southern brown bandicoot • Australian Grayling • Dwarf galaxias (movement only) • Growling grass frog (movement only) 	
OS-07	Dam	Gum Scrub Creek	<p>Dam next to Gum Scrub Creek, fenced from cattle. The dam has relatively steep banks, with a combination of muddy and vegetated banks (tussocks, sedges and grasses). The pond supports a high cover of floating and emergent vegetation. Water was very turbid, and small fish were evident, most likely eastern gambusia.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	

Site	Type	Catchment	Site description	Photo
OS-08	Dam	Gum Scrub Creek	<p>Dam next to Gum Scrub Creek, not fenced from cattle. Supports some fringing vegetation (mostly blackberry) but perimeter is predominantly muddy, with pugging. The water was shallow and turbid.</p> <p>Habitat has the potential to support the following target species, but is generally of low quality.</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	
OS-09	Creek	Gum Scrub Creek	<p>Gum Scrub Creek just upstream of Lecky Road. A series of well vegetated runs and small more open pools. Runs support some submergent and emergent vegetation (primarily Persicaria), and fringing vegetation on runs and pools is a mix of tussocks, sedges and grasses. The water is turbid, and there is some cow pugging particularly in the pool sections. Creek flowing well.</p> <p>Habitat has the potential to support the following target species but is generally of low quality.</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	

Site	Type	Catchment	Site description	Photo
OS-10	Dam	Gum Scrub Creek	<p>Farm dam next to Gum Scrub Creek. Approximately 50% cover of fringing vegetation including grasses, sedges and reeds, with the rest muddy. Some floating vegetation on fringes. Patches of emergent vegetation, predominantly Typha. Water relatively clear compared to other sites.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	
OS-11	Stormwater wetland	Gum Scrub Creek	<p>Constructed stormwater wetland next to Gum Scrub Creek. Supports very high cover of floating and submergent vegetation (primarily water ribbons) and dense tall fringing vegetation. Some emergent vegetation. Relative dense cover of surrounding trees and tall shrubs. The water appears deep and relatively clear.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog • Southern brown bandicoot (but quite isolated from other habitat) 	
OS-12	Instream stormwater wetland	Officer South Drain	<p>Newly constructed wetland on Officer South Drain. Edges jute matting, currently being revegetated. Some rocks and boulders. At the junction of Officer South Drain and other smaller drains.</p> <p>Habitat has the potential to support the following target species, especially once revegetated.</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	

Site	Type	Catchment	Site description	Photo
OS-13	Creek	Cardinia Creek	<p>Cardinia creek on Melbourne Water land. Deeply incised and narrow section of the creek with reasonable flow. Lots of fringing and riparian vegetation including some larger old gums that overhang the creek. Lots of small snags in the river, quite turbid.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Southern brown bandicoot • Australian Grayling • Dwarf galaxias (movement only) • Growling grass frog (movement only) 	
OS-14	Creek	Cardinia Creek	<p>Cardinia Creek just downstream of drop structure. Creek is quite broad (5 – 10 m wide), with low steep banks in a shallow valley. Lots of fringing and emergent vegetation, including an expansive dense patch of phragmites. Creek flowing well. large area of phragmites. High cover of riparian vegetation, primarily small trees and large shrubs. Mid-storey quite patchy.</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Southern brown bandicoot (however groundcover is patchy) • Australian Grayling • Dwarf galaxias (movement only) • Growling grass frog (movement only) 	
OS-15	Creek	Cardinia Creek	<p>Cardinia Creek at Chasemore Road crossing. Creek is quite broad (5 – 10 m wide) in a shallow valley. Lots of fringing vegetation (mostly grass). Creek flowing well, and there is a riffle and fishway section just downstream of the crossing. High cover of riparian vegetation, primarily small trees and large shrubs. Mid-storey denser than OS-14..</p> <p>Habitat has the potential to support the following target species</p> <ul style="list-style-type: none"> • Southern brown bandicoot • Australian Grayling • Dwarf galaxias (movement only) • Growling grass frog (movement only) 	

Site	Type	Catchment	Site description	Photo
OS-16	Farm dam	Gum Scrub Creek	<p>Farm dam immediately adjacent to Gum Scrub Creek. Fenced off from cattle. Lots of fringing vegetation that extends into the water. Some small patches of emergent Typha and Phragmites. Fish present - most likely gambusia and carp.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	
OS-17	Farm dam	Gum Scrub Creek	<p>Farm dam immediately adjacent to Gum Scrub Creek. Fenced off from cattle. Lots of fringing vegetation that extends into the water in places.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog 	
OS-18	Creek	Gum Scrub Creek	<p>Gum Scrub Creek at driveway crossing sampled both upstream and downstream of a small 1.5 m high sloped barrage. Lots of fringing vegetation (combination of blackberry and swamp paperbark, and some emergent Persicaria. At driveway culvert the creek was a wider slower flowing pool, creek then flows over the sloped barrage into a narrow (<1 m wide), fast flowing section with lots of Persicaria.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog • Southern brown bandicoot 	

