

Nungatta Feral Predator Free Area, South East Forest National Park: Aquatic Fauna Surveys



Austral Research and Consulting
ABN 73 007 840 779
15 Buntings Rd
Kirkstall Vic 3283 Australia
Web: www.austral.net.au

DISCLAIMER This report was prepared by Austral Research and Consulting in good faith exercising all due care and attention, but no representation or warranty, express or implied, is made as to the relevance, accuracy, completeness or fitness for purpose of this document in respect of any particular user's circumstances. Users of this document should satisfy themselves concerning its application to, and where necessary seek expert advice in respect of, their situation. The views expressed within are not necessarily the views of the Department of Planning and Environment (DPE) and may not represent DPE policy. © Copyright State of NSW and the Department of Planning and Environment.

Contents

1. Introduction	4
2. Study Area	5
3. Methodology	7
3.1. Desktop Analysis	7
3.1.1. Key Fish Habitat	7
3.2. Fish Surveys	8
3.2.1. Environmental DNA (eDNA)	9
3.2.2. Electrofishing	10
3.2.3. Bait Traps	10
3.2.4. Dip netting	10
3.2.5. Site observations	11
3.2.6. In-situ water quality	11
3.2.7. Limitations	11
4. Results and Discussion	12
4.1. Desktop Analysis	12
4.1.1. DPI Fisheries NSW Spatial Data Portal	12
4.1.2. Key Fish Habitat	12
4.1.3. Bionet	12
4.1.3.1. Platypus	13
4.1.4. Protected Matters Search Tool	14
4.1.4.1. Listed Threatened Ecological Communities	14
4.1.4.2. Listed Threatened Species	15
4.1.4.3. Listed Migratory Species	15
4.2. Key Fish Habitat	15
4.3. Site Description	16
4.3.1. Crossing 1 – Reef Creek	16
4.3.2. Crossing 42 – Surveyor’s Gully	17
4.3.3. Crossing 6 – unnamed waterway	18
4.3.4. Crossing 9 – Sandy Creek	19
4.3.5. Crossing 11 – Donald Laing’s Creek	20
4.1. Water Quality	21
4.2. Aquatic Survey	22
4.3. Platypus eDNA results	22
4.4. Fish eDNA results	24
4.4.1. Likelihood of aquatic fauna	27
5. Barrier and Exclusions	29
5.1. Culvert barriers	29
5.2. Bridge Barriers	30
5.3. Potential impacts	31
6. Conclusions	33
5. References	34

Abbreviations

The following abbreviations have been used:

Austral	Austral Research & Consulting
CPOM	Coarse Particulate Organic Matter
DAWE	Department of Agriculture, Water and the Environment
DELWP	Department of Environment, Land, Water and Planning
DO	Dissolved Oxygen
DPI	Department of Primary Industries
DPIE	Department of Planning, Industry and Environment
eDNA	Environmental deoxyribonucleic acid
EPBC	Environment Protection and Biodiversity Conservation
FM	Fisheries Management
IPC	Internal Positive Control
km	Kilometres
LoO	likelihood of occurrence
LWD	Large Woody Debris
m	Metre
mg	Milligrams
mg/L	Milligrams per Litre
ml	Millilitres
mm	millimetres
NFPFA	Nungatta Feral Predator Free Area
NSW	New South Wales
ntu	Nephelometric Turbidity Units
PCR	Polymerase Chain Reaction
PL	Provisional Management List.
PMST	Protected Matters Search Tool
qPCR	quantitative Polymerase Chain Reaction
Sp.	Species
TEC	Threatened Ecological Community
µm	micrometers
µS/cm	Microsiemens per centimeter
°C	Degrees Celcius

1. Introduction

Austral Research and Consulting has been engaged by Parks NSW to undertake Aquatic Fauna surveys along the proposed route of the predator proof fence for the Nungatta Feral Predator Free area (NFPFA). Feral predators (cats and foxes) are a recognised threat to Australia's native fauna and cats are thought to impact 117 threatened species in NSW (NSW DPE 2021) with 50-60% of the surviving mammals species facing extinction in NSW. A parliamentary enquiry recommendation to combat the problem of cat predation is to increase the area of predator free safe zones across a wide range of ecosystems. To date the current network of predator free areas has contributed to the protection of 13 mammal species from extinction and protected populations of 40 mammal species from predators (NSW DPE 2021).

The proposed NFPFA is one of four sites that will result in the exclusion of predators from a total of 45500 hectares of habitat. The NFPFA will cover an area of 2000 hectares and locally extinct species considered for reintroduction will include eastern bettong, smoky mouse, eastern quoll and long-footed potoroo. The construction of the predator exclusion fence will include the crossing of 24 (1st – 4th order streams) recognised waterways including four third order streams and one fourth order streams. The establishment of the NFPFA will require barriers (i.e. grills, nets and fencing) to be constructed on the waterways and has the potential to impact the movement of native aquatic fauna. Therefore the waterways require assessment to identify aquatic fauna values that may be impacted as a result of the project.

NSW Fisheries is supportive of installing barriers because of the conservation benefit to terrestrial threatened species of the project. NSW Fisheries asked NPWS to survey and monitor aquatic ecosystems to determine if fox and cat exclusion might also benefit them (Ganassin 2022).

To assess the aquatic values of the waterways a desktop assessment, field surveys and eDNA samples were collected. This report details the methods and results of the assessments and surveys with discussion around the aquatic values and the potential impacts of the proposed exclusion measures to be employed at the waterway crossings. The data and findings of this report will inform the Review of Environmental Factors (NSW DPE 2022) for the project.

2. Study Area

The proposed feral free area is located at Nungatta and lies within the South East Forest National Park. The proposed feral free covers an area of approximately 2000 hectares (the study area) (NSW DPIE 2021) (Figure 2-1). Specific sample sites are shown in Figure 2-2 and site coordinates are presented in Table 2-1. The study area is located approximately 36 km southeast of Bombala in NSW and lies within the South East Corner bioregion, the Southern Rivers Catchment and borders the Snowy Monaro Regional and Bega Valley Shire councils (NSW DPI 2022a). Thackway and Cresswell (1995) describe the South East Corner bioregions as “A series of deeply dissected near coastal ranges composed of Devonian granites and Palaeozoic sediments, inland of a series of gently undulating terraces (piedmont downs) composed of Tertiary sediments and flanked by Quaternary coastal plains, dunefields and inlets. The regional climate is strongly influenced by the Tasman Sea and the close proximity of the coast to the Great Dividing Range. Vegetation consists of high elevation woodlands, wet and damp sclerophyll forests interspersed with rain-shadow woodlands in the Snowy River Valley. Lowland and coastal sclerophyll forests, woodlands, warm temperate rainforest and coastal communities occur in the lower areas.” The climate of this bioregion is described as temperate whilst a small area in the southwest (high country) occurs in a montane climate zone. Almost half of the South East Corner bioregion is managed in conservation tenures (NSW DPIE 2003).

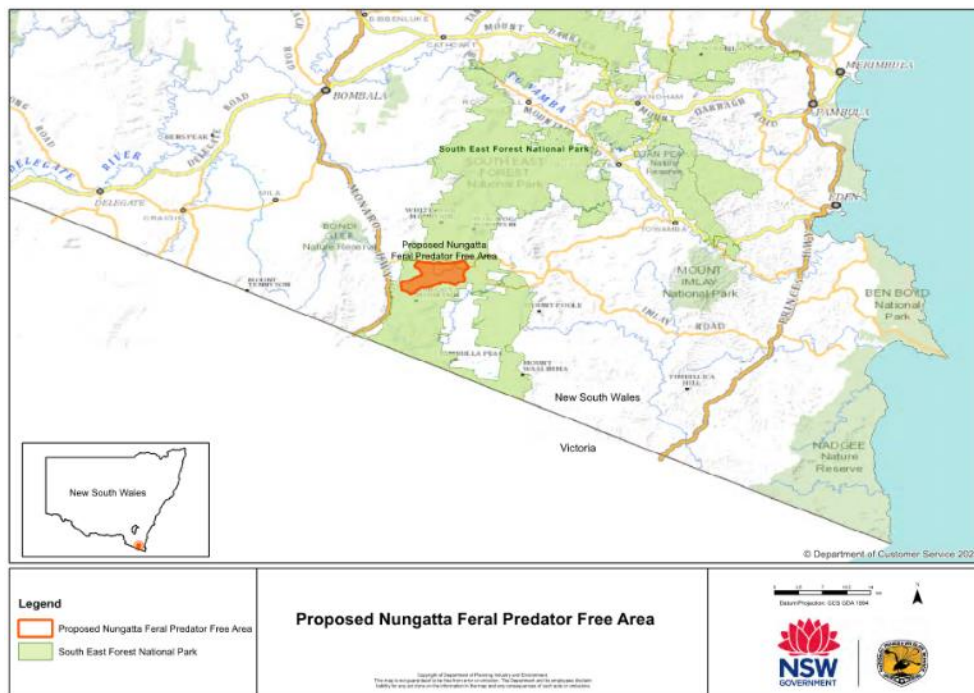


Figure 2-1: Proposed Nungatta Feral Predator Free area (study area) (NSW DPIE 2021)



Figure 2-2: Order 3 and order 4 water way crossings within the study area (Google Earth). Note – White line = boundary fences, Black line = tracks, blue line = waterways.

Table 2-1: Site location data of the Nungatta Feral Predator Free area waterway crossings (GPS - UTM)

Site name	Waterway	Zone	Easting	Northing
Crossing 1	Reef Creek	55	707425	5884477
Crossing 42	Surveyor's Gully	55	710979	5887146
Crossing 6	Unnamed waterway	55	712693	5887135
Crossing 9	Sandy Creek	55	714024	5884274
Crossing 11	Donald Laing's Creek	55	713145	5884175

3. Methodology

3.1. Desktop Analysis

Database searches were undertaken to compile background information on threatened aquatic habitat, threatened ecological community's (TECs) or populations that may inhabit waterways with the potential to be impacted by the project. Databases providing information on aquatic GDEs were also accessed; however, there are no databases available for NSW which specifically catalogue the presence of subterranean fauna.

State and Commonwealth database resources included:

- Freshwater threatened species distribution maps (NSW DPI Fisheries);
- Threatened species lists (NSW DPI Fisheries);
- Key Fish Habitat maps (NSW DPI Fisheries);
- Fish stocking (NSW DPI Fisheries);
- Fisheries NSW Spatial Data Portal (NSW DPI Fisheries);
- BioNet Atlas (NSW DPIE);
- Protected Matters Search Tool (PMST) (DAWE);
- Provisional list of animals requiring urgent management intervention (DAWE); and,
- NSW Fish Passage Database.

All of the database information was then used in conjunction with our site and species knowledge to comment on the likelihood of a species being present and was reported as the likelihood of occurrence (LoO).

3.1.1. Key Fish Habitat

In accordance with Policy and guidelines for fish habitat conservation and management (NSW DPI 2013) habitat sensitivity was assessed at five sites (Figure 2-2 and Table 2-1) by assigning a 'waterway type', while the functionality of the waterway as fish passage was assessed by assigning a 'waterway class'. 'Sensitivity' is defined by '*...the importance of the habitat to the survival of fish and its robustness (ability to withstand disturbance)*' (NSW DPI 2013). Definitions, relevant to the aquatic ecology report, of the waterway types and waterway classes are summarised in Table 3-1 and

Table 3-2, respectively. NSW DPI Fisheries (2013) only recognises native aquatic plants with regard to waterway type classification. Where it was not known as to whether an aquatic plant was native or exotic, a conservative approach was taken, potentially overestimating the native vegetation component of waterway type classification.

Table 3-1: Waterway type definitions for habitat sensitivity

Classification	Characteristics of waterway class
Type 1 – Highly sensitive key fish habitat	Freshwater habitats that contain in-stream gravel beds, rocks greater than 500 mm in two dimensions, snags greater than 300 mm in diameter or 3 metres in length, or native aquatic plants.
Type 2 – Moderately sensitive key fish habitat	Freshwater habitats and brackish wetlands, lakes and lagoons other than those defined in Type 1.
Type 3 – Minimally sensitive key fish habitat	Ephemeral aquatic habitat not supporting native aquatic or wetland vegetation.

Table 3-2: Waterway class definitions for fish passage

Classification	Characteristics of waterway class
Class 1 – Major key fish habitat	Marine or estuarine waterway or permanently flowing or flooded freshwater waterway (e.g. river or major creek), habitat of a threatened or protected fish species or 'critical habitat'.
Class 2 – Moderate key fish habitat	Generally named intermittently flowing stream, creek or waterway with clearly defined bed and banks, semi-permanent to permanent water in pools or in connected wetland areas. Freshwater aquatic vegetation is present. Type 1 and Type 2 habitats present.
Class 3 – Minimal key fish habitat	Named or unnamed waterway with intermittent flow and sporadic refuge, breeding or feeding areas for aquatic fauna (e.g., fish, yabbies). Semi-permanent pools form within the waterway or adjacent wetlands after a rain event. Otherwise, any minor waterway that interconnects with wetlands or other Class 1-3 fish habitats.
Class 4 – Unlikely key fish habitat	Generally unnamed waterway with intermittent flow following rain events only, little or no defined drainage channel, little or no flow or free-standing water or pools post-rain events (e.g., dry gullies, shallow floodplain depressions with no aquatic flora).

3.2. Fish Surveys

All fish surveys were undertaken in accordance with the *Survey guidelines for Australia's threatened fish: Guidelines for detecting fish listed as threatened under the Environment Protection and Biodiversity Conservation Act 1999* (DSEWPaC 2011).

Survey methods and effort was dependent on habitat characteristics at each site and are outlined in Table 3-3.

Table 3-3: Sampling effort at the five crossing locations for the Nungatta Feral Free Area

Method	Site				
	Crossing 1	Crossing 42	Crossing 6	Crossing 9	Crossing 11
Dip netting (min)	-	10	10	-	-
Bait traps (no.)	10	10	10	-	10
Electrofishing (backpack)	900 secs	600 secs	600 secs	420 secs	660 secs
eDNA (sample reps)	3	3	3	3	3

3.2.1. Environmental DNA (eDNA)

Analysis of environmental DNA (eDNA) is a non-invasive method for detecting single species or, more recently, entire taxonomic groups (Rees et al. 2014; McColl-Gausden et al. 2019; Thomsen and Willerslev 2015). Genetic material that an organism leaves behind in its environment is known as eDNA. Quantitative comparisons with traditional sampling methods indicate that eDNA methods are effective at detecting scarce, elusive or cryptic species (Biggs et al. 2015; Lugg et al. 2018; Smart et al. 2015; Thomsen et al. 2012; Valentini et al. 2016), or species at low densities.

During June 2022, water samples were collected from 5 sites (Table 3-3) by Austral staff following EnviroDNA sampling protocols. At each site, water samples were collected in triplicate by passing up to 500 mL of water (average 488 mL) through a 1.2 µm syringe filter. Filtering on site reduces DNA degradation that may occur during transport of water (Yamanaka et al. 2016). Clean sampling protocols were employed to minimise contamination including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. A preservative (approx. 0.5 ml 10xTris-EDTA) was added to the filters after filtering to minimise DNA degradation. Filters were stored out of sunlight and kept at ambient temperature before being transported to the laboratory for processing. Samples were delivered to EnviroDNA for processing.

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Power Soil Pro) that minimizes compounds that can inhibit PCR reactions. Real-time quantitative Polymerase Chain Reaction (qPCR) assays were used to amplify the target DNA, using species-specific markers targeting a small region of the platypus mitochondrial DNA, previously developed and assessed for specificity and sensitivity by EnviroDNA (e.g. Lugg et al. 2018). Positive and negative controls were included for all assays as well as an Internal Positive Control (IPC) to detect inhibition. Assays were performed in triplicate on each sample. At least three positive qPCR assays (out of nine assays undertaken for the site) were required to classify the site as positive for the presence of platypus. To minimise false positives, sites were considered equivocal if only one or two assays returned a positive result, indicating very low levels of target DNA. While trace amounts of DNA may indicate the target species is actually present in low abundance, it may also arise from sample contamination through the sampling or laboratory screening process (minimised through strict protocols and negative controls), facilitated movement of DNA between waterbodies (i.e. water birds, recreational anglers, water transfers, predator scats), or dispersal from further upstream.

Fish and vertebrate biodiversity assessments were performed on all samples using a universal Fish assay (McCull-Gausden et al. 2020) and a universal vertebrate assay (Riaz et al 2011) targeting a small region of the 12S mitochondrial DNA. Library construction involved two rounds of PCR whereby the first round employed gene-specific primers to amplify the target region and the second round incorporated sequencing adapters and unique barcodes for each sample-amplicon combination included in the library. Negative controls were also included during library construction. Negative controls consisted of the extraction negative as well as PCR negatives where nuclease-free water was used in place of DNA during both rounds of PCR. Sequencing was carried out on an Illumina iSeq 100 machine.

Following quality control filtering to remove primer sequences, truncated reads and low-frequency reads, DNA sequences were clustered into Operational Taxonomic Units (OTUs) on the basis of sequence similarity. Taxonomic assignment was performed with VSEARCH software (Rognes et al. 2016) whereby each OTU cluster was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. Where a species could not be assigned (i.e. reference database was deficient and/or taxa were poorly-characterised), taxonomic assignments were manually vetted by first obtaining a list of possible species through BLASTN searches against the public repository Genbank (www.ncbi.nlm.nih.gov), then eliminating species on the basis of their geographic distribution using information from the Atlas of Living Australia (ALA). In cases where an OTU could not be adequately resolved to a single species (due to shared haplotypes for instance), either a list of multiple species was included, or it was assigned to the lowest taxonomic rank without further classification. Similar to the interpretation of qPCR results above, detection of species in multiple replicates from a site increases the confidence that the eDNA detection represents actual presence of the species. Detection of a species in a single replicate may indicate species presence at low abundance but can also arise from site level (in field) or sample level (sampling or laboratory protocols) contamination (Darling et al. 2021).

3.2.2. Electrofishing

Backpack electrofishing was undertaken using an E-fish electrofisher. A range of fish species are sampled using backpack electro fishers. During the survey work the effectiveness of the electrofishing unit is constantly monitored to ensure that other aquatic and semi-aquatic fauna are not affected. Backpack electrofishing was used to target likely fish habitat such as snags, undercut banks and instream vegetation and trailing bank vegetation. The effort used at each site was dependent on habitat availability with the maximum amount of electrofishing on time being 8 x 150 seconds (20 minutes).

3.2.3. Bait Traps

The bait traps consist of a 2 mm mesh and are approximately 250 mm x 250 mm x 450 mm. This size rating complies with restrictions set by Fisheries NSW (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 1 mm and 4 mm). The traps were set overnight and retrieved the next morning.

3.2.4. Dip netting

Dip netting was undertaken in areas of appropriate habitat for a minimum of 10 minutes per site.

3.2.5. Site observations

A visual inspection of each site was undertaken. A description of the habitat was collected and included aquatic vegetation and characteristics of each site and suitability of platypus habitat.