Site	Type	Catchment	Site description	Photo
OS-19	Shallow instream wetland	Gum Scrub Creek	<p>A small branch of Gum Scrub Creek that flowed through a marshy area next to a patch of swamp paperbark scrub. Marsh supports high cover of grasses, sedges and rushes, as well as some patches of blackberry. While the area is fenced, there is evidence of cattle damage. Flow was quite low, and it is likely that the site dries from time to time.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog • Southern brown bandicoot 	
OS-20	Stormwater wetland	Officer South Drain	<p>Narrow constructed stormwater wetland on Officer South Drain. Supports very high cover of emergent floating and submergent vegetation and dense tall fringing vegetation. Relative dense cover of surrounding trees and tall shrubs. The water appears deep and relatively clear.</p> <p>Habitat has the potential to support the following target species,</p> <ul style="list-style-type: none"> • Dwarf galaxias • Growling grass frog • Southern brown bandicoot 	
OS-21	Gum Scrub Creek	Gum Scrub Creek	Narrow section of creek along Gum Scrub Creek at Patterson Road. Supports a linear strip of dense riparian and fringing vegetation	N/A

Appendix B. Fishing survey catch data summary

Fishing survey data summary with site number, habitat type and waterway (shaded).

Site	Type	Catchment	Fish survey
OS-01	Creek	Cardinia Creek	1 flathead gudgeon
OS-02	Farm dam	Cardinia Creek	Multiple exotic species
OS-03	Farm dam	Cardinia Creek	Common and spotted galaxias. Equivocal eDNA for dwarf galaxias
OS-04	Dam	Cardinia Creek	None (not fished?)
OS-05	Large farm dam	Cardinia Creek	Goldfish only
OS-06	Creek	Cardinia Creek	1 Australian grayling , common galaxias, 1 flathead gudgeon, eel and tupong
OS-07	Dam	Gum Scrub Creek	Common galaxias, short-finned eel, goldfish and Eastern gambusia
OS-08	Dam	Gum Scrub Creek	Common galaxias, goldfish and Eastern gambusia
OS-09	Creek	Gum Scrub Creek	Common galaxias, goldfish and Eastern gambusia
OS-10	Dam	Gum Scrub Creek	Eastern gambusia, short-finned eel
OS-11	Stormwater wetland	Gum Scrub Creek	Eastern gambusia, short-finned eel
OS-12	Instream stormwater wetland	Officer South Drain	None (not fished)
OS-13	Creek	Cardinia Creek	Common galaxias, flathead gudgeon, shortfinned eel, southern pygmy perch
OS-14	Creek	Cardinia Creek	Common galaxias, eastern gambusia, flathead gudgeon. Equivocal eDNA for dwarf galaxias.
OS-15	Creek	Cardinia Creek	Australian grayling , brown trout, common galaxias, shortfinned eel, tupong
OS-16	Farm dam	Gum Scrub Creek	None (not fished)
OS-17	Farm dam	Gum Scrub Creek	None (not fished)
OS-18	Creek	Gum Scrub Creek	None (not fished)
OS-19	Shallow instream wetland	Gum Scrub Creek	Common galaxias, eastern gambusia, shortfinned eel
OS-20	Stormwater wetland	Officer South Drain	>700 Eastern gambusia
OS-21	Gum Scrub Creek	Gum Scrub Creek	Common galaxias, shortfinned eel

Catch data (site and number of individual caught from netting/e-fishing)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Australian Grayling						1									7						
Brown Trout*															6						
Common Galaxias			1			7	2	2	1				21	1	3				1		2
Dwarf Galaxias																					
Eastern Gambusia*		>60					>700	>300	39	>500	>150			76					1	>700	
European Carp*		3																			
Flatheaded Gudgeon	1					1							1	16							
Goldfish*		31			28		1	3	1												
Short-finned Eel						3	2			1	2		2		5				1		2
Southern Pygmy Perch													1								
Spotted Galaxias			1																		
Tupong						3									4						