3.2.6. In-situ water quality

In-situ water quality parameters were measured at each site including dissolved oxygen (mg/L), temperature (°C), specific conductivity ($\mu\text{S}/\text{cm}$) and pH using a YSI ProPlus water quality meter. Turbidity (ntu) was measured using a Hach 2100Q turbidity meter.

3.2.7. Limitations

These surveys gather data in the form of a 'snap shot in time'. Results may vary depending on the time of year surveys are undertaken. Data collected is considered appropriate to inform on those ecological values located within the study area that may be impacted by the proposed predator exclusion measures installed on the waterway crossings.

4. Results and Discussion

4.1. Desktop Analysis

4.1.1. DPI Fisheries NSW Spatial Data Portal

A review of the NSW Department of Primary Industries' (NSW DPI 2022a) revealed that the freshwater fish community is considered generally 'poor' within the vicinity of the study area. No threatened, listed freshwater species are modelled to occur within the study area (NSW DPI 2022). Fish stocking occurs in the Bombala area to the northwest with stocked species limited to Brown trout (*Salmo trutta*) and Rainbow trout (*Oncorhynchus mykiss*) (NSW DPI 2022b).

4.1.2. Key Fish Habitat

Key Fish Habitat is identified as "*those aquatic habitats that are important to the sustainability of the recreational and commercial fishing industries, the maintenance of fish populations generally, and the survival and recovery of threatened aquatic species*" (DPI 2022a). A review of the NSW Department of Primary Industries' (DPI 2022a) revealed that key fish habitat is modelled to occur within the study area (Table 4-1).

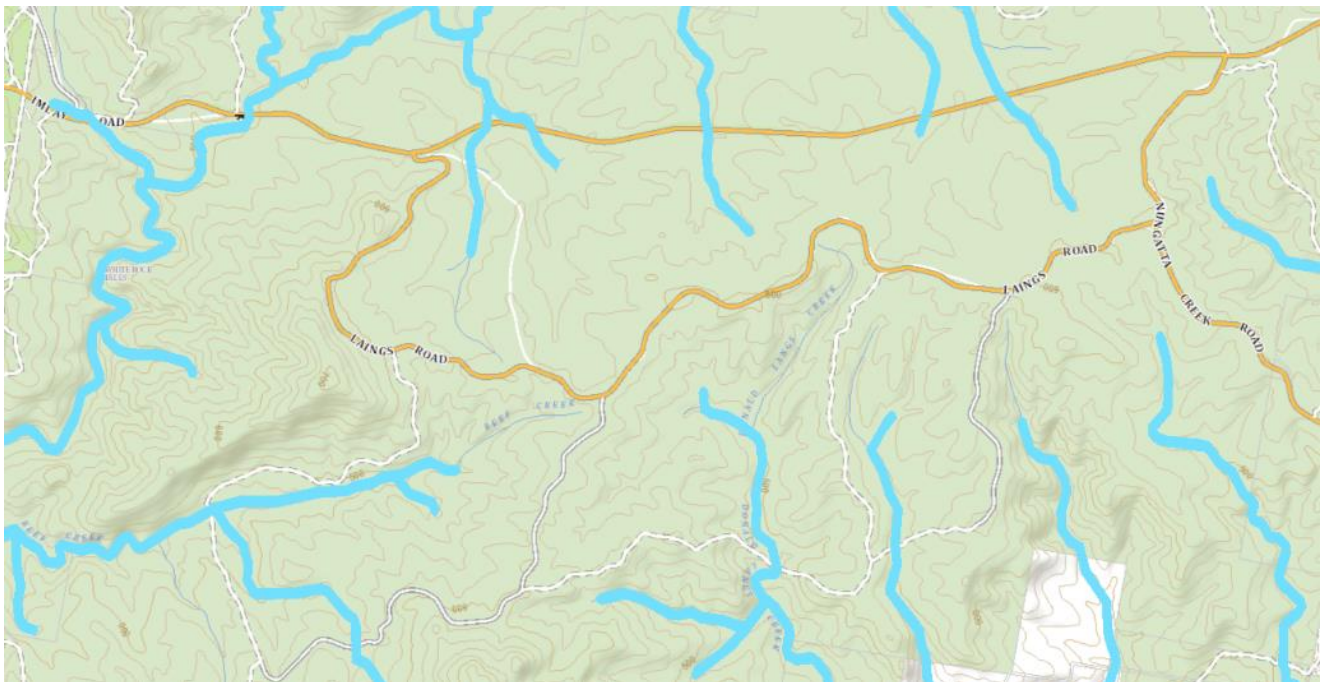


Figure 4-1: Key Fish Habitat occurring within the study area as indicated by the thick blue line (NSW DPI 2022a)

4.1.3. Bionet

A search of the NSW Department of Planning and Environment Bionet Atlas revealed 5 278 species have been recorded within the Southern Rivers Catchment (NSW DPE 2022). Non-aquatic species are beyond the scope of this report and were subsequently excluded. The results of the Bionet search includes species, conservation listing and likelihood of occurrence (LoO) (Table 4-1).

Table 4-1: Results of the Bionet search including species, conservation listing and likelihood of occurrence (LoO).

Common Name	Species Name	Conservation Listing	LoO
Native Fish Species			
Australian bass	<i>Macquaria novemaculeata</i>	-	Low
Common Galaxias	<i>Galaxias maculatus</i>	-	Moderate
Cox's gudgeon	<i>Gobiomorphus coxii</i>	-	Low
Empire Gudgeon	<i>Hypseleotris compressa</i>	-	Low
Firetail Gudgeon	<i>Hypseleotris galii</i>	-	Low
Flathead gudgeon	<i>Philypnodon grandiceps</i>	-	Moderate
Long-finned Eel	<i>Anguilla reinhardtii</i>	-	High
Macquarie Perch	<i>Macquaria australasica</i>	EPBC Act; FM Act	Low
Mountain Galaxias [#]	<i>Galaxias olidus</i>	-	High
Short-finned Eel	<i>Anguilla australis</i>	-	High
Striped Gudgeon	<i>Gobiomorphus australis</i>	-	Low
Exotic Species			
Common Carp	<i>Cyprinus carpio</i>	-	Low
Eastern mosquitofish	<i>Gambusia holbrooki</i>	-	Low
Reptiles			
Eastern Snake-necked Turtle	<i>Chelodina longicollis</i>	-	Low
Macquarie River Turtle	<i>Emydura macquarii macquarii</i>	-	Low
Mammals			
Platypus	<i>Ornithorhynchus anatinus</i>	PL	Low - moderate

Note: PL = DAWE (2000) provisional management list. # the Mountain galaxias complex has recently been redescribed and included as Roundsnout galaxias (*Galaxias terenasus*) throughout this report.

Macquarie Perch is Listed as Endangered under the EPBC Act and Endangered under the FM Act.

While the Platypus is not currently listed under the FM Act or the EPBC Act, there is currently a lack of knowledge regarding species abundance at a local catchment level (Australian Museum, 2019) and the species is subject to similar impacts as threatened fish, including waterway bank erosion, channel sedimentation, regulated waterways, barriers to water flow (eg dams and weirs), riparian zone degradation and loss of riparian vegetation (Bino, et al., 2019; Temple-Smith & Grant, 2003). The Platypus was included on the DAWE (2020) provisional list of animal species identified as requiring immediate urgent management intervention in February 2020, following the 2019/2020 bushfire season in southern and eastern Australia.

4.1.3.1. Platypus

Platypus inhabit a variety of freshwater streams throughout eastern Australia (Grant, 1992). Platypus were recently listed as Near Threatened by the International Union for the Conservation of Nature (IUCN) (Woinarski & Burbidge, 2016; Woinarski, et al., 2014) and Vulnerable in Victoria. As an apex predator in Australian aquatic ecosystems, platypuses are vulnerable to a number of threatening processes which may degrade their habitats or reduce the availability of macroinvertebrate food resources. Threats include a reduction of available surface water through drought, water extraction or

diversion, changes to flow regimes, clearing riparian and broader catchment vegetation, poor water quality, barriers to dispersal, entanglement in litter or fishing equipment, and predation (Grant & Temple-Smith, 2003; Grant & Temple-Smith, 1998). Platypuses are highly mobile with individual home ranges and daily movements encompassing several kilometres (Gust & Handasyde, 1995; Griffiths, et al., 2014; Kelly, et al., 2012; Serena, et al., 1998; Otley, et al., 2000) with densities roughly estimated at 1-2/km in a small creek around Melbourne (Serena, 1998). Platypuses require adequate surface water, flow regimes and habitat to support sufficient food resources of macroinvertebrate prey. Platypuses are adapted to feed exclusively in water where they forage for a range of benthic macroinvertebrates with adults consuming approximately 15-30 percent of their bodyweight daily (Krueger, et al., 1992; Holland & Jackson, 2002). Other habitat variables known to be important for platypuses include large riparian trees, overhanging vegetation, pools 1-3m deep, and near vertical or undercut banks at least 0.5 m above the water (Serena, 1998; Ellam, et al., 1998; Bethge, et al., 2003; Grant, 2004; Serena, et al., 2001; Worley & Serena, 2000). Reproduction in *Platypus* has been linked with rainfall (and presumably reliable flows) in the months preceding breeding (August – December) (Serena, et al., 2014; Grant & Temple-Smith, 1998). Whilst various individual councils across Australia have prepared Recovery Plans for platypus no formal, overarching recovery plan has been prepared at the state or federal level for platypus.

4.1.4. Protected Matters Search Tool

A review of the Protected Matters Search Tool (PMST) (DAWE 2022) revealed that seven Listed TECs, 66 Listed Threatened species and 13 Migratory species have the potential to occur within the vicinity of the study area.

4.1.4.1. Listed Threatened Ecological Communities

Floristic survey of NFPFA found no listed threatened ecological communities in or adjacent to the study area (Miles 2021).

A review of the PMST revealed seven Listed TECs are considered as ‘likely’ or ‘may’ occur within the vicinity of the study area (Table 4-2) (DAWE 2022). Of the seven TECs one has the potential to contain aquatic habitat: “Upland Wetlands of the New England Tablelands (New England Tableland Bioregion) and the Monaro Plateau (South Eastern Highlands Bioregion)” (EPBC Act, Endangered). This TEC is recorded within the New England Tableland Bioregion and South Eastern Highlands Bioregion whereas the study area is situated within the South-east Corner bioregion. The study area occurs partially within one TEC: “Brogo Vine Forest of the South East Corner Bioregion” however this TEC is not reported as containing aquatic habitat (DAWE 2021). It is considered unlikely that any TECs will be impacted by the aquatic components of the proposed action.

Table 4-2: Listed Threatened Ecological Communities occurring within the vicinity of the study area

Community ID	Community Name	Threatened Category	Rank
43	White Box-Yellow Box-Blakely's Red Gum Grassy Woodland and Derived Native Grassland	Critically Endangered	May
82	Lowland Grassy Woodland in the South East Corner Bioregion	Critically Endangered	Likely
39	Upland Wetlands of the New England Tablelands (New England Tableland Bioregion) and the Monaro Plateau (South Eastern Highlands Bioregion)	Endangered	Likely
55	Brogo Vine Forest of the South East Corner Bioregion	Endangered	Likely
32	Temperate Highland Peat Swamps on Sandstone	Endangered	May
152	Natural Temperate Grassland of the South Eastern Highlands	Critically Endangered	Likely
154	River-flat eucalypt forest on coastal floodplains of southern New South Wales and eastern Victoria	Critically Endangered	May

4.1.4.2. Listed Threatened Species

A total of 66 Listed Threatened species have the potential to occur within the study area (DAWE 2022). Of the 66 species reported only one is considered truly aquatic and is considered in this report. Australian Grayling (*Prototroctes maraena*) may occur within 10 km of the study (DAWE 2022).

4.1.4.3. Listed Migratory Species

A total of 13 migratory species are reported as having the potential to occur within the study area. None of the reported migratory species are considered aquatic. As such migratory species are not considered further within this report.

4.2. Key Fish Habitat

Key Fish Habitat was assessed at all sites during the surveys. General habitat characteristics are reported in Section 4.3 and the results of the key fish habitat assessments are summarised below and in Table 4-3.

All sites were classified as Type 1 highly sensitive key fish habitat as they contained either boulder and bedrock features or extensive instream vegetation.

Four sites (Table 4-3) were classified as Class 2 moderate key fish habitat due to either the ephemeral nature of the stream or low flow conditions resulting in the presence of pool habitat. Additional characteristics of Class 2 moderate key fish habitat is the presence of freshwater aquatic vegetation and the absence of habitat considered critical to the survival of threatened or endangered species (

Table 3-2). One site was classified as Class 3 minimal key fish habitat due to its ephemeral nature, lack of critical habitat and lack of instream vegetation (Table 4-3).

Table 4-3: Stream order, Key Fish Habitat waterway class and waterway type

Site name	Strahler (1952) order	Key Fish Habitat: Waterway Type	Key Fish Habitat: Waterway Class
Crossing 1	4 th Order	Type 1	Class 2
Crossing 42	3 rd Order	Type 1	Class 2
Crossing 6	3 rd Order	Type 1	Class 2
Crossing 9	3 rd Order	Type 1	Class 3
Crossing 11	3 rd Order	Type 1	Class 2

4.3. Site Description

All sites are located in the South East Forest National Park at an altitude of 400 – 650m above sea level. The park was extensively burnt in the 2020 bush fires and the impacts were observable at all sites. The bush fire impacts included extensive burning of the overstory, mid story and understory. There was also evidence of direct fire impact to the waterways with minimal organic matter present amongst the riparian sedges and burnt logs in the waterways. There was also evidence of high flows with debris dams present in most waterways. Brief site descriptions are provided below for the five survey sites.

4.3.1. Crossing 1 – Reef Creek

Reef Creek is a 4th order stream that supports an intact riparian zone with the overstory dominated by *Eucalyptus* sp. (Figure 4-2 and Figure 4-3). The understory and ground cover supported good coverage with the ground cover dominated by sedges. Reef Creek is 3-5 m wide at the base and 7-10 m wide at the top of the bank. Habitat in Reef Creek is a series of pools, runs and cascades. The pools are up to 1.5m deep with long runs present. Reef Creek was flowing at the time of the survey. Substrate consisted of gravel and boulder with areas of granite bedrock. A natural barrier to fish passage in the form of a granite cascade is located downstream of the proposed crossing location. There was an extensive cover of algae (possibly brown algae) across all aquatic habitat where slow flowing water was present. The source of the algae is not known however it may be a consequence of the bushfires with a more open canopy, greater sunlight penetration to the waterway and increased nutrients associated with the sediment runoff. Fish habitat was present in the form of pools, runs and trailing bank vegetation with undercut banks present.



Figure 4-2: Reef Creek pools – Crossing 1
(Source: Austral Research and Consulting)



Figure 4-3: Natural fish barrier on Reef Creek – Crossing 1
(Source: Austral Research and Consulting)

4.3.2. Crossing 42 – Surveyor’s Gully

Surveyor’s Gully is a 3rd order stream that supports an intact riparian zone which is dominated by *Eucalyptus* sp. demonstrating extensive regrowth after the bushfires (Figure 4-4 and Figure 4-5). The mid story and understory were intact and regenerating well, groundcover was dense and supported thick beds of vegetation that was dominated by Mat Rush (*Lomandra* sp.). The site supported little flowing surface water with the stream consisting of a series of pools up to 30 m in length and four metres wide. Pools were typically 0.3 – 0.6 m deep. Submerged vegetation was present and substrate consisted of sand and silt. Fish habitat was present in the form of large woody debris (LWD), submerged and trailing bank vegetation with undercut banks.



Figure 4-4: Surveyor's Gully pools – Crossing 42 (Source: Austral Research and Consulting)



Figure 4-5: Surveyor's Gully sedge cover – Crossing 42 (Source: Austral Research and Consulting)

4.3.3. Crossing 6 – unnamed waterway

The unnamed waterway is a 3rd order stream that was relatively dry compared to other sites. The riparian zone was intact however the understory was more open compared to other sites (Figure 4-6 and Figure 4-7). Groundcover was dominated by Mat Rush, sedges (*Carex* sp.) and rushes (*Juncus* sp.). Surface water was limited at the time of the survey with the stream consisting of small pools. The largest pool was 20 m long and 3 m wide. Submerged vegetation was present and was dominated by River Buttercup (*Ranunculus* sp.) with the colonial green algal also present (*Chara* sp.). Fish habitat is present in the form of small pools with instream and trailing vegetation with limited LWD.



Figure 4-6: Pool on the unnamed waterway – Crossing 6 (Source: Austral Research and Consulting)



Figure 4-7: Vegetation on the Unnamed waterway - Crossing 6 (Source: Austral Research and Consulting)

4.3.4. Crossing 9 – Sandy Creek

Sandy Creek is a small 3rd order stream with an intact riparian zone (Figure 4-8 and Figure 4-9). The overstory is dominated by *Eucalyptus* sp. supporting large amounts of post fire regrowth. The mid story was dominated by Wattle sp. and the understory and ground cover consisted of ferns and sedges along the banks of the creek. The stream is ephemeral and the bed is up to 2 m wide and ~7 m wide at the top of the bank. There was little flow in the creek at the time of the survey. Sandy Creek consisted of low flow pool and run habitat with pools supporting high algal growth up to 0.2 m deep. A small waterfall is present downstream of the proposed crossing site and forms a natural fish barrier. Fish habitat consists of pool and run habitat, some LWD, trailing bank vegetation and undercut banks.

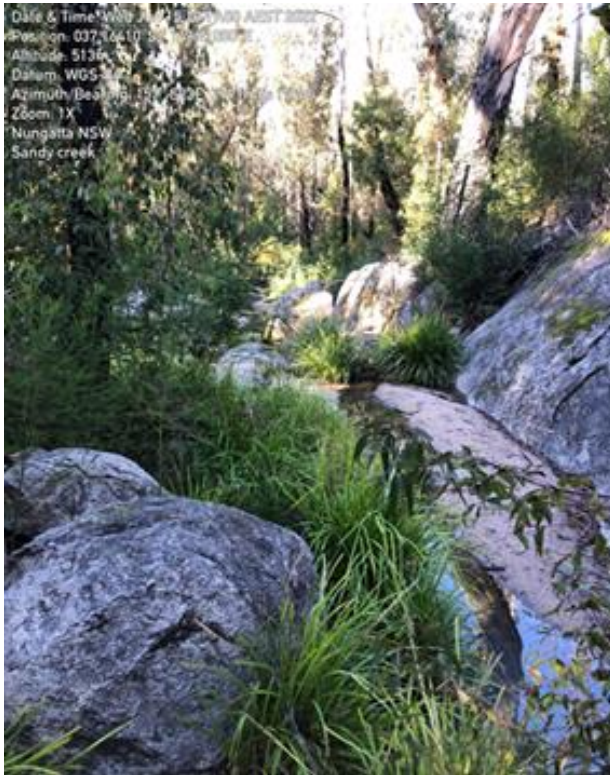


Figure 4-8: Sandy Creek with granite boulders and bedrock (Source: Austral Research and Consulting)



Figure 4-9: Sandy Creek with sandy substrate and training bank vegetation (Source: Austral Research and Consulting)

4.3.5. Crossing 11 – Donald Laing’s Creek

Donald Laing’s Creek is a 3rd order stream that supports an intact riparian zone (Figure 4-10 and Figure 4-11). The overstory was dominated by *Eucalyptus* sp. with an intact mid story and understory layer. Flows were low in Donald Laing’s Creek and the stream consisted of pool and run habitat with granite cascades. Pools were typically small with the largest approximately 20 m x 5 m and ranged from 0.4 m to 0.7 m deep. Trailing bank vegetation was present in the form of ferns and sedges with some instream vegetation present in the form of River Buttercup. Substrate consisted of sand and gravel with granite boulders. Fish habitat was present in the form of small pool and run habitat, undercut banks, LWD and coarse particulate organic matter (CPOM). There was an extensive cover of algae (possibly brown algae) across all aquatic habitat where slow flowing water was present.