Site fishing methods summary

Site	eDNA	Dip Netting (10 min)	Bait traps (#)	Fyke nets (#)	Electrofishing (time/mins)	Seine (2mm x 20m)
OS-01	Yes			1		
OS-02	Yes					yes
OS-03	Yes		8	2		
OS-04	Yes	yes	8			
OS-05	Yes					yes
OS-06	Yes	yes	8	2	6	
OS-07	Yes	yes	8	2		
OS-08	Yes					yes
OS-09	Yes	yes	8			
OS-10	Yes					yes
OS-11	Yes	yes	8			
OS-12	Yes					
OS-13	Yes	yes	8	2		
OS-14	Yes	yes	8	2		
OS-15	Yes		8	2	6	
OS-16	Yes					
OS-17	Yes					
OS-18	Yes					
OS-19	Yes	yes	8	1		
OS-20	Yes	yes	8			
OS-21	No	yes	8	1		

Appendix C. Summary eDNA results

eDNA summary results (N = negative, E = equivocal)

	Site number																			
Species detection	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Australian Grayling	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Dwarf Galaxias	N	N	E	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N
Growling Grass Frog	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Low sample volumes may have compromised detection

Appendix D. Water quality summary data

Site	temp (°C)	DO%	DO (mg/l)	SPC (us/cm)	Cond (us/cm)	pH	Turb (ntu)	time
OS-01	12	93.6	9.89	273.1	205.5	7.47	12.1	1100
OS-02	16.1	64	6.27	273.4	227.2	7.3	209	1130
OS-03	11.5	13	1.31	519.3	345.2	7.5	8.19	1030
OS-04	14.2	45	4.56	437.6	347.6	7.1	36.9	1200
OS-05	17.9	96.1	8.98	259	224.1	7.86	158	1440
OS-06	14.5	106.4	10.76	407.6	321.7	13	7.7	1600
OS-07	16.5	87.2	8.33	499.8	417.1	7.34	31.8	1500
OS-08	19.7	125.2	10.96	639	575	7.09	92.5	1600
OS-09	15.8	104.1	10.12	931	767	7.17	31.1	1330
OS-10	19.4	127.2	11.57	369.9	333.9	7.73	22.2	1400
OS-11	16.3	101.1	9.76	2468	2058	7.41	14.2	1300
OS-12								
OS-13	11.5	89.8	9.56	387.3	288.1	7.06	13.3	1400
OS-14	13.0	42.7	4.49	238.2	183.9	6.96	28.7	1200
OS-15	11.6	84	8.88	279.9	208.6	7.18	15.8	1000
OS-16								
OS-17								
OS-18								
OS-19	12.7	68.8	7.34	1088	829	7.62	35.5	1000
OS-20	17.8	110.6	10.38	548.7	470.3	8.06	36.4	1400
OS-21	12.8	73.5	7.56	1173	899	7.64	23.7	900
Water quality objective*		70-130			<500	6.4 – 7.9	<35	

* As set out in the EPA's Environment Reference Standard, Table 5.8. For Rivers and Streams, Urban segment, which includes Western Port catchment. Values do not apply to constructed stormwater, irrigation or agricultural drains, wetlands, or off-stream private dams and are provided for high-level comparison only.

Appendix E. Site description and location summary

Site	Type	Catchment	Latitude	Longitude
OS-01	Creek	Cardinia Creek	-38.073895	145.377597
OS-02	Farm dam	Cardinia Creek	-38.077245	145.378284
OS-03	Farm dam	Cardinia Creek	-38.071145	145.379722
OS-04	Dam	Cardinia Creek	-38.074548	145.390384
OS-05	Large farm dam	Cardinia Creek	-38.088544	145.391526
OS-06	Creek	Cardinia Creek	-38.096405	145.392495
OS-07	Dam	Gum Scrub Creek	-38.082747	145.419343
OS-08	Dam	Gum Scrub Creek	-38.082567	145.418464
OS-09	Creek	Gum Scrub Creek	-38.081934	145.417386
OS-10	Dam	Gum Scrub Creek	-38.084449	145.418166
OS-11	Stormwater wetland	Gum Scrub Creek	-38.070852	145.415017
OS-12	Instream stormwater wetland	Officer South Drain	-38.072552	145.407744
OS-13	Creek	Cardinia Creek	-38.083958	145.380387
OS-14	Creek	Cardinia Creek	-38.091219	145.383339
OS-15	Creek	Cardinia Creek	-38.119182	145.400879
OS-16	Farm dam	Gum Scrub Creek	-38.094445	145.423775
OS-17	Farm dam	Gum Scrub Creek	-38.093736	145.421082
OS-18	Creek	Gum Scrub Creek	-38.094682	145.423583
OS-19	Shallow instream wetland	Gum Scrub Creek	-38.125707	145.43003
OS-20	Stormwater wetland	Officer South Drain	-38.065175	145.407507
OS-21	Gum Scrub Creek	Gum Scrub Creek	-38.114896	145.422036

Appendix F. Pre-existing species records