Figure 4-10: Debris piles in Donald Laing's Creek (Source: Austral Research and Consulting)

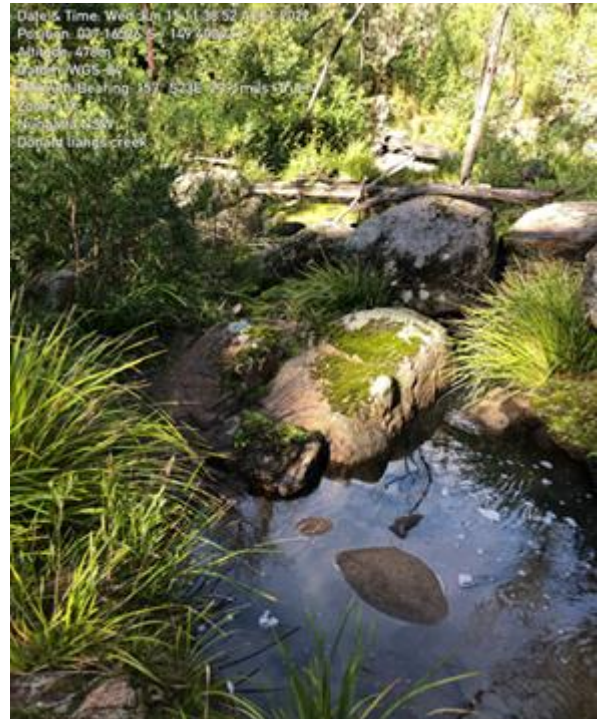


Figure 4-11: Boulders and small pools in Donald Laing's Creek (Source: Austral Research and Consulting)

4.1. Water Quality

In-situ water quality was collected at all sites and the results are presented below (Table 4-4). Temperature was relatively consistent and low across all sites ranging from 5.6°C at Crossing 11 to 7.5°C at Crossing 9 (Table 4-4) and are a reflection of seasonal variation. Dissolved oxygen (DO) was low at three sites (Crossing 6, 9 and 42) and ranged from 4.06 mg/L (33.9 percent saturation) to 5.8 mg/L (48.7 percent saturation) (Table 4-4) and was likely due to low flow conditions. DO was higher at Crossing 11 (10.16 mg/L) and Crossing 1 (8.02 mg/L) and is likely due to the increased flows observed at these sites. Conductivity was low and was below 266.4 ($\mu\text{S}/\text{cm}$) at all sites. pH values indicated that the water chemistry was slightly acidic to neutral ranging from 6.22 – 7.15 across all sites which is typical of head water streams and granite based catchments that generally have a lower acid neutralising capacity compared to limestone based catchments (Krueger and Waters 1983) resulting in slightly acidic waters. Turbidity was low across all sites ranging from 4.16 - 27.6 NTU with low turbidity expected in headwater streams and protected areas such as the National Park.

Table 4-4: Water quality results for the Nungatta Feral Predator Free area waterway crossings

Site	Temp (°C)	DO (mg/L)	DO %	Specific Conductivity @25°C (µS/cm)	Conductivity (µS/cm)	pH	Turbidity (NTU)
Crossing 1	6.0	10.16	81.7	183.9	117.2	6.95	4.16
Crossing 42	6.7	4.88	39.8	164.6	107.0	6.33	24.9
Crossing 6	7.1	4.06	33.9	114.8	75.5	6.22	11.6
Crossing 9	7.5	5.8	48.7	147.9	98.4	7.15	27.6
Crossing 11	5.6	8.02	63.7	266.4	165.7	6.74	6.98

4.2. Aquatic Survey

Abundance was low (75 fish observed) and diversity was low (three species) across all sites with a total of fish recorded at three of the five sites surveyed (Table 4-5). Reef Creek (Crossing 1) was the largest (4th order stream) waterway surveyed and supported the highest abundance and diversity with a total of 73 individuals observed from three species, Roundsnout galaxias, Australian smelt (*Retropinna semoni*) and Short-finned eel. Distribution was partially influenced by natural fish barriers (ie granite cascades) located on Reef Creek. Natural fish barriers may influence species distribution with Australian smelt only located downstream of the granite cascade. Roundsnout galaxias and Short-finned eel were both detected above the fish barrier which are not likely to restrict eel species, and galaxias species to a lesser extent.

The remaining waterways were 3rd order streams and were significantly smaller in physical size and flow volume. Given the reduced capacity and ephemeral flows observed a lower diversity of aquatic fauna was not unexpected. From the remaining sites two Roundsnout galaxias were observed at the unnamed waterway (Crossing 6). No other aquatic species were observed for the sites. The low flows and fragmented habitat connectivity as a result of low flows and natural barriers will restrict fish passage and limit the opportunity and likelihood of upstream colonisation of the waterways by fish and aquatic species.

- **Table 4-5: Fish surveys results for the Nungatta Feral Predator Free area waterway crossings**

Scientific Name	Common Name	Site (Crossing number)					Total
		1	42	6	9	11	
<i>Anguilla australis</i>	Short-finned Eel	1				1	2
<i>Galaxias terenusus</i>	Roundsnout Galaxias	64		2			66
<i>Retropinna semoni</i>	Australian smelt	8					8
Total		73	0	2	0	1	76

4.3. Platypus eDNA results

No platypus eDNA was detected at Crossing 6 and Crossing 9 from the eDNA analysis (Table 4-6). The lack of Platypus at Crossing 6 and Crossing 9 is not unexpected given the ephemeral nature of both waterways. Positive detections of platypus DNA were recorded for Crossing 1, Crossing 42 and Crossing 11 (Table 4-6).

Table 4-6: Platypus eDNA results for the Nungatta Feral Predator Free area waterway crossings

Site	Waterway	Date	qPCRs +ve	Test Result
Crossing 1	Reef Creek	15/6/22	8/9	Positive
Crossing 42	Surveyor's Gully	16/6/22	6/9	Positive
Crossing 6	Unnamed waterway	16/6/22	0/0	Negative
Crossing 9	Sandy Creek	15/6/22	0/0	Negative
Crossing 11	Donald Laing's Creek	15/6/22	5/9	Positive

The presence of Platypus at three sites has implications for the design, operation and maintenance of the predator exclusion structures constructed at the waterway crossing. The stream length and catchment area upstream of the crossing locations is provided in Table 4-7 and indicates that Crossing 1 and Crossing 11 have the largest catchment areas and stream lengths. Understanding platypus utilisation of these waterway and the potential impacts that the barriers may have on the species can be inferred based on the catchment area and stream length based on foraging behaviour and ecological requirements.

Male platypus have larger home ranges (3- 7km) (Gardner and Serena 1995) than females (2-4km long). The male home range is larger to encompass the potential for overlapping with more female home ranges. Based on the home range of males and females it is unlikely that the area within the proposed NFPFA will encompass the home range of a male Platypus but it may be possible to encompass a female platypus home range (approx 30-50km of suitable interconnected waterways is estimated to be required to support a viable platypus population in the medium term estimating 1-2 individuals per km). As the waterways are primarily 3rd order streams (excluding Crossing 1 - Reef Creek) and subject to low flows and cease to flow periods it is most likely that the area upstream of the crossing locations from part of a home range for platypus that supports opportunistic seasonal foraging. Crossing 1 (Reef Creek) is the largest waterway to be encompassed within the NFPFA and may be large enough and have a flow regime (permanent flows) suitable to permanently support one of more Platypus.

Based on the size of the NFPFA and the waterway crossings assessed there are a number of issues regarding risk to platypus that need to be considered;

1. The waterways within the NFPFA area are large enough to support a few platypus however not large enough to support a viable population within the NFPFA.
2. If platypus are excluded from upstream migrations into the NFPFA it will remove foraging habitat for some individuals and this is likely a minor impact.
3. If platypus are trapped in the NFPFA and are not able to migrate downstream the population and/or individuals will become isolated within the NFPFA.
4. Entrapment of platypus in any exclusion structures is a risk (monitoring and mitigation measures detailed in section 5.3).

Table 4-7: Catchment area and length for the Nungatta Feral Predator Free area waterway crossings. Note: shading indicates presence of platypus from eDNA analysis.

Site	Waterway	Date	Stream Length (m)	Area (km ²)
Crossing 1	Reef Creek	15/6/22	4772	7.86
Crossing 42	Surveyor's Gully	16/6/22	1749	1.46
Crossing 6	Unnamed waterway	16/6/22	1304	1.43
Crossing 9	Sandy Creek	15/6/22	2290	1.98
Crossing 11	Donald Laing's Creek	15/6/22	3240	3.95

4.4. Fish eDNA results

A summary of the fish species detected from the metabarcoding analysis is provided in Table 4-8. A total of nine taxa were identified across all sites including seven native and two introduced taxa. A *Galaxias* sp. was detected that could not be identified to species level. Roundsnout galaxias is known to occur in the area but was not present in the DNA database and may be the species detected here.

Three of the species detected were recorded in a single sample at just one site – Golden perch (*Macquaria ambigua*), Southern black bream (*Acanthopagrus butcheri*) (or another species within the genus) and. Pygmy perch sp. (*Nannoperca* sp.). While this may represent actual occurrence of the species at low density, such results may also represent site or sample contamination from vectors such as predator scats, human activities, or laboratory processes (although this is unlikely as all negative controls were negative for any DNA). Therefore, such results should be interpreted as low probability of occurrence. This coupled with the knowledge of the species would suggest that neither Golden perch or Southern black bream are present at these sites while it would be possible for Pygmy perch to be present.

The eDNA results confirm the findings from the field survey (Table 4-5) with the exception of Australian smelt at Crossing 1 (Reef Creek) which was not detected via eDNA. It is not known why there was no eDNA detection in the samples from Crossing 1.

The eDNA analysis suggests that there is a number of additional fish species that are likely to be present across the sites including, Goldfish (*Carassius auratus*) or Common carp (*Cyprinus carpio*) which were detected at Crossing 1, 11 and 42. Climbing galaxias (*Galaxias brevipinnis*) were detected from Crossing 1. Redfin perch (*Perca fluviatilis*) were detected at all Crossings locations except Crossing 6 and Australian smelt was identified at Crossing 11. Climbing galaxias are a species that is known to move long distances into the headwaters of streams past barriers and would be expected to occur in habitats present in the NFPFA and passed on the eDNA detections are considered likely to occur in the area. The habitat available in the NFPFA is less suitable for Redfin perch and Goldfish or Common carp but based on the strong eDNA detections it is considered possible that these species are likely to occur.

The results of eDNA analysis completed need further assessment before species other than Climbing galaxias are considered resident in the NFPFA. Climbing galaxias are considered to be present based on the known habitat of the species and the eDNA results.

Table 4-8: Positive results from the FISH biodiversity assay for each site with explanatory footnotes below. Results presented are the number of positive results from the three samples collected at each site.

Scientific names	Common names	Crossing				
		1	9	6	11	42
<i>Acanthopagrus butcheri</i> (1)	Southern black bream	-	1/3	-	-	-
<i>Anguilla australis</i>	Australian shortfin eel	2/3	-	-	2/3	1/3
<i>Carassius auratus</i> or <i>Cyprinus carpio</i>	Goldfish or Common carp	2/3	1/3	1/3	3/3	2/3
<i>Galaxias brevipinnis</i>	Climbing galaxias	3/3	-	1/3	-	-
<i>Galaxias</i> sp.	genus of small, native freshwater fish	3/3	-	-	-	1/3
<i>Macquaria ambigua</i>	Golden perch, Yellowbelly	-	-	-	1/3	-
<i>Nannoperca</i> sp.	genus of Australian pygmy perch	-	-	-	1/3	-
<i>Perca fluviatilis</i>	Redfin perch	1/3	2/3	-	3/3	2/3
<i>Retropinna semoni</i>	Australian smelt	-	-	-	2/3	-

Note – 1 = this species is the best match from the DNA library but there are other species from the genus that co-occur in the area for which the lab have no genetic data at the marker used.

4.4.1. Likelihood of aquatic fauna

Based on the desktop assessment, field survey results, site inspections and eDNA results the likelihood of the species identified in the bionet search and additional species considered on site is presented below as, unlikely, possible, low, moderate, high and definite (detected). Based on the likelihood of species presence from Table 4-9 the potential impacts and risks have to these species have been considered further in section 5.

Only three native fish species were identified: Australian smelt, Roundsnout galaxias and Short-finned eel (Table 4-9) in field surveys. Other species considered to be present based on eDNA results and the ecology of the species are Climbing galaxias. Long-finned eel (*Anguilla reinhardtii*) have been considered to be possibly present based on the known distribution of the species and confirmed presence of the Short-finned eel.

Common carp, Goldfish and Redfin perch were considered a moderate likelihood of being present based on eDNA evidence only and requires further confirmation (field survey or addition eDNA data).

No turtle species are considered likely to occur based on the habitat available, the substrate type and low and ephemeral nature of many of the waterways surveyed.

No Platypus were observed during field surveys however eDNA analysis confirmed they are present in three of the waterways surveyed.

A range of aquatic invertebrate species (*Euastacas* sp., *Cherax* sp., and *Engaus* sp.) were considered while undertaking the site assessment and surveys. None were identified from the desktop assessment, and none were observed during the field surveys. Site inspections also failed to identify burrows or chimneys that are evidence of the presence of burrowing crayfish species (*Engaus* sp.). The presence of crayfish species is still considered possible based on the habitat values and hydrology.

Table 4-9: Aquatic fauna identified and/or likelihood of species to be present based on desktop assessments, field surveys, eDNA analysis and site inspections.

Common Name	Species Name	Conservation Listing	LoO
<u>Native Fish Species</u>			
Australian bass	<i>Macquaria novemaculeata</i>	-	Unlikely
Australian grayling	<i>Prototroctes maraena</i>	EPBC Act	Unlikely
Australian smelt	<i>Retropinna semoni</i>	-	Definite
Climbing galaxias	<i>Galaxias brevipinnis</i>	-	Definite
Common galaxias	<i>Galaxias maculatus</i>	-	Unlikely
Cox's gudgeon	<i>Gobiomorphus coxii</i>	-	Unlikely
Empire gudgeon	<i>Hypseleotris compressa</i>	-	Unlikely
Firetail gudgeon	<i>Hypseleotris galii</i>	-	Unlikely
Flathead gudgeon	<i>Philypnodon grandiceps</i>	-	Unlikely
Long-finned Eel	<i>Anguilla reinhardtii</i>	-	Possible
Macquarie perch	<i>Macquaria australasica</i>	EPBC Act; FM Act	Unlikely
Roundsnout galaxias	<i>Galaxias terenusus</i>	-	Definite
Short-finned eel	<i>Anguilla australis</i>	-	Definite
Striped gudgeon	<i>Gobiomorphus australis</i>	-	Unlikely
<u>Exotic Species</u>			
Common carp	<i>Cyprinus carpio</i>	-	Moderate
Eastern mosquitofish	<i>Gambusia holbrooki</i>	-	Unlikely
Gold fish	<i>Carassius auratus</i>	-	Moderate
Redfin perch	<i>Perca fluviatilis</i>	-	Moderate
<u>Reptiles</u>			
Eastern snake-necked turtle	<i>Chelodina longicollis</i>	-	Unlikely
Macquarie River turtle	<i>Emydura macquarii macquarii</i>	-	Unlikely
<u>Mammals</u>			
Platypus	<i>Ornithorhynchus anatinus</i>	PL	Definite
<u>Invertebrates</u>			
Burrowing crayfish	<i>Engaus</i> sp.	-	Possible
Spiny crayfish	<i>Euastacus</i> sp.	-	Possible
Yabbie	<i>Cherax</i> sp.	-	Possible

5. Barrier and Exclusions

The predator proof fencing will be constructed from a fencing mesh using a 30mm mesh. Culverts and bridge crossings provide a range of difficulties when attempting to restrict predator access due to the presence of water flows and an unstable and dynamic substrate in the form of the river or creek bed. A number of options are available for the protection of culverts, floodways and creek crossing (Long and Robley 2004) and the preferred options for Nungatta will be discussed below.

5.1. Culvert barriers

The proposed method for protecting the culverts from predator access is to fit a hinged grill to the culvert with a 30mm aperture and would be similar to Figure 5-1 A and B, noting that the exclusion material will be a 30mm grill.

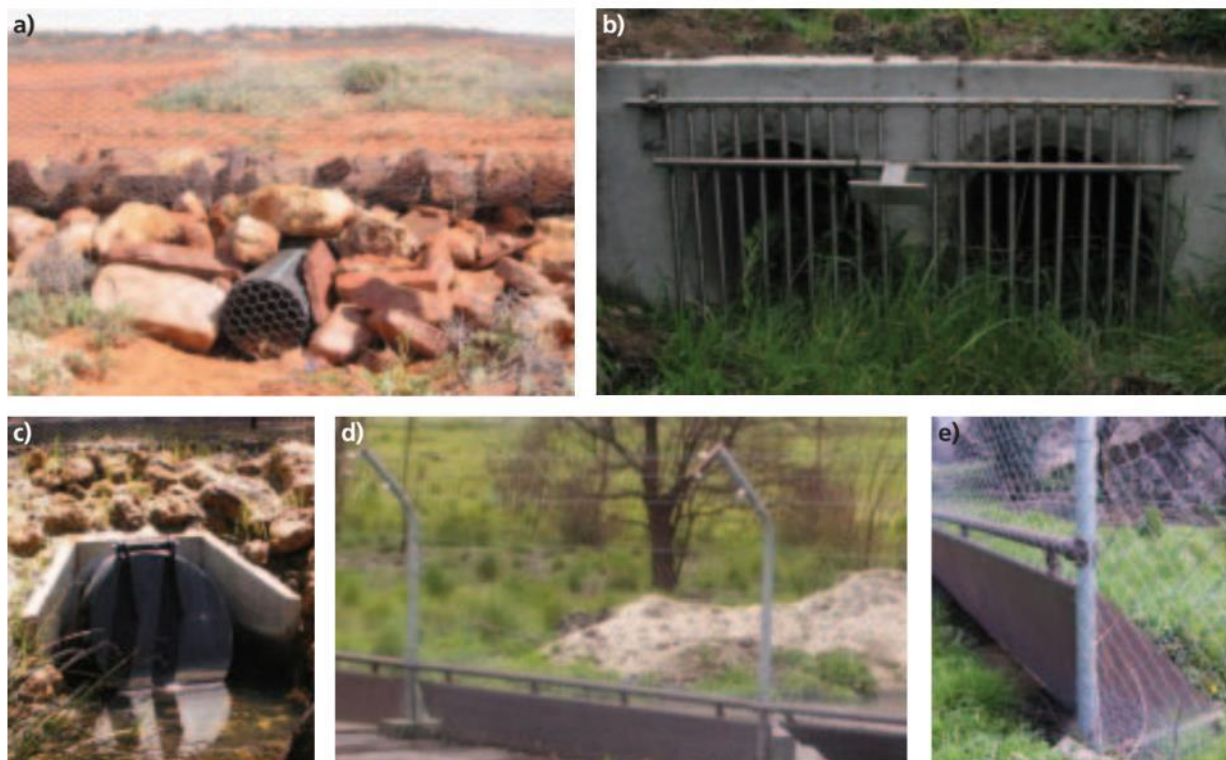


Figure 3. Waterway crossings.

a) Plastic pipes in rock-beds are sufficient for minor ephemeral drainage lines. b) and c) culverts suitable for creeks and drainage lines. Hinged grills b) and covers c) permit variable water flows but exclude feral animals. d) Floodgates, preferably with metal sidings d) can be used to cope with highly variable water flows.

Figure 5-1: Examples of predator proof measures for culverts and waterway crossings (Long and Robley 2004).

5.2. Bridge Barriers

The proposed method for excluding foxes and cats from over passes and bridges is particularly difficult due to the topography of the creek lines and waterways and the potential for this to change with environmental conditions. The current proposal is to have a heavy net fixed to the bridge/overpass (Figure 5-2) that will drape down and lay flat on the creek bed with an apron extending along the bed to prevent the passage of animals larger than the 30 mm aperture. The proposed net will be in a number of panels in an arrangement similar to that pictured in Figure 5-1 D and E. This arrangement will allow for smaller flows to pass through the net/barrier. Under very high flow conditions it's likely that the net may be lifted, and debris washed under the barrier, as flow decrease the barrier will be repositioned on the bed of the creek or onto of debris potentially compromising the integrity of the exclusion barrier and providing opportunities for predators to enter the NFPFA. Floats attached to the netting or sumps under the stream bed may be used to allow larger fish passage though permanent waterholes.



Figure 5-2: Mock-up of a net exclusion barrier as described for the proposed bridge crossings with floats and bracing in the middle of the net to maintain the structure of the excluder and weighted rope at the bottom (Source: Austral Research and Consulting).

5.3. Potential impacts

The greatest risk associated with the culverts and bridge exclusion barriers is the entrapment of material and or fauna within the grill or netting. The specific impacts can be summarised as;

- 1) Entrapment of fauna – The fish fauna present included Eels and small bodied species such as Australian smelt, Roundsnout galaxias and Climbing galaxias. These species will be able to pass through the proposed 30mm mesh sized materials. The natural topography of the NFPFA limits the likelihood of turtle species being present. While no individuals or evidence of any invertebrate or crayfish species was detected crayfish species are still considered to possibly be present and should be considered when assessing risks to fauna. Burrowing crayfish species (*Engaus* sp.) and yabbies (*Cherax* sp.) are unlikely to be impacted by the exclusion grills or netting based on their smaller size. Some species of spiny crayfish (*Euastacus* sp.) can grow to a large size and the proposed 30mm grills could exclude the movement of larger individuals. Smaller Spiny crayfish individuals would be able to pass through the exclusion grill.
- 2) Entrapment of fauna – the identified exotic species including Goldfish, Common carp, and Redfin perch would likely be entrapped in the exclusion barriers as adults. Juveniles of all three species will be able to pass the exclusion barrier.
- 3) Impacts to platypus – the construction of the NFPFA has the potential to exclude some platypus from what is most likely seasonal and opportunistic foraging habitat in Surveyors Gully (Crossing 42) and Donald Laing's Creek (Crossing 11). The construction of the NFPFA has the potential to exclude and isolate platypus in Reef Creek (Crossing 1) in the short term and will likely result in the long term loss of the species from the area within the NFPFA if movements are restricted. Movement of adults past the barriers proposed for culverts is likely to be limited with downstream passage possible during high flows when the hinged excluders open. Barriers proposed for the bridge crossings are likely to prevent upstream movements of adults entirely, while downstream movements may be possible in high flow periods. Entrapment of adult platypus in the excluders will need to be considered. It is possible that juvenile platypus are able to pass through the 30mm netting.

If permanent exclusion of platypus from the NFPFA is desired and or deemed an acceptable outcome, monitoring, trapping and relocating to waterways outside of the NFPFA would be possible. This should not be undertaken during breeding season (Oct – March). A trapping and relocation plan would need to be developed.

- 4) Entrapment of debris in culverts - The capture of material in the grill or net could result in damage to the structure or the development of a debris dam that allows water to backup behind the culvert or bridge resulting in flows being bypassed around the structure with a risk of erosion. Culverts should be appropriately sized and potentially oversized to offset the risk of a culvert blocking. The hinges on the grills will allow for debris to be swept from the culvert as the water

pressure behind the build up becomes too great and the grill is lifted. This should allow for the debris to be swept away with the flow.

Post event inspections (immediately after the event when it is safe to inspect) and routine inspections and maintenance (every 2-3 days) of the culverts are proposed. This inspection regime should minimise the risk of blockages of the grills with debris.

- 5) Loss of fauna exclusion post event – the integrity of the fauna exclusions on both the culverts and the bridges could fail, partially or completely, during and after high rainfall events. It is likely that the construction of the proposed bridge excluders is more prone to temporary failure post event due to the separate independent sections that make up the structure and the likelihood of larger snags becoming entangled in the net. While not reducing the risk to aquatic fauna from an entanglement perspective the consideration of including a composite construction method may improve the integrity of the bridge excluders. Upstream large debris traps may reduce the potential for failures during very high flow events. A barrier that included some solid panels that would force the net up during moderate to high flows (as water can't pass through the material) this may reduce the potential for the entanglement of the LWD and snags which could compromise the integrity of the excluders as flows reduce to normal.

6. Conclusions

Assessments included classification of Key Fish Habitat for each site and fish surveys to determine abundance, diversity and distribution of fish species within the study area. eDNA analysis was undertaken for all sites to both determine the presence/absence of platypus and further inform on fish species utilising the study area.

The assessment revealed that all sites supported high value key fish habitat and included good fish habitat in the form of pools, trailing bank vegetation, undercut banks, and LWD. While habitat values were high, the ephemeral nature of the water ways inspected with the exception of crossing 1 (Reef Creek) had a reduced likelihood of supporting fish due to connectivity and the presence of many natural barriers. The 2020 bushfires and low flows associated with this period also likely resulted in many refuge pools drying out with the loss of any fish species present. Natural fish barriers were also present on Reef Creek (Crossing 1) and likely limit fish and aquatic fauna passage.

Fish surveys revealed the study area supports a low diversity and abundance of fish with only three fish species observed. No fish species supported an elevated conservation listing warranting further investigation. The presence of the proposed exclusion barriers on culvert or bridge crossing are not expected to impact the fish community identified during the current investigation.

The presence of Platypus was confirmed via eDNA in three of the waterways inspected and their presence and utilisation of the NFPFA will need to be considered further. There are risks to the health of individual platypus' with respect to movement. However, it is considered unlikely that a significant population of platypus would be impacted. These risks will need to be considered when finalising the designs of the exclusions for both the culverts and bridge crossings.

Spiny Crayfish species were not identified but are possibly present and larger individuals may become entangled in the proposed barrier material.

Turtles are unlikely to be present due to the geology and ephemeral nature of the waterways in the area.

While identified in the PMST search the presence of Australian grayling is unlikely based on the natural fish barriers present in the study area due to the natural geology.

The routine monitoring and maintenance of the exclusion barriers in the waterways should specifically include the inspection for aquatic fauna. If any aquatic fauna are observed to be entangled then appropriate measures to free the individual should be undertaken. If an aquatic species that was not identified during the current surveys is found to be impacted by the exclusion barriers mitigation measures should be considered. Details of all trapped animals should be recorded and impact of the barriers reviewed regularly.

5. References

- Australian Museum, 2019. Platypus. [Online] Available at: <https://australianmuseum.net.au/learn/animals/mammals/platypus/>
- Bethge, P., Munks, S., Otley, H. & Nicol, S., 2003. Diving behaviour, dive cycles and aerobic dive limit in the Platypus *Ornithorhynchus anatinus*. *Comparative Biochemistry and Physiology Part A. Molecular and Integrative Physiology*, Volume 136, pp. 799-809.
- Biggs J., Ewald N., Valentini A. et al., 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.029. [online]
- Bino, G. et al., 2019. The platypus: evolutionary history, biology, and an uncertain future. *Journal of Mammalogy*, pp. Volume 100, Issue 2, pp 308–327.
- Darling J. A., Jerde C. L. & Sepulveda A. J. (2021) What do you mean by false positive? *Environ. DNA* n/a. [online]. Available from: <https://doi.org/10.1002/edn3.194>.
- Department of Agriculture, Water and the Environment, 2022. Protected Matters Search Tool. [WWW Document]. (Accessed 16/6/2022). URL: <https://pmst.awe.gov.au/#/map?lng=131.50634765625003&lat=28.671310915880834&zoom=5&baseLayers=Imagery>.
- Department of Agriculture, Water and the Environment, 2021. Brogo wet vine forest in the South East Corner Bioregion - endangered ecological community listing. [WWW Document]. URL: <https://www.environment.nsw.gov.au/topics/animals-and-plants/threatened-species/nsw-threatened-species-scientific-committee/determinations/final-determinations/2000-2003/brogo-wet-vine-forest-in-the-south-east-corner-bioregion-endangered-ecological-community-listing>.
- Department of Agriculture, Water and the Environment, 2020. Wildlife and threatened species bushfire recovery research and resources. [WWW Document]. URL: <https://www.environment.gov.au/biodiversity/bushfire-recovery/research-and-resources>.
- DSEWPaC (2011). Survey guidelines for Australia's threatened fish: Guidelines for detecting fish listed as threatened under the Environment Protection and Biodiversity Conservation Act 1999. The Australian Department of Sustainability, Environment, Water, Population and Communities. <https://www.awe.gov.au/sites/default/files/documents/survey-guidelines-fish.pdf>.
- Ellam, B. A., Bryant, A. & O'Connor, A., 1998. Statistical modelling of platypus, *Ornithorhynchus anatinus*, habitat preferences using generalised linear modelling. *Australian Mammalogy*.

- Ganassin, C. (30/3/2022). NSW Fisheries acceptance of crossing types proposed for the Nungatta Feral Predator Free Area, NSW Department of Primary Industries. [Email].
- Gardner, J. L and Serena, M., 1995. Spatial-Organization and Movement Patterns of Adult Male Platypus, *Ornithorhynchus-Anatinus* (Monotremata, Ornithorhynchidae). *Australian Journal of Zoology* 43 (1) 91 – 103.
- Grant, T., 2004. Depth and substrate selection by Platypuses, *Ornithorhynchus anatinus*, in the lower Hastings River, New South Wales. *Proceedings of the Linnean Society of New South Wales*.
- Grant, T. R., 1992. Historical and current distribution of the platypus, *Ornithorhynchus anatinus*, In Australia. *Platypus and Echidnas*, pp. 232-254.
- Grant, T. R. & Temple-Smith, P. D., 2003. Conservation of the Platypus, *Ornithorhynchus anatinus*: Threats and challenges. *Aquatic Ecosystem Health and Management*, Volume 6, pp. 5-18.
- Grant, T. R. & Temple-Smith, P. D., 1998. Field biology of the platypus (*Ornithorhynchus anatinus*): Historical and current perspectives. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, Volume 353, pp. 1081-1091.
- Griffiths, J., Kelly, T. & Weeks, A., 2014. Impacts of high flows on platypus movement and habitat use in an urban stream, Parkville: s.n.
- Gust, N. & Handasyde, K., 1995. Seasonal Variation in the Ranging Behaviour in platypus (*Ornithorhynchus anatinus*) on the Goulburn River, Victoria. *Australian Journal of Zoology*, Volume 43, pp. 193-208.
- Holland, N. & Jackson, S. M., 2002. Reproductive behaviour and food consumption associated with the captive breeding of platypus (*Ornithorhynchus anatinus*). *Journal of Zoology*.
- Kelly, T., Griffiths, J. & Weeks, A., 2012. Development of a novel tracking technique for Platypuses using acoustic telemetry., Parkville: CESAR.
- Krueger CC. and Waters TF., 1983. Annual production of macroinvertebrates in three streams of different water quality. *Ecology* 64(4):840–850.
- Krueger, B., Hunter, S. & Serena, M., 1992. Husbandry, diet and behaviour of Platypus *Ornithorhynchus anatinus* at Healesville Sanctuary. *International Zoo Yearbook*.
- Long, K. and Robley, A. 2004. Cost Effective Feral Animal Exclusion Fencing for Areas of High Conservation Value, Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, Victoria. Report prepared for the Australian Government, The department of Environment and Heritage.

Lugg W. H., Griffiths J., van Rooyen A. R., Weeks A. R. & Tingley R., 2018. Optimal survey designs for environmental DNA sampling. *Methods Ecol. Evol.* 9, 1049–1059.

McColl-Gausden E. F., Weeks A. R., Coleman R. A. et al. (2020) Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Mol. Ecol.* n/a. [online]. URL: <https://doi.org/10.1111/mec.15644>.

McColl-Gausden E. F., Weeks A. R. & Tingley R., 2019. A field ecologist's guide to environmental DNA sampling in freshwater environments. *Aust. Zool.* doi:10.7882/az.2019.025.

Miles, J. (2021). Nungatta Feral Predator Free Area Flora Survey. Report prepared for NSW Department of Planning and Environment.

NSW Department of Planning and Environment, 2021. National Wildlife Parks and Service. Feral Free Area, South East Forest National Park – Fact Sheet.

NSW Department of Planning and Environment, 2022. BioNet. [WWW Document]. (Accessed 16/6/2022). URL: https://www.environment.nsw.gov.au/atlaspublicapp/ui_modules/atlas_atlassearch.aspx.

NSW Department of Planning, Industry and Environment, 2021. Feral Free Area: South East Forest national park.

NSW Department of Planning, Industry and Environment, 2003. Bioregions of New South Wales: South East Corner. Published by: National Parks and Wildlife Service. URL: <https://www.environment.nsw.gov.au/research-and-publications/publications-search/south-east-corner-bioregion>.

NSW Department of Primary Industries, 2022a. Fisheries NSW Spatial Data Portal. [WWW Document]. (Accessed 16/6/2022). URL: https://webmap.industry.nsw.gov.au/Html5Viewer/index.html?viewer=Fisheries_Data_Portal.

NSW Department of Primary Industries, 2022b. Fish Stocking. [WWW Document]. Accessed 16/6/2022). URL: <https://www.dpi.nsw.gov.au/fishing/recreational/resources/stocking>.

NSW Department of Primary Industries, 2013. *Policy and guidelines for fish habitat conservation and management*, Sydney: NSW Department of Primary Industries.

Otley, H. M., Munks, S. A. & Hindell, M. A., 2000. Activity patterns, movements and burrows of Platypuses (*Ornithorhynchus anatinus*) in a sub-alpine Tasmanian lake. *Australian Journal of Zoology*, Volume 48, pp. 701-713

Rees H. C., Maddison B. C., Middleditch D. J., Patmore J. R. M. & Gough K. C., 2014. REVIEW: The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* 51, 1450–1459. [online]

- Rognes T., Flouri T., Nichols B., Quince C. & Mahé F. (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ*.
- Riaz T., Shehzad W., Viari A., Pompanon F., Taberlet P. & Coissac E. (2011) ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Res.* 39, e145–e145. [online]. Available from: <https://pubmed.ncbi.nlm.nih.gov/21930509>.
- Serena, M., 1998. Use of time and space by Platypus (*Ornithorhynchus anatinus*: Monotremata) along a Victorian stream. *Journal of Zoology*, Volume 232, pp. 117 - 131.
- Serena, M., Williams, G. A., Weeks, A. R. & Griffiths, J., 2014. Variation in Platypus (*Ornithorhynchus anatinus*) life-history attributes and population trajectories in urban streams. *Australian Journal of Zoology*, Volume 62, pp. 223-234.
- Serena, M., Worley, M., Swinnerton, M. & Williams, G. A., 2001. Effect of food availability and habitat on the distribution of Platypuses (*Ornithorhynchus anatinus*) foraging activity. *Australian Journal of Zoology*, Volume 49, pp. 263-277.
- Serena, M., Thomas, J. L. & Williams, G. A., 1998. Status and habitat relationships of Platypus in the Dandenong Creek Catchment: II. Results of surveys and radio-tracking studies, September 1997 - March 1998, Whittlesea: Australian Platypus Conservancy.
- Smart A. S., Tingley R., Weeks A. R., Van Rooyen A. R. & McCarthy M. A., 2015. Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol. Appl.* 25, 1944–1952
- Strahler, A., 1952. Dynamic Basis of Geomorphology. *Geological Society of America Bulletin*, Volume 63, pp. 923-938.
- Temple-Smith, P. D. & Grant, T. R., 2003. Conservation of the Platypus, *Ornithorhynchus anatinus*: Threats and Challenges. *Aquatic Ecosystem Health & Management*, pp. 6(1):5-18.
- Thackway, R. & Cresswell, I., 1995. An interim biogeographic regionalisation for Australia: a framework for setting priorities in the National Reserves System Cooperative Program. Australian Nature Conservation Agency. Canberra, ACT.
- Thomsen P. F., Kielgast J., Iversen L. L. et al., 2012. Monitoring endangered freshwater Biodiversity using environmental DNA. *Mol. Ecol.* 21, 2565–2573.
- Thomsen P. F. & Willerslev E., 2015. Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.019. [online].
- Valentini A., Taberlet P., Miaud C. et al., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25, 929–942.

Woinarski, J. & Burbidge, A. A., 2016. *Ornithorhynchus anatinus*. The IUCN Red List of Threatened Species. s.l., s.n.

Woinarski, J. C., Burbidge, A. A. & Harrison, P. L., 2014. The action plan for Australian mammals 2012. Collingwood: CSIRO Publishing.

Worley, M. & Serena, M., 2000. Ecology and conservation of Platypuses in the Wimmera River catchment. IV. Results of habitat studies, Whittlesea: Australian Platypus Conservancy.

Yamanaka H., Motozawa H., Tsuji S., Miyazawa R. C., Takahara T. & Minamoto T., 2016. On-site filtration of water samples for environmental DNA analysis to avoid DNA degradation during transportation. *Ecol. Res.* 31, 963–967.